Pediatric Endocrinology

FIFTH EDITION

Volume 2
Growth, Adrenal, Sexual, Thyroid, Calcium, and Fluid Balance Disorders

EDITED BY

Fima Lifshitz

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"Gracias a la vida que me ha dado tanto"
and has given me health to enjoy my life
and wisdom to do right.

Fima Lifshitz, M.D.

Foreword

As Osler once said, ''Read with two objects: first, to acquaint yourself with the current knowledge on a subject and the steps by which it has been reached; and second and more important, read to understand and analyze your cases.'' The fifth edition of the textbook of *Pediatric Endocrinology* provides the reader with an opportunity to meet both objectives, combining an update on the latest developments in the field of pediatric endocrinology with providing practical information on how this knowledge can be applied to patient care.

Books are a reflection of their times. It is not surprising, therefore, that the evolution of pediatric endocrinology over the past two decades is reflected by the changes that have occurred in this textbook since its initial printing in 1985. The first edition of *Pediatric Endocrinology* consisted of 27 chapters with 668 pages; the current edition consists of two volumes containing 53 chapters with a total of more than 1300 pages. The expansion in the size of the book reflects the rapid expansion of knowledge that has occurred in the field over the last 22 years. In 1985, only three chapters with 60 pages were devoted to the diagnosis and treatment of Type 1 diabetes mellitus and ketoacidosis, while Type 2 diabetes and obesity were not addressed. The current edition has been split into two separate but complementary volumes; the first volume covering disorders of carbohydrate metabolism (obesity, T1DM, T2DM, insulin resistance, and hypoglycemia) in 20 chapters with 510 pages and the second volume dealing with ''traditional'' endocrinology (growth, adrenal, sexual, thyroid, calcium, and fluid balance) in 33 chapters with 768 pages. The increase in the number of chapters and pages devoted to obesity and diabetes reflects the increased prevalence of these disorders in the pediatric population and is concordant with the patient distribution in many pediatric endocrine practices.

This book, therefore, provides an in-depth coverage of the disease states seen in the early 21st century. It provides the readership with an opportunity to explore the wonders of the science and the clinical breadth of pediatric endocrinology by just turning the pages. Osler wisely said, ''To study the phenomena of disease without books is to sail an uncharted sea, while to study books without patients is to not go to sea at all.'' This book provides the sail, the boat, and the rudder; the clinicians must determine how to apply it to their patients.

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The previous four editions of *Pediatric Endocrinology*, progressively, have been a dominating educational tool for subspecialists in pediatric endocrinology, genetics, nutrition, etc., and for pediatric generalists. The fifth edition exceeds my expectations and will exceed yours. The expansion of the text into two volumes could be discouraging to the prospective owner. It should not be. The educational leadership and organizational talent of Dr. Lifshitz have provided us with a text that now has necessarily expanded into two volumes as the scope of the specialty expanded. The new organization enhances the use of this textbook, permitting the reader initially to be very focused in obtaining the information he/she seeks, but it subsequently supplements that information as time permits by going to other chapters, each written by different well-known authors. Examination of Dr. Lifshitz's preface of this text and the indexed outline of chapters permits confirmation of my conclusion.

The multiple contributing authors also deserve strong commendations for the excellent content of this two volume text. The collaboration of multiple authors for each subsection permits the presentation of broader perspectives than if a single author had been responsible.

I have considered the presence of each of the previous four editions of *Pediatric Endocrinology* a necessity on my shelf because of their high quality as a diagnostic and therapeutic tool in the practice of pediatrics and pediatric endocrinology. The fifth edition similarly will be a necessary and welcomed addition on the shelf reserved for my favorite textbooks. I personally extend my thanks and congratulations to Dr. Lifshitz and to each of his contributors in producing this excellent and timely textbook.

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Foreword

Dr. Fima Lifshitz's expansive view of the scope of pediatric endocrinology is reflected in the fifth edition of his classic textbook *Pediatric Endocrinology,* first published in 1985. Each edition has improved upon its predecessor by adding new chapters on topics relevant to the rapidly developing science and changing the practice of pediatric endocrinology. Unlike other textbooks of pediatric endocrinology, which tend to be largely devoted to classical pediatric endocrine subjects, Lifshitz's *Pediatric Endocrinology* has an expanded scope as it covers not only these subjects but is equally devoted to what might be referred to as Metabolic Endocrinology thereby reflecting the true practice of the specialty. The latter includes the various forms of diabetes mellitus, hypoglycemia, obesity and its related disorders, insulin resistance, lipid disorders and various genetic disorders of metabolism.

With 53 fully inclusive chapters, the fifth edition of *Pediatric Endocrinology* has expanded to two volumes. A substantial portion of volume one is devoted to metabolic endocrinology, whereas volume two contains chapters on all the classical topics in pediatric endocrinology including growth, adrenal, sexual, thyroid, calcium, and fluid balance disorders as well as other miscellaneous endocrine alterations. This textbook also provides valuable information on a variety of topics relevant to pediatricians, pediatric endocrinologists and academic clinicians throughout the world. The two volumes complement each other and together provide comprehensive coverage of the contemporary practice of the expanded scope of pediatric endocrinology and metabolism.

Dr. Lifshitz has carefully selected pertinent topics and superb authors, all experts in their respective fields, who are both investigators and clinicians. As a result, *Pediatric Endocrinology* is characterized by an exceptional blend of rigorous scholarship and pragmatism, which undoubtedly contributes to its broad appeal and will ensure its continued success.

I congratulate Dr. Lifshitz on admirably accomplishing the monumental task of editing, unassisted, a textbook of this magnitude and complexity and ensuring that each chapter meets his exacting standards of scholarship, clinical relevance and clarity of exposition. It has been an honor and a privilege to serve as a contributor to *Pediatric Endocrinology* and to write this Foreword.

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In this age of molecular science, our knowledge of the genetic basis of endocrine disorders is rapidly expanding. As we come to understand the etiology of the disease with pinpoint accuracy, our treatments become more effective and tailored to our patients' needs. Current molecular research impacts treatment when clinicians can use such findings to guide their therapies. This textbook serves as a crucial bridge between the molecular laboratory and the clinical practice, encouraging translational research.

Dr. Fima Lifshitz has again put together an authoritative, current and important text to serve the burgeoning field of pediatric endocrinology. I know that it will continue to be an invaluable tool for both clinicians and researchers.

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Preface

> The good life is one inspired by love and guided by knowledge. —*Bertrand Russell*

The fifth edition of *Pediatric Endocrinology* marks the 22nd anniversary of this textbook. This edition has built upon the accumulated experience of the previous versions and every one of the chapters has been thoroughly updated; thereby its content enhances the reputation that *Pediatric Endocrinology* has enjoyed as ''the classic book in the field.'' Each of the topics of the chapters of the fifth edition of this book addresses one of the many potential alterations of patients referred to the pediatric endocrinologist for evaluation and treatment. Together, they provide the most updated information needed by the physician caring for these children, yet written with the detail required by the subspecialist in academic settings. The chapters are written in a didactic manner, containing practical information, with comprehensive discussions that address all clinical situations. Thereby, the book serves to increase the knowledge of both the practitioner and the subspecialist. The fifth edition of *Pediatric Endocrinology* constitutes a state-of-the art textbook, written by mature, well-established contributors who transmit their knowledge in an erudite manner, covering the theoretical and the clinical considerations of each entity.

Since the first edition of *Pediatric Endocrinology* published in 1985, the field has grown and has evolved. The state of knowledge and the scientific basis of the practice of the specialty are markedly different from that of two decades ago. This edition encompasses the current status of the specialty and the care of patients with pediatric endocrine diseases. The ever-increasing scope of the science of endocrinology and the rapid acquisition of new knowledge are captured and synthesized in each chapter by experts in all aspects of the specialty. The clinical care and practical aspects of pediatric endocrinology are written by those who are committed to the practice of the specialty.

The fifth edition of *Pediatric Endocrinology* comprises two volumes—each one dealing with major areas in the field. The expanded version of the textbook allows a comprehensive review of the multiple advances and provides the reader the factual information to address all the concerns that arise when caring for children with endocrine-related alterations. Each one of these two books contains comprehensive chapters of specific entities that contain sufficient detailed information to cover the topic in its entirety. Thereby, each volume constitutes a book in its own right, yet both complement each other and together they form the resource in the field in an integrated easy-to-read and clearly written manner.

Volume 1 of *Pediatric Endocrinology* is devoted to obesity, diabetes mellitus, insulin resistance and hypoglycemia, with a special section on private practice and clinical research. Currently, caring for patients with these diseases constitutes a major part of the pediatric endocrinologist's time and effort. Thus, the expanded review of these topics reflects the true state of the specialty. Whereas previous editions of this book already contained chapters dealing with obesity and diabetes in children, long before these entities attracted the full attention of pediatric endocrinologists; other texts in the field have barely addressed these topics. However in this fifth edition of *Pediatric Endocrinology,* these entities are fully expanded to provide the reader with a substantive appraisal of the subjects and of the current issues. The major public health problem of obesity is most blatantly visible; yet it is often a neglected disease. In this book, obesity is discussed from a pediatric endocrinologist's perspective, with attention given to all aspects of the disease; including the epidemic and the mechanisms of the illness. The genetics and the single gene disorders that are manifested with obesity are reviewed as are the prevention and treatment of this disorder and the comorbidities. Included are also the chapters that address the current state of knowledge of the insulin resistance/metabolic syndrome and the diseases that often result from insulin resistance, such as hypertension and hyperlipidemia. The long-term endocrine alterations that follow the birth of a small-for-gestational-age infant are reviewed, with particular detail to the development of the insulin resistance syndrome, appearing later on in the life of such children.

There was a time when pediatric endocrinologists were not involved with the care of children with diabetes or with the teaching and research of this disorder; that was the past. Currently, the care for such patients demands the attention of the specialist; pediatric endocrinologists are now intimately involved in providing care and advancing the knowledge of the disease through clinical and basic science research. This is evident in each of the chapters of this book, which pertain to all aspects of diabetes mellitus. Included in the book is an update of the new clinical multicenter research programs designed to address the causes of Type 1 diabetes and chapters dealing with the theoretical and practical aspects of the care of such patients. Also, there is an expanded chapter dealing with Type 2 diabetes mellitus because this disease has become a more prominent area for the pediatric endocrinology specialty. In the section on hypoglycemia, the disorders that produce this alteration are reviewed with attention paid to the pathophysiology, its causes and the treatment, both in children and in neonates. The emergencies that pediatric endocrinologists deal when consulted for patients with inborn errors of metabolism are thoroughly addressed and the norms for the assessment of newborn screening alterations are provided. Finally, there are new chapters dealing with the current realities in the field, namely the private practice of the subspecialty and the performance of clinical trials by both the academic pediatric endocrinologist and the physician committed to patient care. There is also a comprehensive reference resource containing frequently used charts and tables needed for the assessment of endocrine patients.

Volume 2 of *Pediatric Endocrinology* is devoted to growth, adrenal, sexual, thyroid, calcium and fluid balance disorders, with a special section on radiation terrorism. The web resources available to the pediatric endocrinologist and the dynamic and genetic tests utilized in the care of patients with endocrine diseases are also contained therein. The diseases reviewed in this volume have been traditionally included within the realm of the pediatric endocrinology specialty and were included in previous editions of the book. However in the fifth edition of *Pediatric Endocrinology*, the specific chapters dealing with each of these entities are thoroughly reviewed and completely updated with attention given to the clinical and pathophysiological aspects of the disease. The book contains sections devoted to (i) growth and growth disorders, (ii) adrenal disorders, (iii) sexual development abnormalities, (iv) thyroid disorders, (v) calcium and mineral metabolism disorders, (vi) miscellaneous endocrine entities and (vii) endocrine testing protocols. In these sections, the diseases that afflict children cared for in a pediatric endocrine service are discussed. In the fifth edition of *Pediatric Endocrinology,* there are new chapters dealing with specific advances in the field of growth hormone insufficiency, the molecular basis of growth disorders and the integrity of the IGF system for appropriate growth. Also, there is a chapter on the transition from adolescence to adulthood of the growth hormone–deficient patient and the deficiency of this hormone in adults. The skeletal dysplasias leading to short stature and the syndromes leading to overgrowth and tall short stature are thoroughly reviewed.

The neonatal screening program is now widely used for the diagnosis of multiple inborn errors of metabolism, hypothyroidism and congenital adrenal hyperplasia. Thus, a readily available resource of the standards and guidelines for the care of newborns with abnormal newborn screens is found in the book. The chapters on traditional diseases of the adrenal cortex and medulla, as well as the sexual differentiation disorders and thyroid and parathyroid alterations, provide great detail in comprehensive reviews of all the alterations of patients with diseases of these endocrine glands. Also, there are new chapters dealing with rickets and osteoporosis and brittle bone syndromes, as the scope of the specialty has demanded that pediatric endocrinologists deal with patients with these entities. A major source of concern to pediatricians in practice is also addressed in this book, namely the patient with nonendocrine diseases associated with abnormal endocrine tests, often causing referrals to the pediatric endocrinologist. In this era, a chapter of radiation terrorism was necessary to bring to the pediatric endocrinologist the necessary information ''to be aware and prepared.'' Additionally, the chapter dealing with the use of the web provides an important practical update to the practicing physician for the recognition of genetic syndromes in pediatric endocrinology. Finally, all the chapters address the diagnostics of endocrine function and disease with algorithms and updated tables, special growth charts, dynamic endocrine testing protocols and interpretation of the data. Altogether, the book provides the necessary information to facilitate the care of the pediatric endocrine patient and the understanding of the diseases that they present.

Sophocles said it long ago: ''Look and you will find it—what is unsought will go undetected.''

Fima Lifshitz, M.D.

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Fima Lifshitz, M.D.

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1

Worrisome Growth

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INTRODUCTION

Growth is the fundamental physiologic process that characterizes childhood. It should be closely monitored by pediatricians and families alike as a benchmark of a child's health. Similarly, secular trends in growth patterns are followed as indicators of children's health on a population level. The American Academy of Pediatrics reiterated the importance of growth in March 2000 "Recommendations for Preventative Pediatric Health Care," stating that a child's height and weight should be measured at least at birth, age two to four days, 1, 2, 4, 6, 9, 12, 15, 18, and 24 months, and every year thereafter through age 21 (1), and the measurements plotted on a growth chart.

People come in all different shapes and sizes, and much of the distribution represents normal variability. The physician's first step is to distinguish normal variants from worrisome growth. Thus, some definitions are in order. Growth can be worrisome along two variables: height (short stature) and velocity (growth failure). Height involves a measurement of linear stature at a single point in time and compares it to expected norms. The norms are usually provided by the general population as depicted in growth charts (i.e., height range of healthy children of the same age and gender). Because the normal range within the population is a fairly large distribution, one can get an expected norm that is more specific for a particular child by comparing his or her height to those of the parents (i.e., an indication of that child's genetic potential). Normal ranges in medicine are frequently defined as ± 2 standard deviations (SDs). Thus, short stature can be defined as: (i) height below -2 SD for age and gender within the population, or (ii) height more than 2 SD below the midparental target height. Dwarfism refers to more severe short stature, defined as height below -3 SD for age and gender norms. A midget is a dwarf who retains normal body proportions. Because the more commonly used growth charts depict the fifth percentile (-1.6 SD) or third percentile (-1.9 SD) as the bottom line of the normal range, many parents and pediatricians use "below the curves" as their cut-off for defining abnormal height.

Growth can also be worrisome in regards to velocity. Growth velocity looks at the change in height measured on two separate occasions, relative to the time that lapsed between the two measurements. It is annualized, by the following equation, to enable comparisons:

$$\text{Growth velocity (cm/yr)} = \frac{\text{Height (cm) measured at time}_2 - \text{Height (cm) measured at time}_1}{\text{Number of months between time}_2 \text{ and time}_1} \times 12 \text{ (months per year)}$$

Normal growth velocities are dependent on age and pubertal stage, and are detailed later in the chapter. If growth velocity is abnormally slow for a considerable period of time, it manifests as a fall in percentiles when plotted on the growth chart. Thus, the two other definitions of worrisome growth are: (iii) abnormally slow growth velocity, or (iv) height dropping across two major centile lines on the growth chart.

Short stature and growth failure frequently, but not always, occur together. For example, a healthy child of short parents will have short stature but not growth failure; he or she will grow at normal velocity towards a lower genetic potential. Conversely, a child of very tall parents can have growth failure but still be taller than the cut-off for short stature of the general population.

SIGNIFICANCE OF WORRISOME GROWTH

The Social Problem

Many families seek medical attention for their short child because he or she is now shorter than their younger sibling, the shortest in their class, gets teased, bullied or treated differently in school, because size does not meet their expectations or is an impediment to their participation in sports, or because they want to make sure there is nothing wrong that will prevent their child from attaining normal adult height. Short stature is a cause of psychosocial stress, and the extent to which this is a problem depends on the severity of the height deficit, the degree of tolerance in the local population, and the child's coping skills.

Prejudice against short individuals is a pervasive phenomenon, termed heightism. The reader is referred to the book, *The Height of Your Life*, by Ralph Keyes, for a very comprehensive and interesting description of heightism (2). Using a wry and humorous approach, this book highlights facts regarding height so basic to our relationships with others that we have ceased to think about them. For example, heightism has been incorporated into the English language. ''Feisty'' tends to describe shorter people, ''distinguished'' is used more often for taller individuals, and the association of height with social desirability is inherent to the phrases, ''looks up to'' and ''looks down upon.'' The question is always, ''how tall are you?'' instead of the neutral, ''what is your height?''

More concretely, multiple studies have found associations between tallness and markers of financial and social success. Height has been linked to occupational success (3), achievement as an academic (4), perceptions of presidential candidates (5) and perceived performance and leadership in military service (6).

The association between tallness and power is ingrained since childhood (with few exceptions, heroes are always big and strong) and is exploited in Hollywood and politics. The dominant figures in advertisements and the movies are usually represented by tall people, and when they are played by shorter actors, contrivances are used to correct the deficit, such as standing on hidden boxes, standing on higher levels of the set or using platform shoes. In televising the latter debates during the 2004 United States presidential elections, George W. Bush and John Kerry were shown—busts only—at equal level. Indeed, George W. Bush is only the fourth major presidential candidate in U.S. history to succeed over a taller opponent (Calvin Coolidge was 1 in. shorter than John Davis in 1924, Richard Nixon 1 in. less than George McGovern in 1972, and Jimmy Carter was 7 in. shorter than Gerald Ford in 1976). All but seven U.S. Presidents were above the present average height, with James Madison, at 5 ft 4 in., the shortest. Voting ''by the inch'' may not be a uniquely American phenomenon. The 2000 election marked the first time in the history of Mexico that the opposition candidate, Mr. Vicente Fox (who is very tall), defeated the official presidential candidate of the PRI party, an especially noteworthy accomplishment since the PRI party had held power for over 75 consecutive years.

The social pressures favoring tallness start early and seem to particularly affect males. In a study of elementary school teachers' perceptions, height and size were positively correlated to many aspects of competence in the male students (7). Stature appears to be important in female selection of a mate, with taller males having the social advantage (8). At present, both genders seem to feel that in romantic relationships the male should be taller than the female. Even Sandy Allen, who at 7 ft 7-1/2 in. was certified by *The Guinness Book of World Records* as the tallest woman in the world, was quoted as saying, ''I've got this old-fashioned idea; I will never marry anyone smaller than I am.'' She never married. Thus, the tall man seems to have all of womankind to choose from, whereas the short man appears to be limited to even shorter women.

The greater social pressures for tallness in males have impacted medical practice. Chart reviews of all new-patient encounters referred to a major academic growth center for short stature or poor growth evaluations during 2001 found that girls were referred half as often as boys and were significantly shorter than the boys, relative to both the general population and their expected parental target heights (9). It was unclear how much of the discrepancy observed in this study was due to sex-biased referral practices of the pediatricians, selection bias by the male patients and their families more often requesting specialist referrals from their primary physicians or else seeking specialist care directly, or a combination of the two. In a survey of American pediatric endocrinologists, the specialists were 1.3 times as likely to prescribe growth hormone (GH) treatment for identical hypothetical case scenarios that described boys rather than girls (10). Recombinant human GH (rhGH) registries document male:female treatment ratios of approximately 2:1, depending on the diagnosis, with the highest for idiopathic short stature (11,12).

Although most people agree that short stature imposes psychosocial stress, great controversy wages on two correlates: (i) how short is too short? and (ii) does short stature warrant medical treatment? Height is a continuous variable, and the cut-off between normal and abnormal height is arbitrarily chosen by statistical means. With the availability of rhGH, some challenge withholding treatment from children whose heights are just above the currently accepted thresholds; for example, if rhGH works for children with heights at the third percentile, why not help those at the fifth percentile? A treatment approach based on suffering, rather than height, has been proposed (13).

Despite the social pressures demonstrated on short individuals, other studies have countered that short stature does not preclude normal psychosocial adjustment in children or adults (14). Similarly,

some argue that many studies overestimate the impact of short stature on psychosocial outcome due to methodological flaws; Sandberg and Colsman provide a systematic critique of study design issues in this field (15). This subject is extensively argued in the literature (13,16–24), and we must collectively come to grips with whether rhGH treatment for healthy short stature children construes medical or cosmetic treatment and whether this is an appropriate means of resource allocation on a societal level (Vol. 1; Chaps. 2 and 5).

The Medical Problem

Regardless of one's views on the social problem, there is a compelling medical reason to heed worrisome growth: growth failure may be the first and only sign of an underlying health problem in a child. Multiple diseases can present solely with growth failure (Table 1), such as celiac disease (25), inflammatory bowel disease (26), cystic fibrosis (27), renal tubular acidosis (28), and human immunodeficiency virus (HIV) infection (29).

Failure to recognize the significance of worrisome growth can lead to unnecessarily delayed or missed diagnoses.

The potential medical consequences of delayed or missed diagnoses are multiple. The clinical outcome of many diseases depends on the timeliness of diagnosis and treatment. Additionally, permanent height deficits may result from intervening too late. Once the growth plates fuse at the end of puberty, no further increase in height can be obtained. When a growth-stunting health problem is corrected, the growth velocity frequently accelerates to return the body to its genetically determined channel. Such catch-up growth requires time to reverse deficits. Similarly, early diagnosis and treatment are associated with better final height outcomes when rhGH is used to treat GH deficiency and Turner syndrome (30), and can obviate the need for delaying puberty (31).

Because the definition of short stature is a statistical one (e.g., all children with height below the third percentile of the general population), most children with short stature are healthy. However, some may have serious growth disturbances that may prevent them from reaching normal adult size, and growth failure serves as a screen for this high-risk subgroup. For example, almost half of the 5000 infants born in Newcastle, U.K. in 1960 were measured for height at age 10. The heights of 111 children fell below the third percentile; 16 were found to have a previously unsuspected organic disease as cause of their short stature (32). Similarly, in an elementary school-based screening program in Utah 20 years later, 114,881 children were measured the first year and of these, 79,495 growth rates were obtained the second year. Five hundred fifty-five children were identified with short stature (< third percentile) and poor growth velocity (< 5 cm/yr). Further evaluation of these 555 revealed an underlying organic disease in 14%, of which 5% were deemed endocrine (GH

Table 1 Causes of Short Stature

- *Normal*
  - Constitutional growth delay
  - Genetic/familial short stature
  - Combined constitutional growth delay and familial short stature
- *Pathological*
  - Nutritional
    - Micronutrient deficiency
      - Zinc deficiency
      - Iron deficiency
    - Macronutrient deficiency: decreased intake
      - Hypocaloric diet
      - Kwashiorkor
      - Anorexia nervosa and other eating disorders
      - Nutritional dwarfing (fear of obesity, fear of hypercholesterolemia)
    - Macronutrient deficiency: decreased absorption
      - Inflammatory bowel disease
      - Celiac disease
      - Cystic fibrosis
      - Malabsorption
  - Endocrine
    - Hypothyroidism
    - Isolated GH deficiency
      - Classic GH deficiency
      - Neurosecretory GH deficiency
      - GH insensitivity (primary insulin-like growth factor deficiency)
    - Hypopituitarism
    - Glucocorticoid excess
      - Iatrogenic
      - Cushing's syndrome
    - Precocious puberty
  - Chromosome defects
    - Turner syndrome
    - Down syndrome
    - Prader–Willi syndrome
  - Low birth weight short stature (intrauterine growth retardation)
    - Sporadic
    - Characteristic appearance
      - Russell–Silver syndrome
      - Cornelia de Lange syndrome
      - Seckel syndrome
      - Dubowitz syndrome
      - Bloom syndrome
      - Johanson–Blizzard syndrome
  - Defects in bone development
    - Achondroplasia, hypochondroplasia
    - Chondrodystrophies
    - Other skeletal disorders
  - Metabolic
    - Mucopolysaccharidoses
    - Other storage disorders
  - Chronic disease
    - Chronic renal disease
    - Chronic liver disease
    - Congenital heart disease (especially cyanotic conditions)
    - Pulmonary (cystic fibrosis, bronchial asthma)
    - Poorly controlled diabetes mellitus (Mauriac syndrome)
    - Chronic infections (including human immunodeficiency virus, tuberculosis) associated with birth defects or mental retardation
      - Specific syndromes
      - Nonspecific defects
  - Psychosocial deprivation
  - Chronic drug intake
    - Glucocorticoids
    - High-dose estrogens or androgens
    - Methylphenidate
    - Dextroamphetamine

Abbreviation: GH, growth hormone.

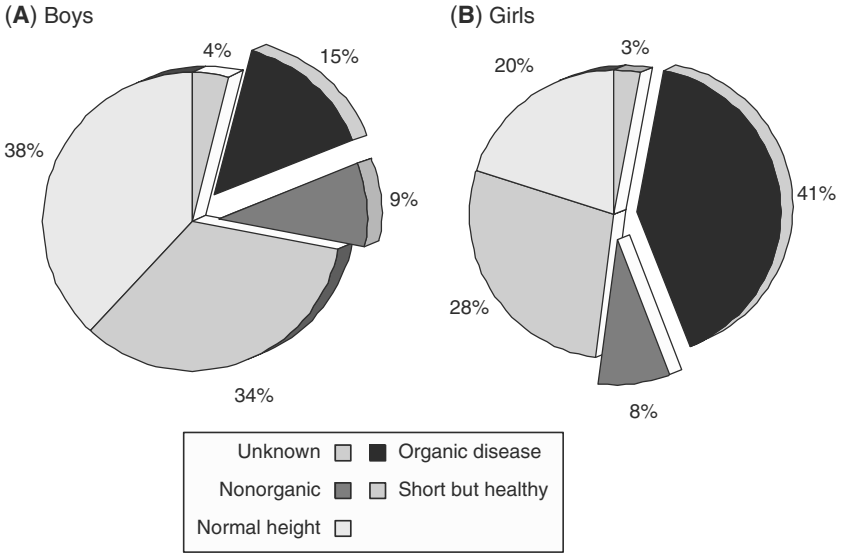

Figure 1 The distribution of each major diagnostic category for (**A**) boys and (**B**) girls referred to an academic Growth Center in 2001. Normal height was defined as healthy children whose height z score was within 2 SD of both the population mean and their mid-parental target. Diagnoses were tabulated for children whose heights did not meet the definition of normal. Short but healthy included children with familial short stature and/or constitutional delay of growth. Nonorganic diseases (failure to thrive/nutritional dwarfing and psychosocial dwarfing) and organic diseases, the two extracted pie slices, represent children with underlying pathology. For both sexes, 3% to 4% had unknown diagnoses due to incomplete evaluations. *Source:* From Ref. 9.

deficiency, hypothyroidism and Turner syndrome). Almost half the children identified with GH deficiency and all the girls with Turner syndrome had been previously undiagnosed (33). Thus, the cause of short stature should always be investigated in all children whose heights fall below the third percentile and more importantly in those who fail to grow at appropriate growth rates.

Growth-related disorders are also the most frequent problems encountered by pediatric endocrinologists. Pediatricians often seek consultation to help in the diagnosis and management of children with growth disturbances. Yet even in a pediatric endocrine referral center, a large proportion of patients with short stature are usually healthy children. At times children are referred for short stature although they are of normal height. This may occur because of either poor, inaccurate measurements, or because of the need for a pediatric endocrinologist to reassure a patient or family when a child is growing in the lower end of the normal range. A pathological condition accounted for poor growth and/or short stature in about one-third of the short stature patients seen in a tertiary referral center (34).

Pediatric endocrinologists believe that short stature by itself may not be of concern for the individual, if he or she is healthy. However, height may play a role in the risk for adult-onset disease. For example, three large population studies—the Physician Health Study (35), the Framingham Study (36), and the Royal Canadian Health Force Study (37)—identified short stature as a risk factor for coronary heart disease and myocardial infarction in adults. The mechanism(s) for the increased risk were not elucidated. Speculation turned to coronary artery size, which would be expected to be smaller in shorter individuals and thus, more prone to blockage by atherosclerotic plaque. Appreciation is growing for the effects of GH and insulin-like growth factor (IGF)-I on the cardiovascular system (38), and future work may reveal subtle defects in the GH/IGF system as the link between stature and coronary disease risk.

If short stature is seen as solely a social problem, then boys are affected more than girls. In contrast, if worrisome growth is seen as a potential sign of underlying pathology, then equal import should be given to evaluating both boys and girls. Although girls were referred to an academic growth center less often and with greater height deficits than the boys, a significantly higher percentage of the girls had underlying pathology that requires intervention (Fig. 1) (9). Thus, worrisome growth in girls should be investigated as rigorously as in boys, even if it may not carry as much social pressure.

DIAGNOSIS OF SHORT STATURE

Measurements

The cornerstone of diagnosing short stature is accurate measurements over time. Although measuring height seems a simple task, studies have shown that it is frequently performed improperly (39). Despite the explicit recommendations from the American Academy of Pediatrics (1), a survey of primary practices throughout the United States found that 10% of the pediatrics practices and 40% of the family practices (259 respondents from1300 surveys originally mailed) do not measure children at every well child visit (40). In a study of an academic pediatric clinic, 35% of well-child encounters failed to plot growth measurements and/or document a growth abnormality (41).

Often, when growth is measured, improper equipment and technique are used (40). Height should always be measured with the shoes off, and

interfering hair accessories removed. Unfortunately, the most frequent method of measuring height, using a flip-up horizontal bar (floppy arm) on a weighing scale, is subject to great errors caused by the child's slumping posture and considerable variation in the angle of the horizontal bar. Children should be measured standing upright and fully extended against a wall or firm vertical structure to which a properly mounted, accurate measuring device is attached. A steel tape measure, properly affixed to the wall, serves this purpose well and economically. The child stands shoeless, heels down, as erect as possible, and with the head directly forward. The back of the head, chest, gluteal area, and heels should touch the vertical surface. A firm object (e.g., a carpenter's angle) is then placed at a right angle over the top of the head and against the wall above the head. A Harpenden stadiometer (Holtain Limited, Crymych, Dyfed, U.K.), which determines height accurately (within 0.1 cm) is the most sophisticated instrument (42). However, other devices are less expensive and/or are comparable in accuracy to the more expensive stadiometer when properly installed (43,44).

Similar supine instruments are available for measuring length in infants and toddlers: a firm platform with an attached yardstick, a fixed head plate, and a moveable footplate. Two people are required for accurate length measurement: one to hold the head, the second to keep the knees straight and bring the measuring board to the feet, which must also be secured. Many practitioners, measuring infants unassisted, lie the patient on the paper covering the examining table, mark off with a pen the positions of the head and feet on the paper, remove the patient, and then use a measuring tape to quantify the distance between the two pen markings. This common technique is highly inaccurate due to incorrect positioning of the infant, movement and crumpling of the paper, and failure to get perpendicular markings by the pen.

Inaccurate measurement can have significant impact on clinical outcome. Observation of 44 pediatric and 11 family practices throughout the United States revealed that only 30% of measurements were accurate (within $\leq$0.5 cm); measurements differed by an average of 1.3 cm from measurements of the same children by study staff (45). Because the average error exceeded the difference between normal (5 cm/yr) and subnormal (4 cm/yr) childhood growth velocities, these errors could lead to inappropriate referrals of normally growing children or failure to detect cases of true growth failure. Thankfully, measurement issues can be readily redressed. When these same 55 practices were randomized to a control group versus an educational intervention group, accuracy rates in the control group remained at 37% and 34% at three and six months, respectively; the intervention group increased its accuracy rates to 55% at three months and 70% at six months, with a decreased mean difference in measurement from study staff of 0.5 cm at six months (45).

Growth Charts

Because growth is a continuous progression in size, serial measurements are best evaluated by plotting on appropriate growth charts to assess the pattern of growth. The population selected for reference is important when judgments are made about the height and growth of an individual. Multiple growth charts have been constructed for American children in past decades, differing in the racial and socioeconomic composition of the reference populations upon which they are based. Growth charts for children of other countries are likewise available, as are charts for children with different genetic syndromes (46). The newest and currently recommended U.S. growth charts (47), published in 2000 by the Centers for Disease Control and Prevention (CDC) (48), are included in the chapter of reference charts in this book (Vol. 1; Chap. 20; included is a version that facilitates reading of the grid and metric axis labels). The CDC growth charts also provide body mass index (BMI) values for ages two years and older, a useful feature in light of the current obesity epidemic (49).

Although the CDC growth charts constitute an important advancement over the previous charts used (1977 National Child Health Survey percentiles), they have some limitations. These percentile charts were based on cross-sectional data that effectively average growth across peers in different developmental periods. This is most pertinent during the peripubertal years, in which individual children grow in different patterns based on their pubertal tempo. Because cross-sectional charts average out and lose this variability, supplemental growth charts for the pubertal periods are available (50). While longitudinal growth charts are helpful to tease apart early and late bloomers, their data were derived from nonrepresentative population samples recorded a long time ago.

A program for monitoring the growth of children from birth to 36 months of age was prepared by the Eurogrowth Study Group (51,52). It allows individual tracking and plotting of growth data, calculates growth velocity, provides BMI centiles, measures influences of breastfeeding on growth, modifies growth by midparental height, corrects growth for gestational age of premature infants, calculates Z scores, and offers multilingual access (52–56). It is a highly recommended tool to assess growth in children up to 36 months of age.

Pathological growth should always be considered in children who do not grow well regardless of height (Fig. 2). Any child who falls behind in growth across major percentiles in the chart should be evaluated, even when the height is not below the third percentile (57). It must be kept in mind that growth is not continuously linear, but instead occurs in steps between saltation and stasis (58). Therefore, growth progression over a long period of time is more informative than extrapolations based on shorter periods of time. Growth velocity also varies with the seasons, generally being fastest in the

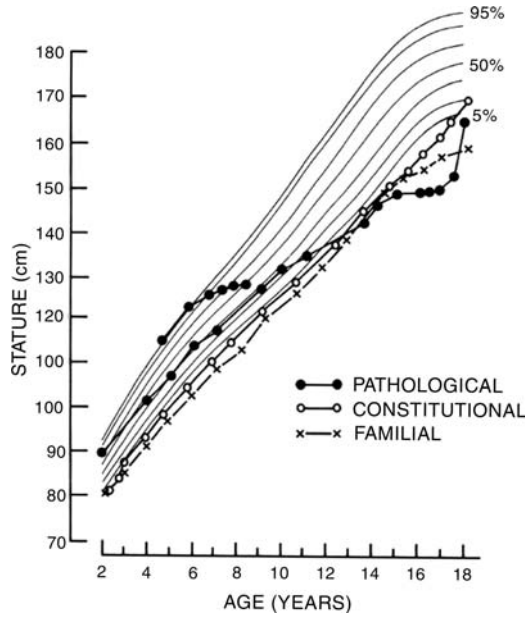

Figure 2 The growth patterns of three patients exemplifying short stature with normal velocity (familial), short stature with delayed growth (constitutional), and pathological growth despite normal height. The patient with pathological short stature received treatment at age 17 and attained catch-up growth. *Source*: From Ref. 34.

spring and summer, due to increased GH secretion in response to the longer periods of light (59). The growth rate in the fastest three month period is two to three times higher, but could be up to seven times the height increment during the slowest growth period in the other months (60,61). Therefore, growth progression should be evaluated over a period of at least six months to one year.

Growth velocity also varies with life stage. It is fastest in the first year of life (about 25 cm/yr overall; 38 cm/yr in the first two months dropping to 12 cm/yr at one year of age), and then declines during childhood (from 12, 10, 7, 6 and 5 cm/yr at ages 1, 2, 2–4, 4–5, and 5 years until puberty, respectively). Growth accelerates again during the pubertal growth spurt, which occurs during Tanner puberty stages II–III in girls (10 cm/yr) and Tanner IV in boys (12 cm/yr). Growth velocity charts are shown in Vol. 1; Chap. 20. A growth velocity of less than 5 cm/yr after age five years is worrisome. Because the growth spurt occurs earlier during puberty and has a lower peak velocity in girls than boys, the mean final height in American women (5 ft 4 in.) is 5 in. shorter than that for men (5 ft 9 in.).

There are commonly made errors in plotting growth charts that result in misleading interpretation of an otherwise accurate series of height measurements. Parallelism is often a cause of plotting the data in the wrong field. Although the ticks on the x-axis for age are labeled by year, age is a continuous variable and points should be plotted according to the precise age in years and months. Rounding down a child's age to the closest year slides the point to the left on the growth chart, resulting in an overestimation of the height percentile; rounding up leads to an underestimation of the height percentile. Another error involves the overlap in charts between ages two to three years (40). Children of this age may be measured standing (height) yet plotted on the length chart because the age is included and that chart already has multiple prior points for comparison. Standing height is always shorter than supine length, so plotting a standing height on the length chart creates an artificial growth deceleration that needlessly causes alarm. Care should always be taken to match the growth chart reference with the measurement technique used.

Monitoring weight gain to assess the growth of patients is as important as following the height progression. Changes in weight progression may precede alterations in height increments in certain conditions such as nutritional growth retardation (NGR) and obesity (62–65). Therefore, monitoring height alone does not provide sufficient information to assess a growth pattern, as discussed in the section of NGR. Accurate weight measurements should be made on a regular hospital weighing scale and plotted on the growth chart. An infant should be stripped of clothes and diapers, and older children should wear a hospital gown or light clothing; this minimizes inaccuracies from variability in clothing weight which occurs with season. Adherence to these rules is important if one is to take note of changes in weight over time.

Genetic Potential

A child's genetic potential should be considered in the evaluation of his or her present growth pattern, and any deviation from the expected height for the family should be worrisome (66). The following formulas provide an easy way of estimating the mid-parental target height (in cm):

$$\text{For males}: \quad \frac{(\text{Mother's height} + 13) + (\text{Father's height})}{2}$$

$$\text{For females}: \quad \frac{(\text{Father's height} - 13) + (\text{Mother's height})}{2}$$

These formulae adjust for the 13 cm (5 in.) difference between the mean adult male and female heights, and provide the midparental height $\pm$ 2 SD (1 SD would be about 5 cm, or 2 in.). It is important to measure the parents' heights rather than rely on guesstimates or self-reports. The target height obtained by this method is then applied to the 20-year line of the gender-appropriate growth chart. The projected height is determined by extrapolating the child's growth along his or her own channel. If the projected final height is within 5 cm of the midparental target height, the child's height is appropriate for the family. On the

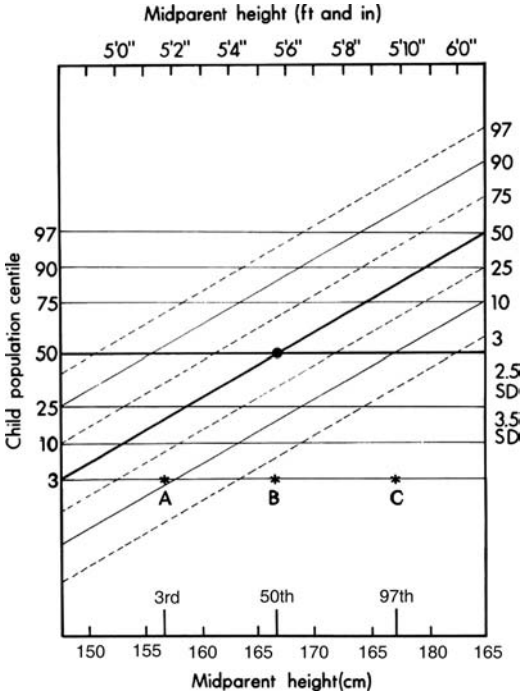

Figure 3 The Tanner standards for height of girls and boys from two to nine years in relation to parents' height. Three patients of the same height are depicted. Patient A has familial short stature, whereas patients B and C have worrisome growth because their heights are significantly shorter than their genetic potentials. *Source*: From Ref. 66.

other hand, if the difference between the projected and target heights is more than 5 cm, a pathological cause should be considered.

Another method for evaluating whether a child is within the normal limits of height for their family is to compare the child's height with the midparental height in special charts that take advantage of the fact that the correlation coefficient of these variables changes little between age two to nine years (66). Figure 3 depicts three patients with different diagnoses using this chart. Patient A's height falls on the third percentile, and his parents' midparental height is 157 cm. The position of this patient's point on the chart is between the 10th and 25th percentiles. Thus, for the population at large, this patient would be small, but he would be appropriate for his immediate family (familial short stature). Patient B, who has the same height as patient A, has parents of average height (midparental height 167 cm). This means that patient B is actually very short for the family and requires further workup. Patient C is more extreme; his parents are tall. Although this patient's height is equal to that of the other two patients, his stature falls more than 3 SD from the family norm.

Skeletal Maturation and Predicted Final Height

In addition to the projected target height, the predicted final adult height should also be considered in the evaluation of the short child. There are three popular methods for calculating a child's predicted adult height. They are based on the fact that in a normal individual, there is a direct correlation between the degree of skeletal maturation and the time of epiphyseal closure, the event that terminates skeletal growth. Predictions of final height take skeletal maturation into account, for the greater the bone age delay relative to the chronological age, the longer the time before epiphyseal fusion ceases growth. However, predictions of final adult height are not very accurate and are of limited value in children with growth disorders, because the predictions vary if children do not grow at normal rates. The data also may not be accurate for short patients from short parents; the children may end up taller as adults, and their target and predicted heights may be underestimated (67).

The most commonly used method to determine predicted adult height is that developed by Bayley and Pinneau over 50 years ago (68). The Bayley-Pinneau prediction tables utilize standards derived from U.S. children living in Cleveland and proportional correlations between the skeletal age [by the Greulich–Pyle method (69)] with final adult height. Thus, final height can be extrapolated by combining a child's current bone age with the height measured at the time of the radiograph study. This correlation is more accurate after nine years of age. The Tanner–Whitehouse (TW) method, derived from British children, utilizes TW standards for bone age assessment (70). In addition to the bone age and measured height, this method factors in chronological age, parental heights, and, in girls, the occurrence of menarche. The third method for predicting adult height is the Roche–Wainer–Thissen (RWT) method (71). This method pays attention to the weight or nutritional status of the child, and uses recumbent length instead of standing height. The five predictor variables in the RWT method are recumbent length, weight, bone age, chronological age, and parental heights.

The main sources of inaccuracy in final height prediction are the inaccuracy of the bone age estimation itself as well as the inability to predict pubertal tempo. A small difference in bone age determination can lead to a great difference in height prediction, especially during the pubertal growth spurt (72). Comparability studies of the various methods of final height prediction suggest that the RWT method is the most accurate, but it involves the greatest amount of calculation. Its inaccuracy increases with age, and should not be used when more than half the bones are adult (71,72). In general, height prediction methods differ in their tendencies to overestimate or underestimate adult height (72), and are useful only in children with normal growth velocities. Computer programs have been developed for the calculation of both midparental target heights and predicted adult heights (by all three methods) (ARC Software). Several rhGH manufacturers currently provide software

at no cost to the clinician with which to evaluate and follow patients with growth problems.

The foundation of predicting adult height is accurate reference for bone maturation patterns (73). In the G–P method, a radiograph of the frontal view of the left hand and wrist are compared with given standards in the G–P atlas (69). The TW2 method assigns maturity scores to each of the 20 hand bones (radius, ulna, carpals, metacarpals, and phalanges), and the total score determines the bone age. The advantages of the TW2 over the G–P method are that it appears to be more objective, and it can differentiate bone age up to one-tenth of a year; the G–P method gives only a rough approximation, with intervals of 6 to 12 months between the standards. Thus, the TW2 method is more sensitive in following small changes in bone age, but it is more time-consuming and few clinicians use it.

Studies comparing the two bone age methods in 2 to 24-year-old children in the same ethnic population suggest that the median G–P skeletal ages were markedly greater than the corresponding chronological ages, particularly from six to nine years in boys and from four to eight years in girls (74). The differences between these scales could represent real differences in skeletal maturity rates in the different populations. Studies comparing the bone age methods in relation to the group of bones evaluated showed a high correlation between bone age determinations that examined all the bones with or without the carpals (74). Discordance among centers, with maturity levels differing between bone groups, can be a normal variant. Because bone age assignment is such a subjective measure, it is prudent for pediatric endocrinologists to make their own readings rather than simply rely on radiologists' reports. This will eliminate inter-reader variability, thereby improving the validity of longitudinally following bone age progression in a child.

The pattern of skeletal maturity is also helpful in differentiating the type of short stature. Bone growth in children with constitutional growth delay is slightly retarded (around 2 SDs of the chronologic age, which is usually two, or at the most, three years delayed), and it is usually proportional to height. When the adolescent growth spurt occurs, the bone age increases proportionally to height. The bone age in patients with familial or genetic short stature is seldom retarded more than one year compared with the chronological age, and it usually follows a normal maturation pattern. In contrast, there may be a marked bone age delay in children with pathological short stature, such as hypothyroidism, GH deficiency or chronic disease. The bone age may be even further behind than that expected for height, and the degree of the delay may reflect the duration of disease. Thus, a short adolescent with sexual infantilism and a bone age delay greater than three years is more likely to have pathological short stature.

Body Proportions

Growth assessments should consider body proportions in addition to body height, weight and skeletal maturity (75). The skeleton does not grow proportionally. The upper to lower body ratio starts at 1.7 at birth, but as the legs grow, the ratio drops to 1.0 by 10 years of age. If the growth plates fuse early, as in precocious puberty, the proportions remain childlike, with short limbs compared to the trunk. On the other hand, if growth is prolonged as in hypogonadism, a long-limbed habitus results (76). Various types of tubular bone alterations are often found among short patients (77). These categorize patients into specific diagnostic groups and potential treatment algorithms (Vol. 2; Chap. 6) (78). Rhizomelia refers to abnormal shortening of the proximal limbs, mezomelia to shortened middle limbs, and acromelia to shortened distal limbs (i.e., hands and feet). Thus, every child who presents with a growth problem should be evaluated for disproportionate limb or trunk shortening as this information helps to narrow the differential diagnosis, including the possibility of skeletal dysplasia.

Determination of the upper to lower body ratio can be accomplished in two ways. The lower body segment is measured by measuring the distance between the upper border of the symphysis pubis and the floor in a patient who is standing against a flat wall in the proper position for height measurement. This measurement is difficult to obtain accurately because the superior border of the symphysis pubis is not easy to locate and palpate, especially in obese patients. Preferably, the sitting height can be measured to represent the upper segment, using a Harpenden sitting table (Holtain Ltd.), though few endocrinologists use such device. The normal absolute and relative sitting heights of the different ages and sexes are listed in the chapter on reference charts (Vol. 1; Chap. 20). Upper and lower body segment ratios are essential in the evaluation of worrisome growth because skeletal dysplasias are usually characterized by disproportionate shortening of the lower limbs or spine (Vol. 2; Chap. 6). Conversely, the trunk may be disproportionately shorter due to scoliosis, spondyloepiphyseal dysplasia, or spinal injury from total body irradiation (79).

Arm span is another helpful measurement in determining body proportions. It should be measured with the patient standing against a flat wall, arms outstretched as far as possible to create a 90° angle with the torso. The distance between the distal ends of both middle phalanges is measured to determine the arm span. Normally, the arm span is shorter than the height in boys before age 10 to 11 years and girls before 11 to 14 years, after which the arm span exceeds the height. The average adult male has an arm span about 5.3 cm greater than his height; the adult female's arm span is 1.2 cm greater than her height (Vol. 1; Chap. 20). Conditions that adversely affect the vertebrae, such as scoliosis or irradiation, may result in growth

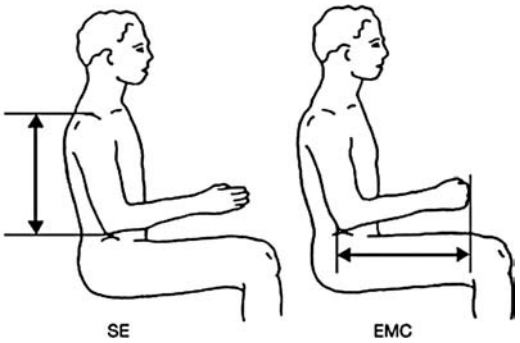

Figure 4 Measurement of SE and elbow-to-metacarpal length EMC is shown using an anthropometer. For SE length, the blades of the anthropometer are positioned from the midshoulder to the distal end of the humerus, with the elbow at a 90° angle and the upper arm next to the lateral side of the chest. To obtain the EMC length, the blades are positioned from the tip of the elbow to the distal end of the third metacarpal of the fisted hand. *Abbreviations*: SE, shoulder-to-elbow length; EMC, elbow-to-metacarpal length. *Source:* From Ref. 77.

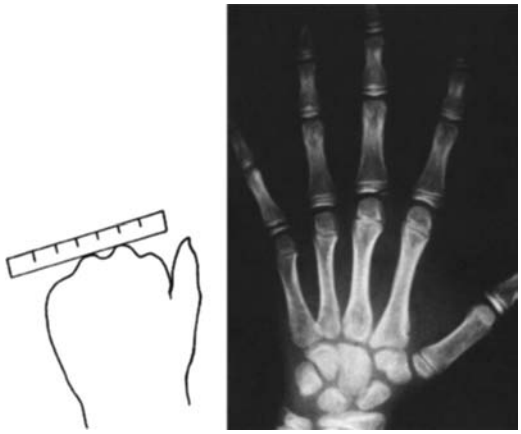

Figure 5 A straight ruler is applied against the distal ends of the third, fourth, and fifth metacarpals of a tightly closed fist. The clinical observation of brachymetacarpia V was confirmed radiologically when the fifth metacarpal bone failed by more than 2 mm to intercept a straight line connecting the distal ends of the third and fourth metacarpal bones. *Source*: From Ref. 88.

retardation and disproportionately long arms. The arm span can sometimes serve as a helpful surrogate for standing height in monitoring the growth of children who are unable to stand straight, such as those with severe cerebral palsy.

When disproportionate short stature is suspected, further measurements of the various limb segments should also be made. Disproportion between the upper arm and forearm lengths may be determined by measuring the shoulder-to-elbow (SE) length and the elbow-to-metacarpal length (EMC) using an anthropometer (Fig. 4). Normally, the SE/EMC ratio is about unity. Rhizomelia is present if this ratio is lower than 0.98 (77). The various disproportions are useful in categorizing the skeletal dysplasias (Vol. 2; Chap. 6), which may present clinically as isolated short stature or short-limbed short stature of genetic or familial nature. Clinically assessed disproportions are confirmed radiographically (80), and newer modalities are allowing improved prenatal diagnosis (81,82). Diagnosis of the different skeletal dysplasias is further enhanced by complementing the morphologic approach with specific biochemical and genetic tests that target the specific causal mutations (83–85).

The presence of shortening of specific bones may likewise lead to the diagnosis of certain syndromes, such as type E brachydactyly (OMIM #113300), Turner syndrome (86), or pseudopseudohypoparathyroidism (87). These patients may seek medical attention for short stature and must be differentiated from patients with other conditions involving shortening of the metacarpals or other tubular bones (77). Metacarpal shortening is detected by placing a ruler in front of the patient's fist; the knuckles of the third, fourth and fifth fingers touch the ruler simultaneously in most normal individuals. In brachymetacarpia V, however, there is a gap of 2 mm or more between the fifth knuckle and the edge of the ruler, as shown in Figure 5. This clinical observation has been confirmed radiologically (88). Fourth metacarpal shortening, a frequent finding in Turner syndrome and pseudopseudohypoparathyroidism, can be similarly detected clinically and radiologically, except the gap occurs between the ruler and the fourth knuckle.

Deficiency of the short stature homeobox (SHOX) gene, recently mapped to the pseudoautosomal region of the sex chromosomes (OMIM #312865), has been identified as the cause of some forms of short stature, including Turner syndrome and Leri-Weill syndrome (dyschondrosteosis) (89,90). It is believed to play a significant role in growth problems with disproportionate short limbs and tubular bone anomalies, particularly Madelung deformity (i.e., shortening and bowing of the radius with dorsal subluxation of the distal ulna, and partial foreleg anomalies) (91). It remains to be established whether SHOX plays a role in other more common forms of short stature, such as children with brachymetacarpia or milder forms of rhizomelia (92). This test is now available for clinicians (93).

Physical and Dental Examinations

Aside from obtaining accurate anthropometric measurements, a detailed physical examination may help elucidate the cause of short stature or growth failure. Specific stigmata are present in common dysmorphology syndromes, such as Russell–Silver syndrome, Turner syndrome, Prader–Willi syndrome (PWS), and Williams syndrome (94). Signs of chronic illness should be looked for, such as pallor, dry skin, abnormal hair texture, splenomegaly, enamel hypoplasia, or dental caries.

Evaluation of dental age is an important part of the physical examination that may provide insight into a child's skeletal maturation (95). The ages at which primary and secondary teeth are expected to erupt are shown in Figure 27 in Vol. 1; Chap. 20. There are wide variations in the time of eruption, which may be affected by local and environmental factors, such as jaw size, position of the unerupted teeth and premature loss of deciduous teeth (96). Children with GH deficiency or untreated hypothyroidism usually have significantly delayed dentition or abnormal teeth (hypodontia, usually of the maxillary incisors). Mild delays in dental progress may occur in constitutional growth delay. Delays in dentition are often associated with delayed closure of the fontanels and delayed bone age.

THE DIFFERENTIAL DIAGNOSES OF WORRISOME GROWTH

A diagnosis is usually made in most instances of short stature, but may remain elusive in some patients. New molecular etiologies are continuously being identified. The different causes of worrisome growth are listed in Table 1. This classification primarily distinguishes two main categories in the differential: short patients who are normal versus patients whose worrisome growth results from an underlying abnormality. This basic concept should be considered in the diagnosis of every short patient. A clinician must determine if a short child suffers from a pathological cause, which must be diagnosed and adequately treated, or if the child may need reassurance only without a major work-up. These two possible categories denote the pathophysiological processes involved and the prognosis for final adult height. The physician should recognize the specific applicable situation before subjecting the patient to expensive and complicated investigations. A notable caveat is that some familial shortness may be due to dominantly transmitted defects, so care should be taken in eliciting the family history and observing for clues to possible pathology in the parents.

Other classification systems have been used by pediatric endocrinologists to categorize short patients. For example, familial or genetic short stature has been referred to as ''intrinsic shortness.'' Other authors have used the term ''idiopathic short stature'' to describe short individuals who are growing poorly, have no demonstrable functional abnormality in GH secretion, and whose parents are normal in height. Idiopathic short stature often implies a continuum of GH insufficiency, not clearly demonstrable by the classic biochemical criteria, that includes defective signaling by GH and IGF-I. However, many of these terms are unnecessary in classifying short patients because the two categories proposed above are inclusive and sufficient to understand and clarify growth problems.

CONSTITUTIONAL GROWTH DELAY

The most common cause of short stature and sexual infantilism in the adolescent is constitutional delay of growth and puberty. This diagnosis constitutes a large proportion of the growth disorders seen by pediatric endocrinologists. The total incidence in the population may be even higher, because pediatricians usually do not refer these patients to an endocrinologist. These patients are the typical ''slow growers'' and ''late bloomers,'' with a familial tendency and likely denote a variant of normal growth. Often it is recognized long before adolescence, when sexual development is not yet a concern. The child with constitutional delay typically is characterized by growth deceleration during the first two years of life, followed by normal growth progression paralleling a lower percentile curve throughout the prepubertal years, until a catch-up growth or pubertal growth spurt occurs late in adolescence (Fig. 6) (97). Fathers frequently report a similar pattern of growth and delayed puberty, though it can be evident on the maternal side as well (98). The pattern of growth usually runs in the family, even though the family may not exhibit short stature. Constitutional growth delay seems to be a polygenic trait and appears to occur more often in boys, though this may reflect ascertainment bias in light of the 2:1 sex ratio of short stature referrals (9). The diagnosis should be made after eliminating other possibilities of pathological growth patterns.

A longitudinal study of patients with constitutional growth delay clearly showed a slowing of growth within the first three to six months of life (99). Both length and weight gain decelerate, and infants destined to have constitutional growth delay downcross percentiles until age two to three years (100). Thereafter, they grow at a normal rate until adolescence. This type of growth recanalization is also seen in infants with familial short stature (see below). Weight progression distinguishes the two conditions; body weight gain slows in patients with constitutional growth delay but not those with familial short stature. Thus, patients with constitutional growth delay appear to fail to thrive with body weight deficits for length, whereas infants with familial short stature maintain a normal, or even an excess, body weight for length. These growth patterns are maintained throughout childhood, but before puberty patients with constitutional growth delay gain weight and recover the body weight deficits for height before exhibiting sexual development (100). These data suggest that in constitutional growth delay, there may be an association with suboptimal nutrition at the time that weight progression decreases in infancy.

The long-term growth effects of early suboptimal nutrition have been corroborated in other models. Suboptimal nutrition in developing countries was shown to produce a growth pattern similar to that of constitutional growth delay (101). In children with primary malnutrition-induced growth failure, growth resumed at a lower percentile when the nutritional intake

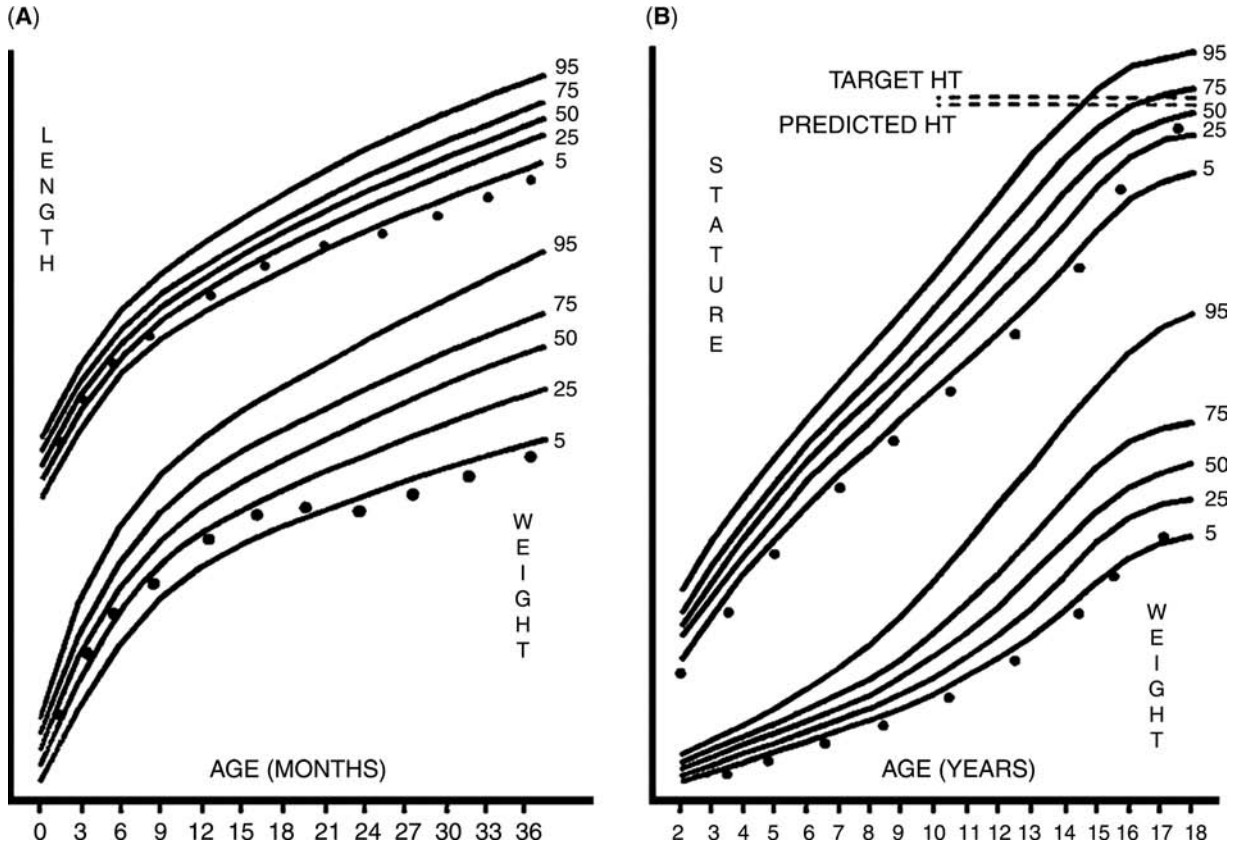

Figure 6 Constitutional growth delay. (**A**) Typical growth in infancy. Note the early readjustment of the length channel and weight percentile. (**B**) Typical growth in childhood and adolescence. Note the progression of height and weight below, but parallel to, the lower percentile. Body weight deficits for height are evident. There is a delayed pubertal growth spurt that eventually leads to normal predicted adult stature, which is in range for the target height. *Source*: From Ref. 97.

improved, similar to constitutional growth delay. Once growth became down regulated, the growth became recanalized at a lower level than before the nutritional insult. A similar pattern of growth retardation was experimentally induced in rats subjected to suboptimal nutrition. Low-protein diet ceased growth, and return to a normal dietary intake led to resumed growth at an appropriate rate, but in a lower percentile (102). The temporary dietary protein restriction in early life led to long-term alternations in GH and insulin secretion in these rats that likely played a role in the lack of catch-up growth. These data suggest that the down-regulation of growth in the early life of patients with constitutional growth delay may also be nutritionally related, although it is not clear why these infants would ingest insufficient nutrients for growth at this stage of life. These patients appear to have failure to thrive (FTT), and the differential diagnosis may be difficult while the recanalization of growth is taking place.

Delayed pubertal onset is a hallmark of constitutional growth delay, with a concomitant second growth deceleration apparent at ages 10 to 14 years. Recent advances in the neuroendocrinology of puberty and its relation to nutritional status also support a possible nutritional role in constitutional growth delay. Pubertal onset has been identified as the initiation of pulsatile release of gonadotropin releasing hormone (GnRH) by the hypothalamus (103). Pubertal development seems to require a minimal threshold level of leptin, the adipocyte hormone that relays central feedback regarding the body's fat stores, though leptin by itself is insufficient to initiate puberty (104). Hypogonadotropic hypogonadism is seen in both leptin-deficient (*ob/ob*) mice (105) and humans (106), a phenotype rescued by treatment with exogenous leptin (107). Exogenous leptin similarly allowed normal pubertal onset in rats despite 80% food consumption and growth retardation; none of the pair-fed animals achieved puberty (108). Leptin only partially overcame the pubertal suppression of more severe food restriction (70% of ad lib) (108), suggesting that other factors are also involved in mediating the suppression of GnRH by undernutrition. Proopiomelanocortin (104) and galanin-like peptide (109) have been proposed as mediating leptin's permissive effects on reproduction, while neuropepetide Y (104) and ghrelin (110) have been implicated in the suppression of reproduction by undernutrition.

The recently discovered KiSS-1 system seems to be the most selective downstream regulatory signal important for hypothalamic GnRH function; it is the only one identified to date that directly stimulates hypothalamic neurons and GnRH release without affecting feeding behavior (111). A 72-hour fast (water only) in prepubertal Wistar rats led to a 16% to 18% reduction in body weight, lower serum leutenizing hormone concentrations, and in the hypothalamus, reduced expression of KiSS-1 with a compensatory increased expression of its receptor, GPR54 (111). 30% chronic food deprivation in these rats lowered body weight by 30% and completely prevented pubertal onset; intracerebral injection of kisspeptin, a KiSS-1 system ligand, rescued the pubertal failure despite the reduced body weights (111). Like leptin, mutations of GPR54 led to hypogonadotropic hypogonadism in mice (112) and humans (113,114).

A study of 80 patients with constitutional growth delay, aged 6 to 15 years, compared to 60 healthy controls found lower body weight and significantly lower plasma leptin levels in the prepubertal patients and lower serum IGF-I levels in the pubertal patients (115). As the neuroendocrinology of puberty continues to be unraveled, future studies will further delineate the specific defects causing constitutional growth delay. Genetic studies trying to identify the puberty clock genes are currently underway (116), and variation in the genes for GnRH and its receptor have already been ruled out as substantial modulators of pubertal timing in the general population (117).

Although children with constitutional growth delay ultimately attain a normal adult height, they generally end up along the lower end of the normal height range for their families (118). Final and predicted adult heights did not differ significantly in boys with untreated constitutional growth delay, but the final height was significantly lower than the measured midparental height (119). This may result from the lower peak height velocity attained by later maturers than normal or early maturers (120), the possible effects of suboptimal nutrition (100), or selection bias in the patients seeking medical attention (118). Nonetheless, it should be noted that an altered pubertal tempo impairs the accuracy of height predictions (121).

Many children with constitutional growth delay are also short for genetic reasons, i.e., they have combined constitutional growth delay and familial short stature. If a child has a two-year delay due to constitutional growth delay and is destined to be an average-sized adult (50th percentile), his height will be at the fifth percentile at age 10 and 5 cm below the fifth percentile at 14 years. Such patients may not come to the attention of the pediatric endocrinologist, especially if one or both of the parents remember that they were late bloomers and realize what is happening. In contrast, if a child with the same delay is destined to reach only the 10th percentile as an adult, then at age 14 he would be about 2 to 3 cm below the third percentile, that is, more than 2 SD below the mean, and therefore likely to be referred for an endocrinological work-up. The typical boy with this syndrome is otherwise healthy, has a delayed bone age and height appropriate for his bone age. Linear and skeletal growth remains consistent, but delayed, until his pubertal growth spurt occurs and secondary sexual characteristics appear. This condition is often difficult to diagnose when the patient is first seen unless measurements at multiple earlier ages are available, and follow-up height increments are assessed. The main concern with these patients is the psychological aspect of the combined short stature with lack of secondary sexual characteristics. Severe cases may develop a defective self-image and social withdrawal.

Treatment of patients with constitutional growth delay with or without familial short stature is controversial. Practicing physicians are now under mounting pressure to prescribe rhGH for short children who are not deficient in this hormone, especially after the U.S. Food and Drug Administration (FDA) approved its use for idiopathic short stature (height ≤ -2.25 SDs) in 2003. However, part of the FDA's indication is a growth velocity that portends an inability to reach normal adult height. Constitutional growth delay does not fit this criterion (Vol. 1; Chap. 2 and 5).

Although puberty eventually occurs spontaneously, treating boys with short courses of testosterone is recommended primarily to ameliorate the psychological problems associated with delayed puberty (122). Treatment is recommended only if the bone age is greater than 12 years, to avoid compromising the final height through inappropriate bone age advancement (122). The recommended dosage is 50 mg intramuscular testosterone enanthate or 170 mg of the cypionate form every month for three to six months. The brief course can be repeated if puberty does not progress spontaneously. Transdermal patch and gel preparations of testosterone are also available, though achieving the needed low doses is trickier than with the injectables. Oral androgens, like methyltestosterone, carry potential hepatic toxicity and should be avoided. Anabolic steroids have been used in constitutional delay to stimulate growth as well as to promote sexual development, and although growth velocity accelerates, final height generally does not improve and could even be attenuated (123). Oxandrolone seems to be the best growth-promoting anabolic compound available; its nonaromatizability minimizes its impact on bone age advancement (124). Treatment with these medications should be reserved for patients who have attained the psychological development appropriate for puberty.

The decision to use pharmacological intervention must depend on the patient's emotional outlook and the severity of delay. Most children with constitutional delay of growth and puberty are able to cope with this condition, if they are properly reassured about their ultimate height and development. This diagnosis, by definition, presages eventual normal maturity and height without medical intervention, provided they ingest appropriate nourishment. Caution is warranted when treating such a benign alteration, although the medical induction of accelerated maturation brings an immediate reward. Careful assessment of the

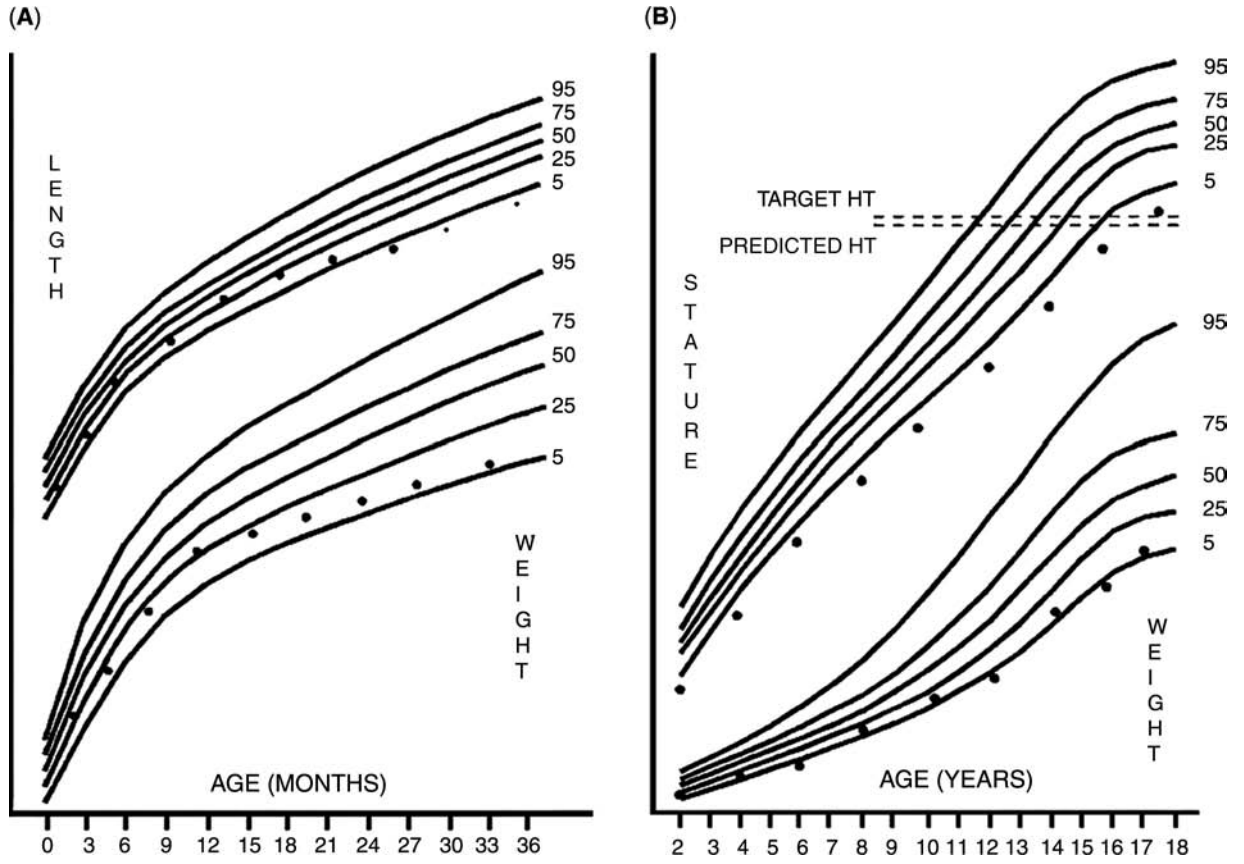

Figure 7 Familial short stature. (**A**) Typical growth in infancy. Note the readjustment of the length channel to one more appropriate for the genetic potential (parental target height). (**B**) Typical growth in childhood and adolescence. The new height percentile is maintained without further fall-off, and the weight is appropriate for height. Short stature is life-long and there is no catch-up growth in puberty. The predicted height of the patient is in range for the target. *Source*: From Ref. 97.

nutritional intake is recommended, with particular attention to deficits in micronutrients, iron, and calcium, because these patients may have decreased bone density as adults (125). If deficits are uncovered, nutritional therapy is recommended.

FAMILIAL SHORT STATURE

Familial short stature, also called genetic short stature, refers to individuals with life-long shortness but normal growth velocity and height that are within normal limits when allowance is made for their parents' heights (Fig. 3) (66). Growth rate decreases some time between 6 and 18 months of age (100), and then follows a steady channel below the fifth percentile after two to three years of age (Fig. 7). This rechanneling reflects the switch in the predominant determinants of size; birth size is mostly determined by maternal and pregnancy health, whereas after six months, the individual's genetic factors predominate. Thus, a child who was born of average size may now shift to lower percentiles because his or her parents are of short stature. In contrast to patients with constitutional growth delay, these infants gain weight at a steady rate, do not exhibit weight deficits for length, and have no bone age delay (100).

Familial short stature can be compounded by other issues, but the diagnosis rests on the current or predicted height's being normal for the midparental target height, which is also short. Frequently, there is a superimposed component of constitutional growth and puberty delay. Tubular bone alterations, including brachymetacarpia V (fifth metacarpal bone shortening; Fig. 5), rhizomelia and disproportionate limb shortening (Fig. 4), were described as significantly more prevalent in children and adults with familial short stature than in the normal height population (77). Most children and adults with familial short stature had two to four types of tubular bone alterations, whereas most individuals with normal stature had either none or only one type of tubular bone defect. A direct linear relationship was observed between the degree of shortening of the fifth and first metacarpal bones, but not of the other metacarpal bones (77). These findings suggest that some patients with familial short stature may have an inherited defect in endochondrial ossification, the major process involved in tubular bone elongation and increase in stature. This defect may result not only

in decreased overall stature but also in disproportionate limb shortening.

The prevalence of SHOX haploinsufficiency has been estimated at approximately 2% of individuals with idiopathic short stature and normal karyotype (89,126,127). However, the prevalence may be higher in patients who exhibit tubular bone alterations frequently found in individuals with familial short stature, such as decreased carpal angle, angulation of the distal ulna or brachymetacarpia (77). Furthermore, the prevalence of SHOX may differ between sexes and across age groups, because mild skeletal features are found mainly in males and prepubertal girls (128).

Familial short stature likely represents a heterogeneous group of conditions. Some manifest as short stature alone, while others may present with minor tubular bone alterations, with or without disproportionate limb shortening. For example, patients with type E brachydactyly have no other skeletal abnormalities except short stature and metacarpal and metatarsal shortening (129). Hypochondroplasia, particularly when mild, may only manifest as short stature with a slight, disproportionate limb shortening and brachydactyly (130). Unless careful observations and measurements of the different body segments are made, these patients can be underdiagnosed as simple familial short stature. In these cases, a detailed radiological study and segregation analysis of the family members must be done.

Although it is important to consider the parents' heights in evaluating a child's short stature, it should be remembered that a parent's stature is not necessarily familial or genetic (67). Stature also depends on a multitude of environmental factors that may have affected a parent's growth, including nutrition, drugs, and illness (131). Thus, considering the heights of the parents' siblings and parents, as well as obtaining a medical history of the parents, are also important before making a diagnosis of simple familial short stature (66). For example, sometimes short stature in a child and parent may actually be autosomal dominantly inherited isolated GH deficiency (132).

Like children with constitutional growth delay, there is pressure to consider growth-promoting treatment for children with familial short stature in the hopes of increasing their final height. As mentioned above, the FDA approved rhGH therapy for idiopathic short stature. This has spurred greater research interest in the unrecognized molecular defects that cause GH resistance and hence, non-GH deficient short stature (133). Some of these, if dominantly inherited, may currently be diagnosed as familial short stature. Response to rhGH treatment in idiopathic short stature has been variable (134,135), and the next challenge is to identify the different underlying mechanisms and their respective GH responsiveness (Vol. 2; Chap. 12). In the meantime, the cost of prolonged rhGH therapy to gain a very modest height increment should always be kept in mind, as well as other potential side effects (Vol. 2; Chap. 5).

PATHOLOGICAL (SYNDROMIC) SHORT STATURE

Pathological short stature is the least frequently occurring but most serious cause of short stature. Pathological short stature should be suspected in children who do not grow normally, with a growth velocity of less than 4.5 cm/yr after age five years and/or marked short stature. Bone maturation is usually quite delayed, often behind that expected for height. These patients usually fail to develop sexually, and the prognosis for ultimate height is dependent on the specific diagnosis (Table 1). Pathological short stature accounted for over one-third of the short patients referred to a pediatric endocrinology center (9,34). This incidence is high compared with the general population (32,33), but appropriate for a referral center.

It is essential to recognize these patients, because abnormal growth is frequently the only sign of underlying disease (34,136). A precise diagnosis must be established for timely treatment, and the differential diagnosis is as broad as the field of general pediatrics. Pathological short stature includes systemic diseases and hormonal abnormalities, which are reviewed in other chapters, so this section will focus on the syndromic causes of pathological short stature.

Turner syndrome is reviewed in detail in Vol. 2; Chap. 12, though warrants mention here. Girls with Turner syndrome have sex chromosome abnormalities, either pure 45 X, 46 XX with a variety of X chromosome abnormalities, or mosaicism. Short stature is the most frequently occurring physical finding, so many patients can present without the other classic dysmorphic features of the syndrome (86). Chart reviews of the University of North Carolina Turner Syndrome Clinic found that short stature was the key to diagnosis beyond infancy. Despite this, there was an average 5.2-year delay in diagnosis from the time the height had fallen below the fifth percentile (137). The pathogenesis of short stature in Turner syndrome was identified as haploinsufficiency of the SHOX gene (89). Turner patients are responsive to rhGH treatment, with final outcomes better the earlier rhGH treatment is initiated (30,31,138).

Down syndrome, another chromosomal defect leading to short stature, is the most common malformation in humans, with an incidence of 1:600. These children follow a typical growth pattern (specific growth charts, Vol. 1; Chap. 20) and have obvious dysmorphic features. The average adult female height is about 57 in. and the average adult male, 61 in. Patients have a tendency to be overweight beginning in late infancy and throughout the remainder of the growing years (139). Growth and weight gain may also be affected by a concomitant congenital heart defect or hypothyroidism. Adequacy of the GH/IGF axis in Down syndrome is controversial, because obesity is known to suppress GH secretion and may be a confounder in these studies. Thus, caution is encouraged in interpreting the results of GH testing in these patients. There are reports of accelerated short-term linear growth

in children with Down syndrome when treated with rhGH (140,141), though this treatment is controversial and currently limited to research. Careful evaluation of the safety, efficacy and ethical ramifications of rhGH treatment of children with Down syndrome is recommended before embarking on this form of intervention (141,142). However, regular monitoring of thyroid functions and thyroxine replacement for thyroiditis is established standard of care for these patients.

In contrast to Down syndrome, rhGH treatment received FDA approval in 2003 for ameliorating the short stature of PWS (Vol. 2; Chap. 5). PWS is caused by loss of expression of paternally inherited imprinted genes on chromosome 15q11-q13, and many of its features—growth failure, hyperphagia and obesity, temperature instability, hypogonadotropic hypogonadism—have implicated a hypothalamic defect as the primary pathogenic mechanism (143). In addition to increases in linear growth and final height, rhGH therapy has been shown to improve body composition, physical strength and agility in patients with PWS (144). However, reports of sudden death in children with PWS on rhGH treatment have caused alarm. It remains unclear if rhGH played a causal role or if the rhGH surveillance programs led to an ascertainment bias, as data on sudden death in untreated PWS were previously lacking. Subsequent reviews, of both rhGH-treated and untreated fatalities, revealed common themes: sudden death in PWS tends to be respiratory failure in younger years (central apnea as well as obstructive apnea exacerbated by hypotonia, respiratory infections or obesity), and related to complications of obesity and diabetes mellitus (DM) in later years (145–147). It is still unclear whether rhGH may help with the respiratory fatality risk by improving the hypotonia, or whether rhGH worsens the respiratory risk by inducing lymphoid hyperplasia. In any case, polysomnography, otorhinolaryngologic examination and tonsillectomy if indicated, and aggressive treatment of upper airway infections should be provided to all PWS children. Even if future evidence clears rhGH from the sudden death scare, rhGH treatment should always be provided as part of multidisciplinary care that includes programs to address the cognitive, behavioral and hyperphagia aspects of PWS (148).

Another category of genetic defects leading to pathological short stature is the skeletal development disorders, which result in disproportionate short stature (Vol. 2; Chap. 6). The primary defect may affect either the cartilaginous or the bone-forming stage of bone development. More than 250 different types of skeletal dysplasia are known, and many of their causes were not identified until recently. Traditional classification schemas were therefore based largely on morphological criteria (149), though newer molecular ones have been proposed (Vol. 2; Chap. 6). The disproportion seen in many skeletal dysplasias may have therapeutic implications. Because some bones grow better than others, growth-promoting therapies may exacerbate the disparities among the various bones.

The term, primordial dwarfism, has been used to refer to a group of conditions characterized by severe short stature and typical dysmorphic features, that are not associated with an identifiable skeletal dysplasia or other specific cause for the short stature. Short stature is prenatal in onset; these children are born small for gestational age (SGA), and skeletal age is retarded (94). The best known condition among the primordial dwarfisms is Russell–Silver syndrome, described independently by Silver in 1953 and Russell in 1954. Skeletal asymmetry is a distinct feature of this disorder, as are clinodactyly of the fifth finger and small triangular facies with down-turning of the corners of the mouth. Cafe-au-lait spots are usually present, and responses to rhGH therapy have been promising (150).

Cornelia de Lange syndrome typically features growth failure, mental retardation, microbrachycephaly and characteristic facies that include bushy eyebrows and synophrys, hirsutism, delayed dentition, micromelia, phocomelia and/or oligodactyly, and hypoplastic external genitalia, hypospadias and undescended testes. There may or may not be associated endocrinopathies (151). Mutations in the *NIPBL* gene have recently been identified as the cause, though the function of the *NIPBL* gene product in humans is still unknown (152).

Bloom syndrome is characterized by mild microcephaly with dolichocephaly, malar hypoplasia, and facial telangiectatic erythema. There may be a mild mental deficiency and immunoglobulin deficiency. Death is usually caused by a lymphoreticular malignancy (94). Johanson–Blizzard syndrome is characterized by varying degrees of intellectual impairment, hypoplastic or aplastic alae nasi, hypoplastic deciduous teeth, and absent permanent teeth. They may have cryptochoridism, micropenis, imperforate anus, hydronephrosis, septate or double vagina, primary hypothyroidism, and/or pancreatic insufficiency (153).

Seckel syndrome is characterized by microcephaly, mental deficiency, premature synostosis, central nervous system abnormalities, clinodactyly of the fifth finger, and dislocation of the radial head and/or hips (94,154). These patients had been referred to as bird-headed dwarfs because of the disproportionately large nose relative to the mandible and face (94), but this term has fallen out of favor. Finally, Williams's syndrome is characterized by a specific neurocognitive profile, typical facies, infantile hypercalcemia, growth and developmental retardation, and cardiovascular anomalies that include supra-valvular aortic stenosis, pulmonary artery stenosis, ventricular or atrial septal defects, renal artery stenosis, hypertension, and hypoplasia of the aorta (155,156). It is caused by deletion of chromosome 7q11.23 that spans approximately 17 contiguous genes including the one for elastin.

INTRAUTERINE GROWTH RETARDATION

Intrauterine growth retardation (IUGR) is a pathological condition in which infants do not reach their

genetic growth potential as a result of various genetic and/or environmental influences during gestation. They have low birth weight (LBW) for their gestational age, with a cut-off of 10th percentile usually defining SGA (157). The Third National Health and Nutrition Examination Survey showed an overall prevalence of IUGR as 8.6% of U.S. live births (158). Elsewhere, the prevalence was found to approximate 3% (159). IUGR is especially important because of the higher incidence of morbidity and mortality and the potential for long-term complications in childhood and adulthood. Infants with LBW are 5 to 10 times more likely to die in the first year of life than are normal birth weight infants (160,161). Those who survive have increased risk for later complications, some of which are reviewed in the following sections. The long term endocrine dysfunctions of SGA in postnatal life are reviewed in Vol. 1; Chap. 12.

IUGR is frequently, though inaccurately, used synonymously with SGA, as a birth weight below the 10th percentile for gestational age (157,162). This weight cut-off has been criticized for allowing an overestimation of the real incidence of this disorder, because it implies that 10% of normal infants will have a birth weight that qualifies for the diagnosis. Standards for fetal growth were developed many years ago by Usher and McLean (163) and Brenner et al.(164). Risk for fetal death is significantly increased in those with birth weights between the 10th and 15th percentiles (165). Up to 70% of all SGA infants may be constitutionally small fetuses expressing their genetic potential, and may not be at risk for perinatal morbidity or mortality. The remaining 30% are growth-restricted infants because of various pathological conditions, and are at risk for an adverse outcome. Thus, SGA should refer to infants not meeting a birthweight threshold (usually 10th percentile), while IUGR refers to pathologically growth restricted infants who are at risk for lifelong sequelae (157).

Assessment of Growth in Fetuses and Infants

In utero detection of fetuses with IUGR may reduce their chances of morbidity and mortality. Ultrasound can detect up to 80% of fetuses with SGA with great precision, but false-positive results may reach an incidence of 20% (166). Several ultrasonographic measurements have been used to diagnose IUGR: fetal abdominal circumference, biparietal diameter, head circumference and skeletal length. In order to detect abnormalities in fetal growth, at least two serial ultrasound measurements should be taken ideally before the 26th week of gestation. Doppler velocimetry of fetal cardiac output, systemic blood flow, and organ supply (especially with respect to placental circulation) is a powerful tool in distinguishing IUGR fetuses at risk of acidemia from constitutionally small but otherwise normal fetuses (167). Cordocentesis to measure lactate concentration in fetal blood is one of the earliest markers of fetal distress. A significant correlation has been found between elevated midtrimester β-core fragment levels of human chorionic gonadotropin and IUGR, comparable to third-trimester ultrasound and superior to maternal serum analytes (168).

At birth, the ponderal index (PI) [birth weight (g) $\times$ 100/length $(cm)^3$] is a simple and easily available measurement of proportionality. It has been used to determine the thinness of infants with IUGR. Infants with low PIs have presumably been exposed to short periods of malnutrition that compromised weight gain but not length or head circumference (disproportionate IUGR). On the other hand, infants with ''normal'' PIs are proportionate at birth and may have been exposed to more chronic injury in utero. However, a recent study found that body weight alone was a better predictor than PI of anthropometric ratios in full-term singleton births, organ asymmetry and distinction between chronically versus acutely anoxic stillborn fetuses (169).

Etiology

Normal growth in utero depends on the genetic potential of the fetus modulated by environmental, hormonal, and other biological factors, including maternal health and nutrition (170). Infants of small parents tend to be small, with maternal size having the greatest infiuence (171). The pathogenesis of IUGR can thus be divided into extrinsic factors, mainly maternal, and to intrinsic fetal growth retardation. Identifying fetuses whose IUGR results from placental insufficiency is important clinically because appropriate diagnosis and timely delivery can alter the outcome (170).

The extrinsic factors that cause IUGR occur later in pregnancy and, as a result of placental disorders or maternal disease, compromise the delivery of oxygen and nutrients to the fetus. Of special importance is the nutritional status of the mother. Chronic undernutrition, more prevalent in developing countries, is responsible for a large population of infants with IUGR worldwide. Different outcomes are observed according to the stage of fetal development at which maternal malnutrition takes place (172,173). Early fetal malnutrition may affect growth permanently by reducing cell proliferation and size, while later malnutrition impairs growth by decreasing cell size despite preservation of the cell population. Infants exposed to early fetal malnutrition have LBW and are symmetrically small (proportionate IUGR). Undernutrition in late pregnancy results in an asymmetrically growth-retarded infant whose head circumference is preserved by a physiological adaptation (brain-sparing phenomenon) in which a major selective blood flow is directed to the brain (174).

There is undoubtedly an association between maternal weight gain during pregnancy and infant birth weight (175). Adequate prenatal care and improved maternal nutrition, through balanced

calorie or protein supplementation, leads to an overall increase in infant birth weight and to a decreased rate of LBW deliveries in at-risk populations (176). These guidelines have been endorsed by the American College of Obstetricians and Gynecologists (177) and used by the supplement food programs for Women, Infants and Children (178). Previous nutritional guidelines recommended a gain of 22 to 27 lb for women of all weight categories. Currently, the Institute of Medicine recommends a weight gain of 29 to 40 lbs for underweight women (BMI < 19.8 kg/m^2); 25 to 35 lbs for average women (BMI between 19.8 and 26 kg/m^2); and 15 to 25 lbs for overweight women (BMI between 26 and 29 kg/m^2). These weight gain recommendations have been associated with a decreased incidence of LBW (173,175–178). In addition to overall weight gain, fetal growth and development depend on adequate micronutrient intake, especially iron (179) and folate (180).

Other maternal risk factors associated with IUGR can be divided into supply-limited constraints (e.g., maternal size) and demand-driven constraints (e.g., twinning) (181). Such risks include young maternal age, small maternal size, early menarche, short inter-pregnancy interval and high maternal parity. Maternal constraints resulting in IUGR may be a multigestational effect, requiring several generations to correct (171). Often several conditions overlap, such as chronic malnutrition and substance abuse, tobacco smoking, alcohol ingestion and low socioeconomic status (182–184). A literature review, mainly consisting of population-based studies, found associations of ambient air pollution and IUGR, though the evidence was deemed insufficient to infer causality and further research is needed (185). Mothers who live at high altitudes are at increased risk of LBW, which may be mediated by systemic hypoxemia or compensatory increases in hematocrit that lead to increased blood viscosity (186). Systemic hypoxemia from maternal illnesses can also impair fetal growth, such as cardiac disease (mainly cyanotic types), sickle cell disease or severe asthma. Proteinuric hypertension during pregnancy is often complicated by fetal growth retardation.

Intrinsic fetal factors that can cause IUGR include infectious and genetic reasons. Infectious agents, such as the viruses associated with TORCH syndrome [Toxoplasmosis, Other (syphilis, varicella–zoster, parvovirus B19), Rubella, Cytomegalovirus, Herpes simplex], are usually responsible for early onset IUGR and have more severe consequences (187). Of these, rubella and cytomegaloviruses are the most important identifiable agents associated with marked fetal growth retardation. These viruses reduce cell number and subsequent birth weight by simultaneously inhibiting cell division and producing cell death (188). Meta-analysis of 31 controlled, prospective studies found that maternal infection with HIV had a summary odds ratio of 1.7 (95% CI, 1.43–2.02) for IUGR and 2.09 (95% CI, 1.86–2.35) for LBW (189).

Genetic causes of IUGR include chromosomal abnormalities, single gene defects, and aberrant expression of imprinted genes (190). Some chromosomal abnormalities, such as Down syndrome, Trisomy 13, Trisomy 18, Turner syndrome and other major congenital malformations, impair both prenatal and post-natal growth. Disrupted imprinting, the phenomenon of parent-specific mono-allelic gene expression, is gaining interest as a pathogenetic mechanism of IUGR. Imprinted genes may account for 0.1% to 1% of all mammalian genes, and have evolved to fine-tune fetal growth (191). Paternally expressed genes generally enhance growth, whereas, maternally expressed genes appear to suppress growth. For example, 10% of patients with Russell–Silver syndrome were found to have maternal uniparental disomy (duplication of the maternally derived allele) or inheritance of a mutated maternal allele of the *GRB10* gene on chromosome 7; GRB10 has a suppressive effect on growth through its interaction with either the IGF-I receptor or the GH receptor (192). Alterations in the paternally imprinted *IGF2* gene on chromosome 11 cause Beckwith–Wiedemann syndrome, which includes prenatal and postnatal overgrowth and increased risk for tumors (190,191).

Hormonal Changes

Insulin, IGF-I and IGF-II seem to be the major hormonal mediators of fetal growth. Insulin has a major effect on growth and birth size, as evidenced by the macrosomy of infants born to diabetic mothers and babies with congenital hyperinsulinism. Insulin induces protein synthesis and hepatic glycogen deposition, increases nutrient uptake and utilization, and stimulates fetal lipogenic activity leading to rapid accumulation of adipose tissue, mostly during the third trimester. In general, growth-restricted infants are characterized by fetal hypoglycemia, which limits insulin secretion and fetal glucose production with increased protein breakdown. This reduces protein accretion, which results in slow growth. Additionally, insulin plays a permissive role in the release of different growth factors from placental tissues (193). Placental lactogen, a structurally related placental peptide that has many GH-like actions, also seems to play an important role in fetal growth. Maternal serum concentrations of placental lactogen rise significantly in the third trimester, parallel with a rise in serum IGF-I. Reduced maternal circulating placental lactogen concentrations have been associated with poor fetal growth, though are insufficient to predict it (194–196).

Early evidence for the roles of IGF-I and IGF-II in fetal growth were mostly associative. Serum IGF-1 levels were lower, and GH levels higher, in SGA newborns than newborns whose weights were appropriate for gestational age (197,198). Higher basal and stimulated [with GH releasing hormone (GHRH)] levels of serum GH in SGA infants similarly

suggested GH resistance (199). In a prospective study of IUGR infants, IGF-I levels correlated positively with weight gain during the first three months of life, but birth levels were not predictive of either later growth or short stature at age two years (197).

More compelling evidence has come from genetic mutations in humans and animal models. Mouse models of IGF-I and IGF-II deficiency are born small and have other phenotypic features seen in human IUGR, such as slow postnatal growth, increased insulin resistance and mental dysfunction (200). Likewise, targeted gene mutations in mice have contributed to our understanding of the interactions between insulin, the IGFs and their respective receptors in controlling murine embryonic growth; the type 1 IGF receptor mediates the actions of IGF-I and IGF-II, while IGF-II, rather than insulin, acts through the insulin receptor to stimulate intrauterine growth (201). Case reports of individuals with IUGR and postnatal growth failure have identified IGF-I gene deletion (202), mutations in the IGF receptor gene causing reduced receptor number or function (203), and mutations in the gene for STAT5b, the major mediator of IGF-I gene induction by GH (204). Further, Laron dwarfism, the prototypic syndrome of primary IGF-I deficiency, is caused by GH insensitivity due to mutations in the GH receptor. Laron dwarfs usually have a birth length that is 10% to 30% less than normal (205).

Analysis of human placentas from IUGR versus normal pregnancies found a 33% reduction in protein content of IGF receptors, selective impairment of the IRS-2/phosphatidyl inositol 3-kinase pathway, and reduced p38 and c-Jun N-terminal kinase activation. This suggests that impaired IGF signaling within the placenta, and not necessarily the fetus, can also lead to IUGR (206).

Finally, leptin, a hormone produced by adipose tissue, may play a role in fetal growth and nutritional homeostasis (207). The hormone has been detected in fetal blood as early as the 18th week of gestation (208). No relationship has been found between maternal and fetal serum leptin levels. Birth weight and BMI correlated strongly with serum leptin concentration in infants; serum leptin levels in SGA infants were half those of appropriate-for-gestational age infants and one third those of large-for-gestational age infants (209). The role of this hormone in the postnatal growth of infants with IUGR remains to be elucidated.

Postnatal Growth

Although many IUGR babies grow and develop normally and attain normal stature as adults, about 10% to 15% do not exhibit catch-up growth and remain short throughout life (159,210). Catch-up growth usually occurs during the first two years of life, with most infants achieving this growth within the first six months of life (159). Unlike term SGA infants, LBW infants who are born prematurely usually show poorer progress. In those in whom catch-up growth does not take place, final stature is compromised.

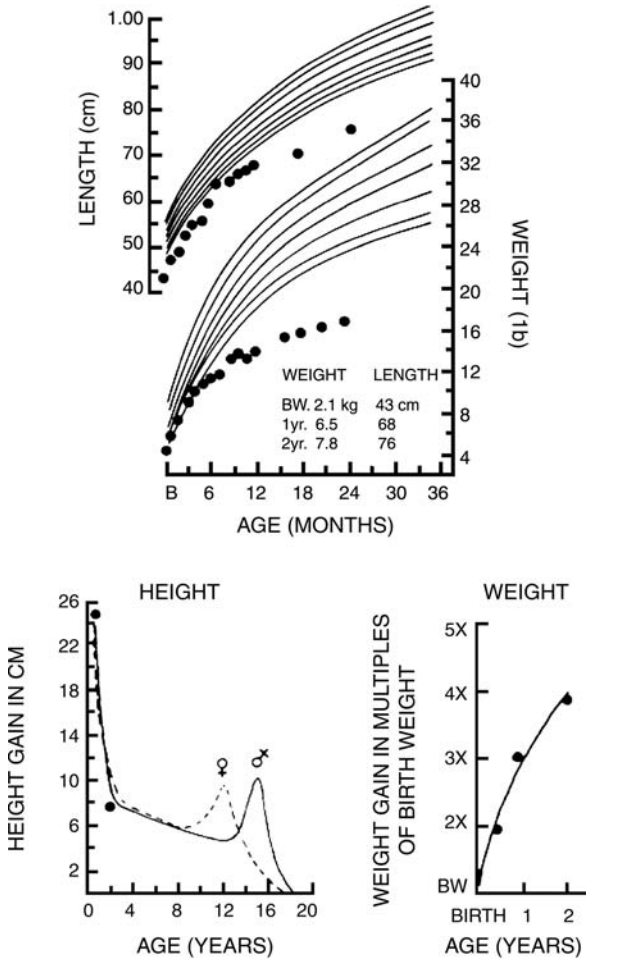

Figure 8 IUGR infant who maintains normal growth rates but does not exhibit catch-up growth. (*Top*). When plotted on a growth chart for normal children, not on a Specific IUGR growth chart, this child's growth appears worrisome for FTT. (*Bottom*). Growth velocity is plotted against normal standards, ruling out the diagnosis of FTT. *Abbreviations*: IUGR, Intrauterine growth retardation; FTT, failure to thrive. *Source*: From Ref. 34.

The determinants of which infants will or will not experience catch-up growth remain unknown. A longitudinal study of 213 SGA infants found that final height was primarily determined by parental height (especially the mother's) and birth length, rather than variables such as gender, birth weight or PI (197). Another study found no differences in environmental risk factors between IUGR infants who showed catch-up growth and those who did not, suggesting that genetic factors account for the persistent effects of IUGR on growth (211). The importance of a genetic contribution is supported by the increased prevalence of IUGR within some families, and the fact that IUGR infants with identified genetic mutations in the IGF pathway continue to have postnatal growth failure.

Frequently, postnatal growth of IUGR infants is compromised by FTT, with many being described by their parents as "picky eaters." The incidence of FTT

in IUGR infants appears to be high, with a reported incidence of 19.7% in a cohort of 914 preterm LBW infants who were evaluated for three years (212). Infants who experienced FTT remained smaller on all growth parameters (weight, length, and head circumference) at three years compared to their matched controls.

IUGR infants may be mistakenly labeled as FTT when they actually maintain normal growth rates but fail to achieve catch-up growth. For example, in Figure 8, the weight and length curves for an IUGR patient appear worrisome; they were plotted on a growth chart for normal children, not on a specific IUGR growth chart. However, examination of the progression in anthropometric measurements, as shown in the bottom panels, ruled out the diagnosis of FTT. The patient tripled his weight by age one year and quadrupled it by two years, and his length similarly progressed well and remained proportional to weight throughout. Thus, this IUGR infant maintained normal growth rates but did not exhibit catch-up growth. Infants with IUGR start with a tremendous size deficit, and those who do not exhibit catch-up growth can be expected to remain proportionally small thereafter. Often these children are forced to increase their caloric intake to no avail as a means of enhancing their linear growth. Aside from the absence of catch-up growth, final height in children with IUGR may also be compromised by early puberty, which is more common among this population (213).

However, if weight gain does not progress at a normal rate, FTT must be considered. IUGR infants often have other associated abnormalities, such as neurological, cardiac or pulmonary disorders, that may contribute to poor growth and/or FTT. Frequently these patients present with oral motor dysfunction (OMD), resulting in "poor feeding" (214). Nutrient intake in LBW infants is difficult at best, and most often does not meet the recommended dietary intakes (215). During the first few weeks of life, there is an accumulated nutrient deficit of energy, protein and other nutrients, that can impact growth long-term and contribute as much as 45% of the postnatal growth variation. Thus, IUGR infants must be carefully monitored to ensure an adequate nutritional intake, which should allow for their maximum growth after birth.

In 2001 the FDA approved rhGH treatment for SGA children who fail to manifest catch-up growth by age two years (usually defined as stature persisting below −2 SD). However, rhGH treatment of SGA infants should not be undertaken without appropriate nutritional intake. This hormone is usually given to these patients at doses up to 0.48 mg/kg/wk (Vol. 2; Chap. 5) (216). Final height data of rhGH treatment for SGA are only recently available. In The Netherlands, 75 prepubertal short children born SGA were enrolled in a multicenter, double-blind, randomized, dose-response rhGH trial. Their mean (SD) age at initiation of therapy was 7.3 (2.1) years, and their mean (SD) final height SDS's were −1.2 (0.7) for those receiving 0.033 mg/kg/day and −0.8 (0.7) for those receiving 0.067 mg/kg/day; the final height SDS were not significantly different between the two rhGH dosage groups (217). In a Swedish study, 77 short children born SGA were treated with 0.033 mg/kg/day rhGH, beginning before the onset of puberty and continuing to final height. Final height was 1.3 SD (9 cm) greater than pretreatment projected height for the entire group, and the growth response was greatest the younger, shorter and lighter the child at the start of treatment (218).

In addition to the paucity of long-term data, some particular concerns have been raised about rhGH treatment for SGA (217). Firstly, children who were born SGA represent a heterogeneous group of underlying disorders, which are mostly unknown. The doses of rhGH used for SGA exceed those for GH deficiency, based on the hypothesis that many of these children have a state of GH resistance that can be overcome with higher doses. In one study that differentiated the underlying condition, rhGH treatment increased height SDS of both 127 children with Russell–Silver syndrome (starting treatment at a median age of seven years) and 593 children with SGA (starting treatment at a median age of 9.2 years) (219).

In light of the higher rhGH doses required to enhance growth, closely monitoring IGF-I levels during treatment is warranted for safety (200). A potential adverse effect of rhGH treatment is especially relevant to children who were born SGA. The metabolic effects of GH increase insulin resistance, and LBW has been associated with impaired insulin sensitivity, type 2 DM, hypertension and cardiovascular disease in later life (Vol. 1; Chap. 12). In a rhGH controlled trial, short SGA children with a mean (SD) age of 6.0 ($\pm$1.5) years were randomly assigned to 0.033 mg/kg/day rhGH ($n = 60$) or control group ($n = 30$) (217). At the start of rhGH therapy, 8% of children had impaired glucose tolerance (IGT) on standard oral glucose tolerance testing, and systolic blood pressure was significantly higher in comparison with healthy peers. Fasting and glucose-stimulated insulin levels were higher after one and six years of rhGH treatment, yet after six years, 4% of children had IGT and none developed diabetes. Fasting and glucose-stimulated insulin levels returned to normal six months after discontinuation of rhGH. During the six years of rhGH therapy both systolic and diastolic blood pressure decreased significantly (217).

Long-Term Effects

IUGR may have important long-term consequences, resulting in increased morbidity and mortality in adulthood (Vol. 1; Chap. 12). An increased incidence of hypertension, cardiovascular and cerebrovascular disease, type 2 DM, and lipid disorders have been reported in older children and adults with clinical antecedent of LBW (220–227). According to the "thrifty phenotype" hypothesis, the fetus adapts to an adverse intrauterine environment by optimizing the use of a reduced nutrient supply to ensure survival; these

developmental changes result in the permanent reprogramming of metabolic pathways and lead to later disease (228). Proposed mechanisms include changes in epigenetic gene regulation, islet development, insulin signaling pathways and activity of the hypothalamic–pituitary–adrenal axis. Supportive data are drawn from epidemiological, clinical and animal studies (229). A recent retrospective longitudinal study of 8760 Finns found that adults with coronary events were characterized by a particular growth pattern: LBW and length, low weight and BMI at age two years, but rapid weight gain between ages 2 and 11 years (230). This growth pattern was also associated with elevated fasting insulin concentrations.

Another sequela of IUGR is an increased risk of poor neurological outcome, including cerebral palsy, cognitive deficits and behavior problems (231). Poor prenatal head growth and increased incidence of major intracranial injury have been implicated. Effects of rhGH treatment on psychological adjustment in very stunted IUGR patients remain to be seen.

FAILURE TO THRIVE

The term FTT is used to describe infants and young children whose body weight and weight gain are substantially less than those of their peers (232). Its definitions include: deceleration in weight gain to a point below the third percentile, a fall in weight across two or more major percentiles, or weight less than 80% of the ideal weight for age. FTT accounts for 1% to 5% of tertiary hospital admissions for patients less than one year of age (233). Many more children, perhaps 10%, are managed as outpatients by physicians throughout the United States (234). Despite its established status in medical terminology, the concept of FTT lacks a clear definition and should be considered a sign or symptom, not a diagnosis or disease (235).

Although all FTT infants have physiologic alterations due to malnutrition, the causes have been subdivided into organic or nonorganic (236), an oversimplistic distinction (237). Organic FTT (OFTT) involves specific diagnosable underlying disorders, and is only identified in 20% to 40% of children hospitalized with FTT, even less frequently in outpatient clinics. In contrast, patients with nonidentifyable underlying disorders account for the majority of infants with FTT, though the percentage varies from institution to institution. Non-OFTT (NOFTT) does not imply a specific etiology, but merely suggests that the cause is primarily external to the infant (238). Children with FTT present with a low rate of weight or length gain, delayed development, abnormal behavior and distorted caretaker–infant interaction. Often OFTT and NOFTT overlap, because there are minor infections, vomiting and diarrhea in children with behavioral problems and altered eating behaviors. Thus, the adequacy of the traditional dichotomous view has been questioned and a third category, called mixed cause FTT, was proposed (239,240). In this section we will limit the discussion to patients without diagnosable organic pathology.

FTT can be caused by a variety of disorders that have little in common except for poor body weight. Each one must be recognized and treated accordingly, though the goals of nutritional rehabilitation are similar regardless of the cause (241). In addition, some patterns of growth in early life can present as factitious FTT and must be distinguished from true FTT (241,242). These patterns include patients with constitutional growth delay and/or familial short stature discussed above. Because birth size is related more to maternal size and intrauterine influences than to genetic factors, recanalization of normal growth in some children manifests as an adjustment in growth velocity greater than 25% (across two major percentile lines). Thus, a significant decrease in growth rate in these conditions may represent a physiological event in the first years of life rather than FTT. Also, patients with IUGR may mimic the symptoms of FTT as described above.

The Breastfed Baby

Caution must also be taken in labeling an exclusively breastfed infant as having FTT. The commonly used growth charts are based on studies of infants who were mostly formula fed (243). The normal growth pattern of a breastfed baby may seem to falter on such growth charts (244) but not on growth charts based on breastfed infants (56,245). Human milk is the ideal and most readily available nutrient, and should therefore be continued and encouraged as much as possible (246) even in an infant whose growth seems to falter on the standard formula-based charts. The World Health Assembly adopted a resolution to recommend exclusive breastfeeding for six months to its member countries (247). The Committee on Nutrition of the American Academy of Pediatrics recommends exclusive breast feedings for four to six months (248).

However, breastfeeding alone may be inadequate for some children who fail to gain weight. Breast feeding must be closely monitored to ensure that adequate lactation is present and that the infant thrives at an appropriate rate (56). As general guides, a healthy, breastfed infant is expected to lose less than 10% of birth weight, return to birth weight by age two weeks, and then gain weight steadily at a minimum of 20 g/day, from age two weeks to three months (249).

Exclusively breastfed infants had slower length velocities after three months of age than infants who were weaned early and given formula plus solids. Although this trend was more obvious at age nine months, there was no deficit found in relative weight for length (244). The DARLING study showed that the mean weight of breastfed infants dropped below the median of the formula-fed group between 6 and 18 months of age, but length and circumference

values were similar between the two groups (250). A retrospective analysis of 13 studies on prolonged breastfeeding found a negative relationship between breastfeeding and growth in eight, a positive effect in two, and mixed results in three studies (250). The lower weight gain of breastfed infants seems to result from infant self-regulation of energy intake rather than nutritional deficits, though the diets of breastfed infants may be limited in vitamin D and minerals, such as iron, zinc, and calcium; these can be provided by sun light and complementary foods (251). Even if prolonged breastfeeding is found to impair weight gain, the protection that breast milk offers against infection and other health issues would argue in favor of preserving the policy of encouraging human milk feedings as the main food (sole nutritional source for the first four to six months of life), particularly where sanitary conditions are poor. Prolonged breastfeeding also offers benefits for birth spacing, mother–child interactions, allergies, other morbidities and infant mortality.

Clinical Findings

In the FTT syndrome, both inadequate nutrition (i.e., nature) and distorted social stimulation (i.e., nurture) contribute to poor weight gain, delayed development and abnormal behavior. These infants are characteristically small for age, thin for length, with wide-eyed expression or gaze aversion, thin chests, wasted buttocks, prominent abdomens, hanging folds under the arms, expressionless face, decreased vocalization, reduced gross motor activity and response to social stimuli, lack of cuddling, and frequently clenched fists.

There is evidence that FTT infants may represent a combination of biological vulnerability with environmental difficulties, and be the products of parents with poor marital relationships (252,253). FTT may be the sole manifestation of child neglect or abuse (254). Observed mother–child play revealed more difficult behaviors (more negative affect, less vocalizing and more gaze aversion) during play by the FTT infants, a reduced likelihood to remain involved during the play interaction with the mothers, and a more chaotic play environment (255). Infants with this type of FTT are more passive, more likely to sleep through meals or take longer to finish their meals, and more likely to present with hypotonia. There is also evidence that they receive less appropriate developmental stimulation at home and have developmental delays.

Developmental delays have also been linked to OMD, which is frequently found in FTT children; the oral motor profiles of children with FTT were remarkably similar to those of children with cerebral palsy of the same developmental age. A careful feeding history, examination of head and neck structures, and observation of feeding may also show signs of dysphagia (256). OMD in children with FTT has been hypothesized to indicate subtle neurodevelopmental disorders (214). At 20 months of age, FTT infants were twice as likely to show mental developmental quotients under 80 and also showed less sociability (252).

Prenatal factors must also be considered that may possibly cause the problem. For example, potential prenatal exposure to psychoactive substances needs to be ascertained, including exposure to alcohol, tobacco and other drugs (257).

Nourishing and Nurturing

Every child who fails to thrive has either not taken, has not been offered, or has not retained adequate energy to meet his or her nutritional needs. However, FTT infants prove that nourishment involves much more than ingestion of food. In most instances FTT results from a disruption in nurturing practices that ultimately impairs the child's ability to obtain proper nourishment. Nurturing factors include parental beliefs and concepts of nutrition, the infant's behavior, and adverse social or psychological environments. Therefore, direct observation of mother–infant feeding and their social interaction is a critical part of the evaluation. A careful nutritional evaluation must also be performed, including a 24-hour dietary recall or, more accurately, a food diary for three to seven days. It should address meal frequency, feeding patterns, and intake of all solids and fiuids. It is also valuable to determine whether any particular food was restricted or promoted, such as ''no junk food'' or excessive fruit juice consumption (258–262), or if there are vegetarian or other practices (263–265).

Often the diet record suggests that the child is receiving adequate calories for weight and length, but not for age. This caloric level allows the infant to maintain current weight but does not provide sufficient nutrients for growth. Sometimes the dietary intake is adequate in calories and protein, but is deficient in specific nutrients, such as iron, zinc and/or other micronutrients, resulting in growth faltering (266). Supplementation studies have demonstrated that improvements in nutrient intake result in improved growth, including bone mineralization and maturation (267–269).

Particular attention should be paid to the presence of nonspecific symptoms, such as intermittent vomiting, spitting up, diarrhea, and frequent upper respiratory tract infections. These may occur in infants with FTT and may also indicate the presence of underlying organic conditions like gastroesophageal refiux (270,271). Behavioral feeding difficulties may also lead to decreased nutrient intake (272). Although apathy and decreased motor activity are recognized behaviors in malnourished infants (273,274), many of the abnormal behaviors of patients with FTT, such as gaze avoidance and fist clenching, are not attributable to malnutrition alone.

Some nutritional alterations may influence the infant's behavior. Iron deficiency has been associated with anorexia, irritability and lack of interest in the surroundings (275,276). Infants with moderate iron

deficiency anemia had poorer mental and motor functioning, and at follow-up in adolescence, performed less well on tests of cognition, attention and motor function even though they were no longer anemic and growing normally (277). Zinc deficiency may likewise compound the course of FTT (277), and excess lead ingestion may complicate the clinical picture even before the blood lead levels reach a toxic concentration. FTT infants were shown to have blood lead levels in a range formerly thought to be safe, i.e., 15 to 20 mg/dL (278). These elements should be monitored, and any detected abnormalities should be treated.

If decreased nutrient intake is found to be the cause of inappropriate weight gain, the question becomes: Why are insufficient amounts of food consumed by infants with FTT? Are these infants simply not offered enough? Do the infants fail to signal hunger or satiety? Do they have a poor appetite or refuse food?

Neglect and Deprivation

Development of a healthy parent–child attachment relationship begins in infancy and has life-long consequences on physical and psychological health. Virtually all infants become attached to their caregivers regardless of the quality of care; although children with disabilities may manifest attachment somewhat differently, they form attachment relationships similarly to nondisabled children (279). Disturbances in the parent–child attachment relationship affect child behavior, including the ability to thrive.

In many cases, parental stress affects the way infants interact with their mothers (242). The quantity and quality of social and emotional stimulation between parent(s) and child may be decreased even before clinical evidence of FTT is apparent. Many mothers of FTT infants are depressed, come from lower socioeconomic groups, lack a support group, and/or are themselves under multiple stresses. Mothers from higher socioeconomic groups may also lack the emotional strength or motivation to interpret or respond to the needs of their infant. As more mothers become engaged in work outside of the home or involved in activities that are independent of family responsibilities, their children may not get the appropriate attention to meet their needs for nurturing (280).

Psychosocial deprivation may also lead to FTT in infants (281,282). Interested in learning the innate language of humans, King Frederick of Sicily isolated infants to learn what language they would speak spontaneously. These children did not thrive and died from lack of communication and attention (283). Similarly, infant mortality and FTT were recognized for over half a century in foundling homes (284–288) and hospitals (289,290). In a classic example of the role of nurturing on somatic growth, Widdowson described two German orphanages run by women of opposite personalities. Despite similar dietary intakes, the children under the care of the unpleasant, aggressive, nonnurturing woman did not thrive, whereas those under the care of the woman with opposite personality traits grew well (288).

When evaluating a patient with FTT, one should carefully explore the possibility of concurrent psychosocial neglect (254). Almost 200 six-year-old children and their families recruited from pediatric clinics serving inner-city, low-income populations were studied longitudinally. FTT was defined as weight-for-age below the fifth percentile prior to age 25 months, and maltreatment was defined as having at least one report to Child Protective Services for neglect, physical abuse and/or sexual abuse. When both conditions occurred, there was a greater risk for behavior problems, worse cognitive performance and poorer school functioning than either risk factor alone (291). Infants accounted for the largest percentage of pediatric victims of abuse or neglect reported to United States Child Protective Services in 2002. Review of the 189,055 children born in 1996 in Florida revealed five risk factors for being part of the 0.85% with verified instances of maltreatment by age one: maternal smoking during pregnancy, more than two siblings, Medicaid beneficiary, unmarried marital status, and LBW infant (292). Measures of child neglect include ascertaining information about precedent neglect, unmet needs, and their consequences (293,294). Systematic review of the evidence regarding the effectiveness of child neglect treatment programs was sparse but indicated that multisystemic therapy was needed for improvement (295).

Infantile Anorexia

Another cause of FTT is infantile anorexia nervosa, characterized by food refusal, extreme food selectivity, and undereating despite parental efforts to increase the infant's food intake. The onset is usually between six months and three years of age, with peak prevalence around nine months (296). These infants are characterized by their willfulness. Mother and infant become embroiled in conflicts over autonomy and control, which manifest primarily during feeding time. This conflict leads to a battle of wills over the infant's food intake. Typically, parents mention that they have tried ''everything'' to get the infant to eat. Thus, mothers of infantile anorexics rated their children as more difficult, irregular, negative, dependent, and unstoppable, than did mothers of picky or healthy eating infants. Meanwhile, the mothers of anorexics showed greater attachment insecurity themselves than did mothers of healthy eaters, but demonstrated neither overt eating pathology nor less marital satisfaction than the other groups (297).

Chatoor (298) hypothesized that this separation-related conflict interferes with the infant's development of somatopsychological differentiation. The process of differentiating somatic sensations, such as hunger or satiety, from emotional feelings, such as affection, anger or frustration, is clouded by noncontingent responses by the parents to cues coming from the infant. As a

result of this confusion, the infant's eating becomes controlled by emotional experiences instead of by physiological needs. The focus of the treatment is on improving communication between the parents and the infant to facilitate the process of separation and individuality. In a behavioral-cognitive approach, the therapist explains to the parents the infant's behavior and suggests ways to positively modify and structure mealtimes to facilitate growth. Further psychotherapy may be needed for the parents struggling with unresolved issues around dependency and control.

Laboratory Evaluation

Poor nutrition and psychosocial factors are by far the most frequent issues leading to FTT. Therefore, laboratory tests offer limited value in determining the cause of growth failure; laboratory tests should only be used when findings from the history and physical examination indicate something organic or possible nutritional abnormalities, or when management of nutritional and psychosocial problems does not result in the expected improvement in growth rate.

In some cases, the child's bone age should be determined to facilitate the process of ruling out systemic chronic disease or a hormonal abnormality. This measurement may also be of help as a baseline for future growth and bone development progression. Laboratory evaluation of the nutritional status should be comprehensive to assess for deficiencies that are not clinically apparent. Such an evaluation should include measures of iron deficiency, which may manifest as anemia, but may also exist with normal hemoglobin and hematocrit levels and be responsible for some of the long-term complications of FTT even when there is no anemia (275–277).

Management

In the past, hospital care was routinely recommended as part of the initial management of FTT patients. The goals were to ensure an adequate dietary intake, to observe the child's behavior and to watch the family–child interactions, especially during meals. Despite today's economic constraints, hospital care is justified when the patient has not responded to appropriate outpatient management, the severity of the malnutrition warrants it, or abuse and/or neglect are suspected. A meta-analysis of FTT found that hospitalization significantly improved growth recovery and sustained catch-up growth (299). However, an aggressive outpatient management program may also be appropriate (300). The use of a multidisciplinary team usually offers special advantages in the rapid correction of undernutrition and developmental progress in children with FTT (301).

Frequent weight check visits to the outpatient clinic are needed to reinforce the behavior modification and to monitor response. If adequate weight gain does not occur soon after advice about feedings is given to the parent(s), the child needs to be evaluated more intensely. An inpatient hospitalization is indicated and may serve three purposes simultaneously: (i) allow observation of parent-child interactions (which may be diagnostically revealing); (ii) facilitate laboratory and radiologic evaluations to rule out organic disease; and (iii) provide appropriate nutritional intake in a highly controlled environment to verify that the patient has the capacity to gain weight and grow well.

Nutritional therapy of children with FTT has several goals: (i) achieving ideal weight for height; (ii) correcting nutrient deficits; (iii) allowing catch-up growth; (iv) restoring optimal body composition; and (v) educating parents about the nutritional requirements and feeding of the child. Regardless of why a child fails to thrive, effective nutritional management primarily consists of providing enough calories to achieve a positive energy balance and growth. The World Health Organization Expert Consultation on Energy and Protein Requirements recommended that ''whenever possible, energy requirements should be based on measurement of expenditure rather than intake'' (302). The standard energy expenditure prediction equations were all derived from data accumulated from healthy children. Thus, they may underestimate about one-quarter of the true energy requirement for infants with FTT (303).

Because nutritional intervention is usually the focus in treating children with FTT, high-calorie, adequate protein feeding has been advocated for many years. With this treatment the child recovers more rapidly, hospitalizations are shorter, and more children can be treated in a given period of time at less cost. Nurses or trained therapists should feed the infant initially to allow identification of a feeding problem and to ensure that intake will be adequate. Proper feeding of the FTT child can be achieved most often with infant formula that is given in sufficient quantities to meet the child's specific nutrient needs. Protein and other types of supplementation are usually not indicated.

Tube feedings are necessary only in cases of severe malnutrition or failure to induce weight gain in the hospital. They may be necessary if the child is severely debilitated, metabolically unstable, or requires immediate restoration of fluid and electrolyte balance. Tube feedings may be useful for children with FTT as a temporary behavior modification modality, or in patients who fail to respond to other methods of nutritional rehabilitation (304).

Many clinical trials have indicated that supplementation with micronutrients improves weight gain in growth-faltering patients. Single-nutrient deficiencies are cumbersome to document, and micronutrient deficiencies commonly coexist. For example, iron deficiency may be present without anemia, and documentation of zinc deficiency is hampered by the lack of a good indicator. Nonetheless, clinical trials of iron supplementation showed positive effects on weight gain, linear growth, and psychosocial behavior (273,305,306). Similar studies have revealed positive

effects of zinc supplementation on growth as well as on morbidity and severity of infections in children (307–311). Vitamin and mineral deficiencies sometimes become evident only after the infant starts growing and gaining weight. Therefore, a multivitamin-mineral preparation that includes iron and zinc is recommended for all undernourished children. UNICEF sponsored an international, randomized, placebo-controlled, double-blind study of micronutrient supplementation during the high risk transition period from breast milk to solid foods (6–12 months). Randomization of 1134 infants from rural populations in Indonesia, Vietnam, Peru, and South Africa showed that a daily multiple micronutrient supplement surpassed weekly multiple micronutrient supplementation, daily iron supplementation, and placebo in reducing weight growth faltering, though it still occurred, and there was no protective effect on stunting (312). Further studies are needed to find the optimal supplementation.

Nutritional rehabilitation for FTT children must accomplish catch-up growth, defined as the growth acceleration that occurs when a period of growth retardation ends and favorable conditions are restored. Catch-up growth in FTT depends on the provision of calories, protein, and other nutrients in excess of normal requirements. Children need 25% to 30% more energy and nearly double the amount of protein for catch-up growth (313). The extent to which nutritional rehabilitation can restore normal body size and composition is a critical subject. Returning to one's previous growth curve does not indicate achievement of a normal body composition (314).

Recovery

During the recovery period, parental nutrition education programs are extremely important. When families with psychosocial maladaptations are revealed to be major contributors to FTT, the physician must discuss these behaviors in a nonjudgmental way, so that guilt is not increased or compliance endangered. Parents should be reassured, and support should be provided for correction of the problems as much as possible.

To improve their infant's eating habits, parents may be introduced to inpatient treatment programs for food-refusing infants (298). Parents and infant may have to be separated at meal times. The nurse must feed the child with structured, time-limited meals. Parents are to be given individual therapy and afterwards be reintroduced to the feeding situation. Parents must be educated regarding the catch-up growth process and long-term growth goals for their child. The baseline appearance of a cachectic child may bias the family's perception of recovery. The misperception that the recovering child is too plump or too active may result in an abrupt diet change and abandonment of high-calorie feedings.

In all instances and at all stages of the evaluation and treatment of FTT, a ''working alliance'' between key family members and professionals must be established (315). Developing such relationships can be a challenge and it requires the availability and commitment of multidisciplinary teams to assist the family in the treatment of the child.

Continued treatment after discharge from the hospital is necessary and the infant should be evaluated at regular intervals for a long time. Growth, development and social behavior must be carefully and continually monitored. Temporary placement in a more favorable setting within the family or in a foster care environment may be necessary if the immediate family is judged as incapable of following through on the recommended management.

Long-Term Outcome

Systematic review of 13 cohort studies or randomized controlled trials in FTT children less than two years old with measures of behavior, development and growth at age three years or beyond found an IQ difference equivalent to approximately three IQ points, and larger differences in height and weight though few children were below the third percentile at follow up (316). The longest follow-up study on growth found that 6 of 14 former FTT children were at least one year below their chronological age for height and weight, in contrast to only 1 of 14 control children (317). Studies of catch-up growth show that FTT children continue to do poorly developmentally despite increased weight. For example, even after extended hospitalization, these infants manifested persistent intellectual delays at a three year follow-up examination, despite maintenance of weight gains achieved during early hospitalization (318). These children remained significantly behind their control group in language development, reading age and verbal intelligence, and scored lower on a social maturity rating.

Special Considerations

The physician faces specific additional problems when dealing with a FTT patient in the managed health care environment. The diagnostic coding of such children is fraught with so-called Catch-22 dilemmas (319). Medical, nutritional, developmental, and/or psychiatric diagnosis may be utilized, but no optimal classification and coding scheme exists for use in these patients. The rapid growth of managed care also has significant implications for access to care, quality of services, reimbursement, and payment for health care. The special needs of these patients amplify the issues and challenges in ensuring that managed care is an effective component of community resources that foster healthy growth and development (320). These patients are at risk for concurrent illness and adverse development outcomes. A healthier child ultimately requires fewer services and indirect benefits may also occur with fewer health care expenditures and lifelong productivity.

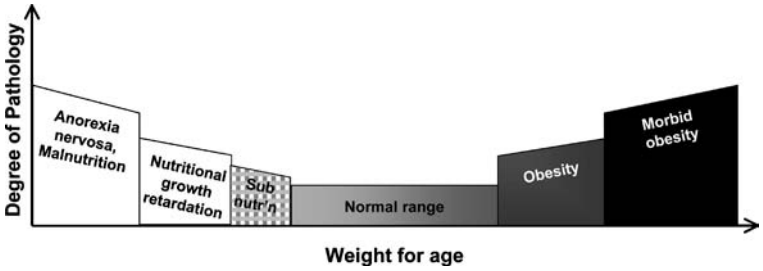

Figure 9 Continuum of weight gain problems. Weight for age is a continuum, with increasing pathology as the deviation from the normal range increases in either direction. Nutritional growth retardation is inadequate nutritional intake to sustain normal growth. More severe weight deficiencies are found in poverty-related malnutrition and in eating disorders, which are further characterized by a distorted body image. In the opposite direction, excessive nutritional intake leads to obesity and morbid obesity, which accelerate growth and pose significant health problems of their own.

NUTRITIONAL GROWTH RETARDATION (NGR)

NGR is a concept similar to FTT—linear growth stunting from inadequate nutritional intake, either organic or inorganic in origin—though at older ages; the term FTT is generally used for children less than two years old, while NGR is used afterwards. The single most important cause of growth retardation worldwide is poverty-related malnutrition (321). When suboptimal nutrition is continued for prolonged periods of time, growth stunting occurs as the main clinical phenotype (322,323).

NGR is a frequently underappreciated entity in pediatric endocrine practices in the United States. Poverty-related malnutrition is thankfully less common than in developing nations, and if anything, our current major health crisis is the obesity epidemic that is overwhelming our pediatric and adult populations. However, partly in response to the obesity around them, a subset of American youths, many suburban upper middle class, restrict their nutrient intake and develop NGR and delayed sexual development (63,324). This decreased intake is on the continuum of weight gain problems; it is insufficient to support normal growth but not as severe as to include a distorted body image as occurs in eating disorders (Fig. 9). Also, poor growth and inadequate nutrition may result from multiple systemic conditions, such as Inflammatory bowel disease (IBD) (26), celiac disease (25), HIV infection (29) and others (136). Children with NGR are generally referred to the pediatric endocrinologist because of short stature or delayed puberty. Therefore, pediatricians and pediatric endocrinologists need to recognize NGR and become familiar with its causes and treatment.

An increasing number of children on stimulant medications are being referred to the pediatric endocrinologist for short stature evaluation. Stimulant medication for the treatment of attention deficit hyperactivity disorder (ADHD) has long been suspected of adversely affecting linear growth, and it is well known that these medications produce anorexia and poor nutrient intake. The first studies concerning growth of these patients were published in the 1970s and since that time there have been numerous reports, which seem to further confuse rather than clarify this relationship. A recent manuscript (325) reviewed 29 cohort studies published through September 2004 of children treated with methylphenidate or dexamphetamine. Twenty-two of the studies involved children, six involved adolescents, and one study included both children and adults. Of the children's studies, nine gave results consistent with growth impairment on stimulants, and 12 had negative findings. The most sensitive studies measured growth progression before and after the period of treatment, and eight of these 16 studies showed an attenuation of growth on stimulants. While many of the studies were deemed of poor quality, those of better quality demonstrated significant associations between treatment and attenuated growth. In the most rigorous study, 540 children with ADHD were randomly assigned to different treatment groups and evaluated by intent-to-treat analyses of the seven- to nine-year-old subjects who were treated for up to 24 months (326). The behavioral effectiveness of medication use was greatest among children who ingested medications throughout the 24 months observation period. Those who stopped taking their medication and those who did not ingest them consistently showed increasing behavioral problems. However, there was significant growth deterioration among children who took the medication for the longest periods. After two years' treatment, height was suppressed by a mean of –1.94 cm and deficits in weight gain were even larger. During the initial phase of treatment the patients lost a mean of 2.5 kg, and during the second year they lost 1.22 kg. The authors concluded that consistent treatment with stimulant medication was associated with maintenance of behavioral effectiveness but continued growth suppression.

The somewhat larger deterioration observed in body weight may be due to the anorexic effects of these medications. Suboptimal nutrition appears to be an underlying cause of stimulant-mediated growth faltering, an aspect that should be thoroughly investigated. For a particular ADHD patient with growth concerns, when the stimulant cannot be interrupted, the physician should attempt to overcome the decreased dietary intake and correct nutrient deficits to foster appropriate growth.

Diagnosis

Although the importance of evaluating the pattern of stature increments throughout life in the differential

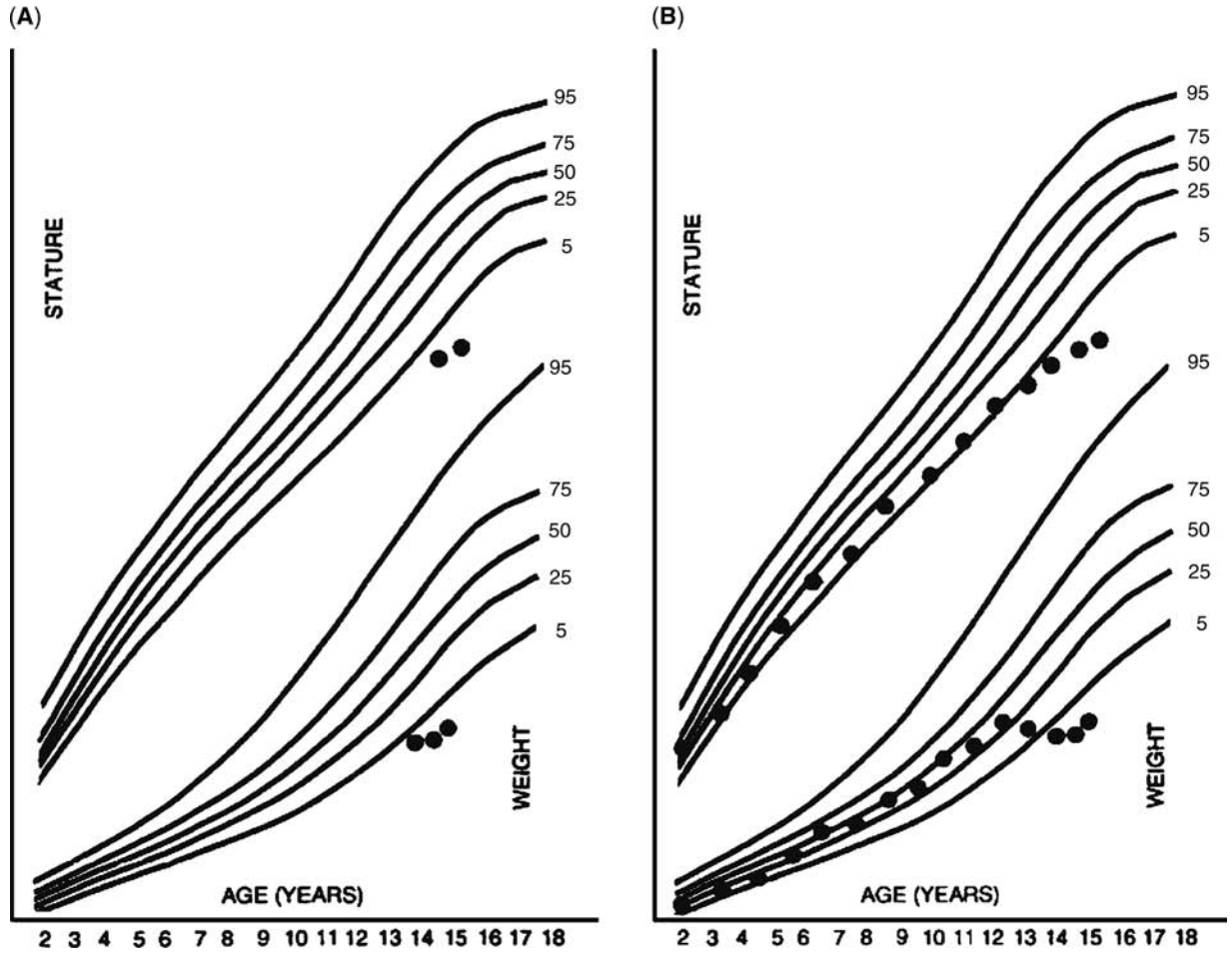

Figure 10 Nutritional growth retardation. (**A**) The patient was referred for short stature. The only available heights and weights were both similarly below the fifth percentile, and signs of sexual development were absent. (**B**) After obtaining complete growth data for the patient, it became clear his growth decelerated after he began dieting at the age of 12 years. *Source*: From Ref. 327.

diagnosis of short stature cannot be overemphasized, carefully assessing the progression of body weight is equally relevant to be able to recognize NGR. Longitudinal assessment of both height and weight is required (327). The CDC BMI curves are helpful to follow the trend.

In an illustrative case (Fig. 10), a healthy 15-year-old boy was referred to the endocrine clinic with the only presenting symptom of deteriorating linear growth. Both his height of 146.9 cm and weight of 37.6 kg were below the fifth percentiles. No body weight deficit for height was evident, and sexual development was delayed (Tanner stage 1). The initial measurements provided by the referring pediatrician indicated a decreasing growth rate with appropriate weight gain that was progressing just below the fifth percentile (Fig. 10A). However, after additional growth data were collected, a typical picture of NGR emerged (Fig. 10B). At 12 years of age, his weight gain ceased, with a subsequent deceleration in linear growth and pubertal delay. Review of his nutritional intake showed that he was consuming approximately 60% of his estimated energy needs based on age and gender. He was an athletic boy who described a desire to remain slim and avoid obesity, a syndrome that was first described in 1983 (62).

The Wellcome Trust classification differentiates NGR from other types of malnutrition characterized by wasting and stunting (328). The anthropometric criteria for NGR stipulate low weight for age with minimal deficit in weight for height. By these cross-sectional criteria, it may be difficult to differentiate NGR children from those with familial short stature or constitutional growth delay. Only the longitudinal progression of body weight and height can more clearly reveal NGR (327), which may occur even when there is weight-for-height excess (63). The distinguishing feature is a delay in linear growth and puberty resulting from inadequate weight gain. Thus, although concern is intensified when weight or height measurements fall below the fifth percentile, deterioration across percentiles of weight and height may also indicate NGR even when they are still above the fifth percentile.

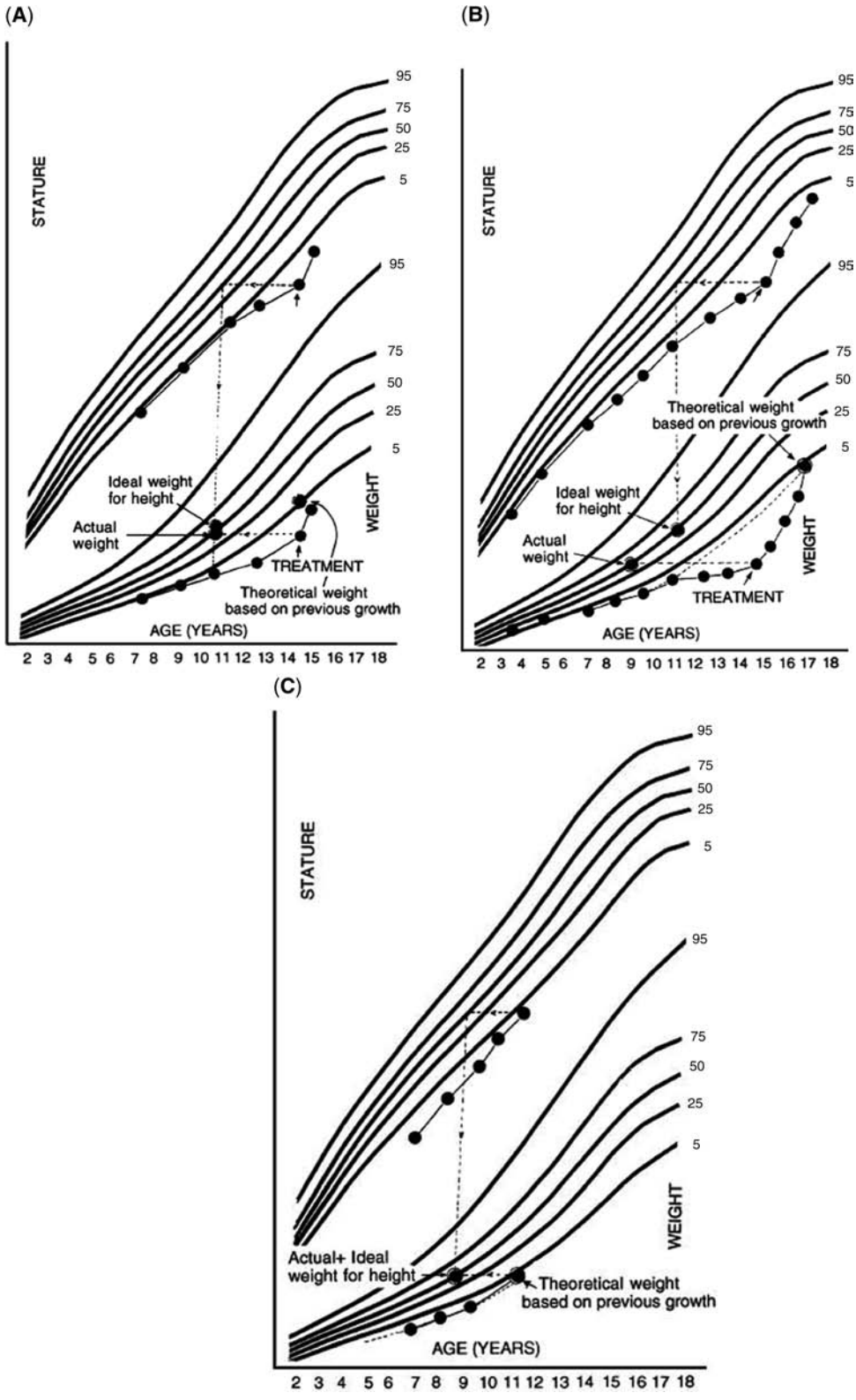

Figure 11 Comparison of nutritional growth delay with constitutional growth delay. (**A**) In one adolescent with NGR, weight gain and height progression decreased after age 10 years. Extrapolated weight after age 14 years revealed a body weight deficit relative to the previous growth percentile, though there was no weight-for-height deficit. With nutritional rehabilitation, there was recovery in weight gain and catch-up growth. (**B**) In another NGR patient, there was a weight-for-height deficit, but the deficit for theoretical weight was even more pronounced. (**C**) In a patient with constitutional growth delay, not NGR, weight gain continued consistently along the lower percentile, with no deviation in growth. There was no weight-for-height deficit, nor weight deficit for theoretical weight based on previous growth. *Abbreviation*: NGR, nutritional growth retardation. *Source*: From Ref. 327.

With nutritional rehabilitation, catch-up growth is usually achieved.

Analysis of body weight progression may be the most important clue for diagnosing NGR in patients with short stature (Fig. 11). Calculation of theoretical weights and heights based on previous growth percentiles may be used to quantitatively compare current anthropometric indices with previously established

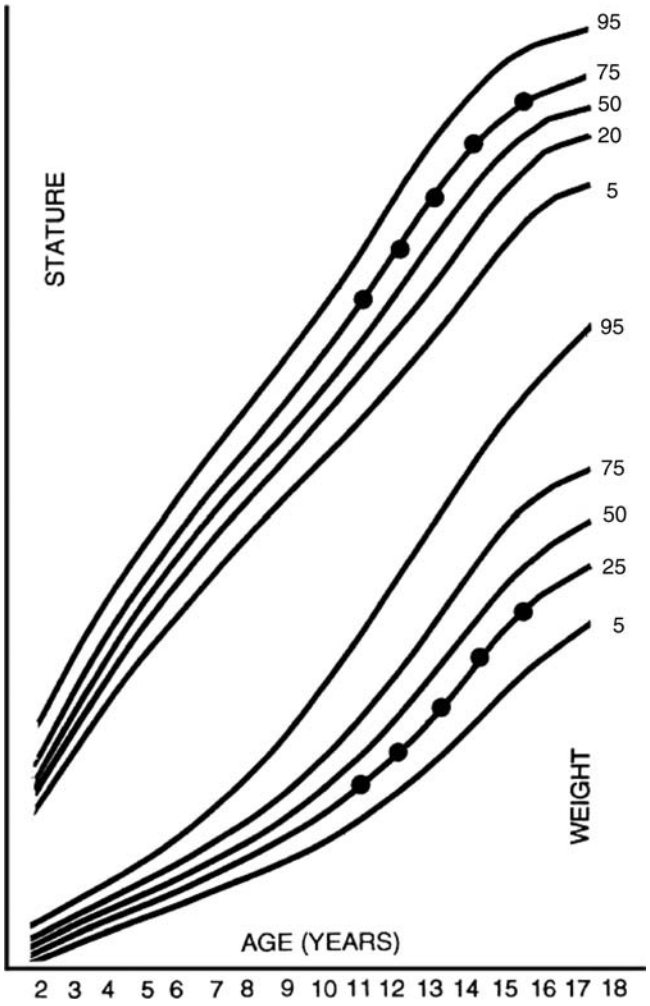

Figure 12 Constitutional thinness. Both height and weight progress consistently in the same percentiles for at least four years. Even though there is a weight-for-height deficit, this cannot be malnutrition because a positive energy balance is required for growth to occur. *Source*: From Ref. 327.

patterns of weight and height progression (Fig. 11A). Theoretical weight is defined as the weight the patient should have had at the time of the examination, if the patient had continued to gain weight along the percentile previously established during the premorbid growth period (327). Body weight-for-height deficits are not common in NGR, but the body weight is often deficient relative to the theoretical weight (Fig. 11A and B). In contrast, short patients without NGR, such as those with constitutional growth delay, continue to gain weight along established percentiles and the body weight at the time of assessment is equal to the theoretical body weight (Fig. 11C).

NGR growth patterns must also be differentiated from normal growth variants, such as variations in frame size, feeding practices or constitutional factors that may resemble NGR. Most normal children grow along established percentiles and exhibit minimal deficits or excesses in body weight relative to height, usually within one or two major percentiles of each other (329). These constitutional variations in body weight do not necessarily refiect over or undernutrition. For example, a child with constitutional thinness (Fig. 12) has a body weight that is two major percentile lines below his height percentile, representing more than 20% body weight-for-height deficit.The adolescent grew and developed normally. A constant weight-for-height deficit that permits normal growth to proceed along a set percentile cannot be construed as abnormal. Children with constitutional growth delay or familial short stature also grow at constant rates, maintaining their weight progression in their respective percentiles after three years of age, as described above. In contrast, a fall in growth associated with a poor rate of weight gain may indicate NGR, even without an appreciable weight-for-height deficit (Figs. 10 and 11A).

Patients with NGR do not appear wasted, and the biochemical parameters of nutritional status, including serum levels of retinol-binding protein, prealbumin, albumin, transferrin, and triiodothyronine (T3) levels, do not differentiate NGR patients from those with familial or constitutional short stature (330). Other indices of malnutrition, such as the urinary creatine-height index or urinary nitrogen/creatinine ratio, do not usually demonstrate abnormalities. The reason is that NGR patients have adapted to their suboptimal nutritional intake and they maintain homeostasis by decreasing growth, thereby reaching equilibrium with preservation of all nutritional markers.

Although fasting and protein–calorie malnutrition have been shown to lower circulating IGF-I levels in humans and rodents (331–335), we found that IGF-I levels could not differentiate NGR patients from those with familial short stature (330). The degree of nutritional insufficiency in NGR is not as severe as that observed in protein–calorie malnutrition or fasting, and may impair growth by altering other cellular mechanisms without affecting the serum IGF-I levels as discussed below. Because the energy restriction is mild, and NGR children consume sufficient dietary protein, IGF-I concentrations may be preserved within a range appropriate for bone age development. Likewise, IGF-I concentrations were maintained within normal ranges or improved rapidly when rats consumed diets containing 15% protein and 90% of the total energy requirements (336,337).

On the other hand, we reported that NGR patients show decreased activity of erythrocyte Na^+,K^+-ATPase compared with familial short stature patients (330). This enzyme is involved in the active transport of sugars and amino acids and in cellular thermogenesis, normally accounting for approximately one-third of the basal energy requirements (338). Reduced energy intake lowers the basal metabolic rate (339) and decreases Na^+,K^+-ATPase activity (340). Thus, it may be a good marker of NGR. Because anthropometric parameters may be lacking or

inaccurate, and biochemical markers may not be sufficient to detect NGR, a more sensitive test is required for diagnosis. Erythrocyte Na^+, K^+-ATPase activity may offer such a diagnostic tool. However, to date, this assay has not been widely available for clinical purposes, is cumbersome, and can be applied only on a research basis.

Pathophysiology

Growth deceleration is the adaptive response to suboptimal nutrition, so patients with NGR have achieved equilibrium between their genetic growth potential and their nutritional intake (341,342). Diminished growth brings the nutrient demands into balance with the nutritional intake without adversely affecting biochemical or functional homeostatic measures. Of course, there are limits to these adaptive possibilities. If nutritional deprivation becomes more severe, or when acute malnutrition is superimposed on the chronic suboptimal state, there will be altered anthropometric measurements, such as weight and skinfold thickness, and biochemical indices reflecting malnutrition.

The biochemical and hormonal changes associated with chronic suboptimal nutrition have been studied utilizing a rodent model. Sodium-potassium ATPase activity was reduced in rats fed 60% of ad libitum energy intake (343). Body weight gain was preserved in suboptimally fed rats treated with rhGH (344,345). Furthermore, simultaneous restriction of both energy and zinc did not augmentthe growth deterioration of chronic suboptimal nutrition (346). Substitution of fat for carbohydrates led to greater body weight gains through reduced energy expenditure and possibly decreased leptin secretion (347). Other changes due to chronic suboptimal nutrition included a reduction of liver weight with an increase in percent total polyunsaturates, *n*-6 polyunsaturates and total unsaturates in mitochondrial lipids (348). Suboptimal nutrition also reduced mandibular and even more so, femur bone growth (349,350). Finally, rats suboptimally fed for three weeks showed decreased T-cell numbers in the thymus that may alter the immune system (351,352). All these studies suggest that minor biochemical and physiological changes occur during chronic suboptimal nutrition.

It has been known for many years that diminished energy intake reduces metabolic rate even before there is a loss of body weight. The rate of protein synthesis may decrease in response to a reduction in energy intake, because this process is energy expensive and accounts for 10% to 15% of the basal metabolic rate (353). Protein catabolism is also sensitive to energy deprivation, such that reduced dietary energy sources may lead to increased nitrogen fiux in which protein breakdown is accelerated to provide energy (354,355). Nitrogen retention markedly increases during nutritional rehabilitation of malnourished children (356); nutritional recovery also normalizes the excretion of amino acids (355) and increases the rate of protein synthesis (357). In NGR, the result of the altered rates of protein turnover and nitrogen retention may be the cessation of normal growth as an adaptive response to the decreased intake. In addition to suboptimal energy intake, various mineral and vitamin deficiencies have been implicated in the etiology of NGR, as discussed below.

More recently we studied the changes in the 24 hours metabolic and physical activity profile of rodents undergoing chronic suboptimal nutrition to assess if the metabolic adaptations contribute to the preservation of body weight gain and growth (358). We utilized the rodent EMTAC (346) to conduct accurate measurements of continuous energy expenditure and physical activity in rats restricted to 80%, 70% or 60% of ad libitum energy consumed by controls. Rats that were restricted to only 80% of their ad libitum energy intake grew at rates comparable to the ad libitum fed controls. Furthermore, they preserved fat-free mass but had reduced energy expenditure and physical activity, along with increased respiratory quotient, during the dark period at night. Thus, these rats utilized their body fat and reduced their physical activity to conserve energy for growth and lean body mass maintenance. However, rats fed only 70% of ad libitum energy intake had reductions in both growth and lean body mass and had a greater magnitude in the reduction of energy expenditure and physical activity. Rats subjected to even greater amounts of energy restriction, such as those fed 60% of ad libitum energy intake, had even greater detrimental effects such as reduced growth, loss of lean body and fat mass along with further decreases in energy expenditure and physical activity yet increased respiratory quotient during both the light and dark periods. Despite consuming only 60% of their ad libitum energy intake, these rats still preserved 26% of their body weight gain as compared to ad libitum fed controls. This suggests that over the course of the experiment, some essential needs of metabolism were still being met and a minimal amount of energy was still available for growth.

The physical activity cycle is controlled by suprachiasmatic nuclei in the hypothalamus (359). Once energy restriction begins, as in the case of a 20% reduction, energy expenditure and physical activity changes begin to appear during the dark cycle period. Other studies have reported similar effects in suboptimally fed rats in which the 24-hour energy expenditure cycle disappeared (360) and the serotonin transporters decreased, affecting the circadian rhythm (361). Similarly, circadian rhythmicity was split during the light/dark cycle in adult rats receiving a diet containing only 6% protein (362). Rats restricted to 50% of their ad libitum food intake for up to two months showed an advancement of their daily pineal melatonin rhythm along with the daily onset of plasma melatonin. These studies suggest that changes in the circadian rhythm may contribute to energy conservation under caloric restriction.

Table 2 USDA Food Guide

	Calorie level							
Food group[a]	1400	1600	1800	2000	2200	2400	2600	2800
Daily amount of food from each group (vegetable subgroup amounts are per week)								
Fruits	1.5 c (3 srv)	1.5 c (3 srv)	1.5 c (3 srv)	2 c (4 srv)	2 c (4 srv)	2 c (4 srv)	2 c (4 srv)	2.5 c (5 srv)
Vegetables[b]	1.5 c (3 srv)	2 c (4 srv)	2.5 c (5 srv)	2.5 c (5 srv)	3 c (6 srv)	3 c (6 srv)	3.5 c (7 srv)	3.5 c (7 srv)
Dark green veg. (c/wk)	1.5	2	3	3	3	3	3	3 c/wk
Orange veg. (c/wk)	1	1.5	2	2	2	2	2.5	2.5
Legumes veg. (c/wk)	1	2.5	3	3	3	3	3.5	3.5
Starchy veg. (c/wk)	2.5	2.5	3	3	6	6	7	7
Other veg. (c/wk)	4.5	5.5	6.5	6.5	7	7	8.5	8.5
Grains[c] (oz-eq)	5	5	6	6	7	8	9 oz-eq	10 oz-eq
Whole grains	2.5	3	3	3	3.5	4	4.5	5
Other grains	2.5	2	3	3	3.5	4	4.5	5
Lean meat and beans	4 oz-eq	5 oz-eq	5 oz-eq	5.5oz-eq	6 oz-eq	6.5oz-eq	6.5oz-eq	7
Milk [c]	2	3	3	3	3	3	3	3
Oils[d] (g)	17	22	24	27	29	31	34	36 g
Discretionary calorie allowance[e]	171	132	195	267	290	362	410	426

Note: The suggested amounts of food to consume from the basic food groups, subgroups, and oils to meet recommended nutrient intakes at 12 different calorie levels. Nutrient and energy contributions from each group are calculated according to the nutrient-dense forms of foods in each group (e.g., lean meats and fat-free milk). The table also shows the discretionary calorie allowance that can be accommodated within each calorie level, in addition to the suggested amounts of nutrient-dense forms of foods in each group. Food group amounts shown in cup (c) or ounce-equivalents (oz-eq), with number of servings (srv) in parentheses when it differs from the other units. See note for quantity equivalents for foods in each group. Oils are shown in grams (g).

[a]Food items included in each group and subgroup. *Fruits*: All fresh, frozen, canned, and dried fruits and fruit juices: for example, oranges and orange juice, apples and apple juice, bananas, grapes, melons, berries, raisins. In developing the food patterns, only fruits, and juices with no added sugars or fats were used. See note 6 on discretionary calories if products with added sugars or fats are consumed; *Vegetables*: In developing the food patterns, only vegetables with no added fats or sugars were used. See note 6 on discretionary calories if products with added fats or sugars are consumed; *Dark green vegetables*: All fresh, frozen, and canned dark green vegetables, cooked or raw: for example, broccoli; spinach; romaine; collard, turnip, and mustard greens; *Orange vegetables*: All fresh, frozen, and canned orange and deep yellow vegetables, cooked or raw: for example, carrots, Sweet potatoes, winter squash, and pumpkin; *Legumes*: All cooked dry beans and peas and soybean products: for example, pinto beans, kidney beans, lentils, chickpeas, tofu (dry beans and peas) (See comment under meat and beans group about counting legumes in the vegetable or the meat and beans group); Starchy vegetables: All fresh, frozen, and canned *starchy vegetables*: for example, white potatoes, corn, and green peas. *Other vegetables*—All fresh, frozen, and canned other vegetables, cooked or raw: for example, tomatoes, tomato juice, lettuce, greenbeans, and onions; *Grains*: In developing the food patterns, only grains in low-fat and low-sugar forms were used. *Whole grains*: All whole-grain products and whole grains used as ingredients: for example, whole-wheat and rye breads, whole-grain cereals and crackers, oatmeal, and brown rice; *Other grains*: All refined grain products and refined grains used as ingredients: for example, white breads, enriched grain cereals and crackers, enriched pasta, white rice; *Meat, poultry, fish, dry beans, eggs, and nuts (meats and beans)*: All meat, poultry, fish, dry beans, and peas, eggs, nuts, seeds. Most choices should be lean or low-fat. See note 6 on discretionary calories if higher fat products are consumed. Dry beans and peas and soybean products are considered part of this group as well as the vegetable group, but should be counted in one group only; *Milk, yogurt, and cheese (milk)*: All milks, yogurts, frozen yogurts, dairy desserts, and cheeses (except cream cheese), including lactose-free and lactose-reduced products. Most choices should be fat-free or low-fat. In developing the food patterns, only fat-free milk was used. See note 6 on discretionary calories if low-fat, reduced-fat, or whole milk or milk products or milk products that contain added sugars are used. Calcium fortified soy beverages are an option for those who want a nondairy calcium source. Quantity equivalents for each food group: Grains—the following each count as 1 ounce-equivalent (1 serving) of grains: 1/2 cup cooked rice, pasta, or cooked cereal; 1 oz dry pasta or rice; 1 slice bread; 1 small muffin (1 oz) one cup ready-to-eat cereal flakes; Fruits and vegetables: The following each count as one cup (two servings) of fruits or vegetables: one cup cut-up raw or cooked fruit or vegetable, one cup fruit or vegetable juice, two cups leafy salad greens; Meat and beans: The following each count as 1 ounce-equivalent: 1 oz lean meat, poultry, or fish; one egg; 1/4 cup cooked dry beans or tofu; one Tbsp peanut butter; 1/2 oz nuts or seeds; Milk: The following each count as one cup (one serving) of milk: one cup milk or yogurt, 11/2 ounces natural cheese such as Cheddar cheese or 2 oz processed cheese. Discretionary calories must be counted for all choices, except fat-free milk.

[b]*Explanation of vegetable subgroup amounts*: Vegetable subgroup amounts are shown in this table as weekly amounts, because it would be difficult for consumers to select foods from each subgroup daily. A daily amount that is one-seventh of the weekly amount listed is used in calculations of nutrient and energy levels in each pattern.

[c]*Explanation of grain subgroup amounts*: The whole grain subgroup amounts shown in this table represent at least three 1 oz servings and one-half of the total amount as whole grains for all calorie levels of 1600 and above. This is the minimum suggested amount of whole grains to consume as part of the food patterns. More whole grains up to all of the grains recommended may be selected, with offsetting decreases in the amounts of other (enriched) grains. In patterns designed for younger children (1000, 1200, and 1400 calories), one-half of the total amount of grains is shown as whole grains.

[d]*Explanation of oils*: Oils (including soft margarine with zero trans fat) shown in this table represent the amounts that are added to foods during processing, cooking, or at the table. Oils and soft margarines include vegetable oils and soft vegetable oil table spreads that have no trans fats. The amounts of oils listed in this table are not considered to be part of discretionary calories because they are a major source of the vitamin E and polyunsaturated fatty acids, including the essential fatty acids, in the food pattern. In contrast, solid fats are listed separately in the discretionary calorie table (appendix A-3) because, compared with oils, they are higher in saturated fatty acids and lower in vitamin E and polyunsaturated and monounsaturated fatty acids, including essential fatty acids. The amounts of each type of fat in the food intake pattern were based on 60% oils and/or soft margarines with no trans fats and 40% solid fat. The amounts in typical American diets are about 42% oils or soft margarines and about 58% solid fats.

[e]*Explanation of discretionary calorie allowance*: The discretionary calorie allowance is the remaining amount of calories in each food pattern after selecting the specified number of nutrient-dense forms of foods in each food group. The number of discretionary calories assumes that food items in each food group are selected in nutrient-dense forms (i.e., forms that are fat-free or low-fat and that contain no added sugars). Solid fat and sugar calories always need to be counted as discretionary calories, as in the following examples:The fat in low-fat, reduced fat, or whole milk or milk products or cheese and the sugar and fat in chocolate milk, ice cream, pudding, etc.; The fat in higher fat meats (e.g., ground beef with more than 5% fat by weight, poultry with skin, higher

Table 2 USDA Food Guide (*Continued*)

fat luncheon meats, sausages); The sugars added to fruits and fruit juices with added sugars or fruits canned in syrup; The added fat and/or sugars in vegetables prepared with added fat or sugars;The added fats and/or sugars in grain products containing higher levels of fats and/or sugars (e.g., sweetened cereals, higher fat crackers, pies and other pastries, cakes, cookies). Total discretionary calories should be limited to the amounts shown in the table at each calorie level. The number of discretionary calories is lower in the 1600-calorie pattern than in the 1000-, 1200-, and 1400-calorie patterns. These lower calorie patterns are designed to meet the nutrient needs of children two to eight years old. The nutrient goals for the 1600-calorie pattern are set to meet the needs of adult women, which are higher and require that more calories be used in selections from the basic food groups. Additional information about discretionary calories, including an example of the division of these calories between solid fats and added sugars.
Source: From Ref. 371.

Other physiological adaptations may contribute to the maintenance of health and body weight gain during chronic suboptimal nutrition. For example, a reduction of body temperature might be another mechanism for energy conservation, because changes in energy expenditure are directly related to body temperature (363,364). It is possible that other factors might also contribute to the preservation of metabolic homeostasis, such as alterations in erythrocyte sodium–potassium–ATPase activity (343).

However, it remains controversial whether decreased body size is an advantageous adaptation to a limited food supply or whether adverse health and functional impairments result (341,342). Mild energy restriction in rodents has been repeatedly shown to extend life span (365–368).

However it is difficult to conceive an appropriate homeostasis that will allow optimal health and prolongation of life with levels of energy restriction that are associated with poor growth and degradation of lean body mass. Physical activity is decreased with a 20% decrease in energy consumption (369), and as mentioned above, energy expenditures in rats promptly decrease with a relatively mild energy restriction (358). Moreover, there are other functional impairments that are more difficult to assess, such as mental capacity and learning ability, that may also be compromised. Decreased growth velocity nevertheless constitutes a functional compromise per se, which should be detected and treated as early as possible.

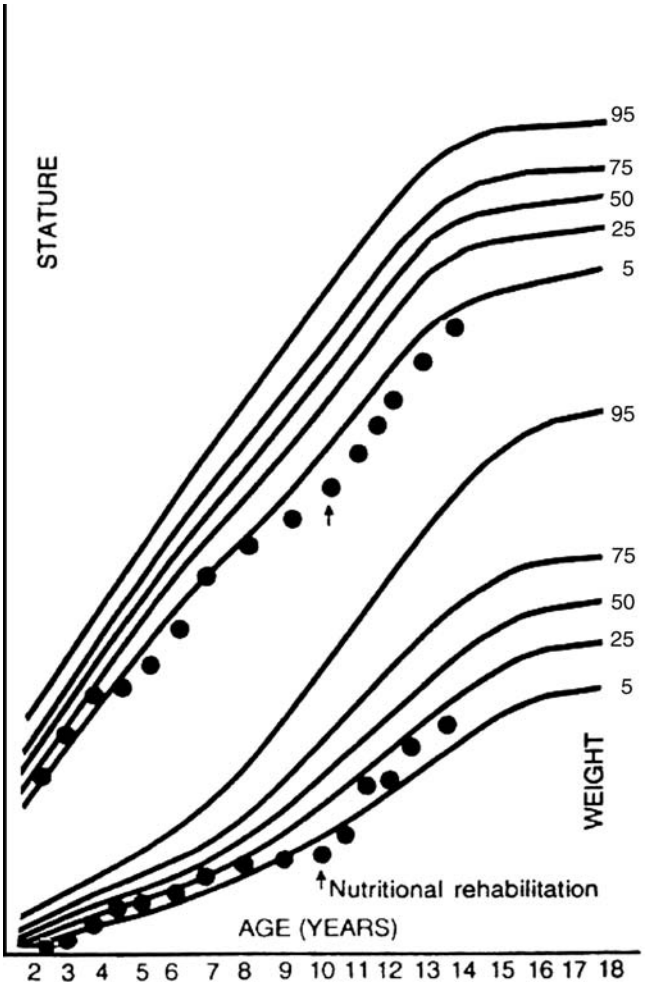

Figure 13 Nutritional dwarfing or growth hormone deficiency? At age 10 years, the patient was believed to have GH deficiency because of poor growth. However, due to the inadequate weight gain, a therapeutic trial with an adequate diet was tried in lieu of GH treatment. Note the catch-up growth after the initiation of nutritional rehabilitation. *Abbreviation*: GH, growth hormone. *Source*: From Ref. 327.

Endocrine Adaptation

Changes in the endocrine system in response to undernutrition are adaptive in nature and largely revert to the ''normal'' state after nutritional status is improved (370). A tabular review of the endocrine alterations in different types of pediatric malnutrition is in Table 2 of Vol. 2; Chap. 25. Undernutrition may involve single or multiple micronutrient deficiencies, and thus any one or a combination of deficits could be the primary problem leading to the endocrine changes. However, it must be remembered that most studies have been conducted in severely malnourished patients, which may not accurately refiect more subtle forms of suboptimal nutrition (371) leading to NGR. For example, although circulating GH levels are increased in severe malnutrition, we have shown that pubertal NGR children show decreased overnight GH secretion and prepubertal subjects have an increased GH response to GHRH stimulation (372). Body composition is a significant determinant of spontaneous GH secretion. In normal short children, the degree of adiposity modifies spontaneous GH secretion, altering the amplitude of GH pulses in

puberty and the number of pulses in prepubertal children (372). Indeed, NGR may be easily confused with GH deficiency or neurosecretory dysfunction if the deterioration in weight progression is overlooked (Fig. 13). These patients respond to nutritional rehabilitation and do not require GH treatment.

Causes

Organic Causes

NGR can result from any pathological condition that causes excess energy expenditures or reduces energy intake, either by malabsorption or decreased nutritional intake (373). Some of the more common conditions include Crohn's disease, celiac disease, cystic fibrosis, and chronic infections like HIV or tuberculosis, cardiac and renal diseases. Sometimes the dietary intake is disturbed as a secondary phenomenon, such as in patients with cleft palate or other developmental disabilities. Poor weight gain and statural growth are frequently the first and only signs of the underlying pathological condition. For example, in HIV infection and acquired immunodeficiency syndrome, short stature and poor growth were shown to precede any other manifestation of disease (29,374), with deceleratioin in body weight progression preceding the growth problem (375). It is very likely that anorexia and decreased intake of nutrients lead to suboptimal nutrition in HIV-infected patients and cause NGR, even before other signs and symptoms of the disease become apparent.

Impaired linear growth, retarded skeletal maturation and delayed sexual development are common in pediatric patients with chronic IBD, with an estimated prevalence of 5% to 10% in ulcerative colitis and 25% in Crohn's disease. Growth failure may be the first indication of IBD, sometimes preceding gastrointestinal symptoms by months or years (26). Therefore, the diagnosis of IBD should always be considered in children who cease to grow adequately, even in the absence of gastrointestinal complaints. Although the pathogenesis is influenced by age at onset, duration of disease, disease activity, and medication use, suboptimal nutrition has long been recognized as the primary factor in the growth failure of Crohn's disease (376). The role of *NOD2* genotype on disease location and severity and on growth retardation is also important (377). The first disease-associated mutations for Crohn's disease were found in the *NOD2/CARD15* gene located in the paracentric region of chromosome 16 (378–380). Mutations within the *NOD2* gene were identified as independent risk factors for the development of Crohn's, though they were not directly correlated with the presence of growth retardation. *NOD2* mutations were related to disease location, present in 43% of patients who had ileal involvement. Patients with ileal disease had height retardation at the time of diagnosis that persisted during follow-up. Genotyping for *NOD*2 single nucleotide polymorphisms was performed in 93 patients who had detailed growth records and disease assessments. *NOD2* mutations were found in 35% of patients; these mutations correlated with ileal disease and height retardation, both at the onset and at follow up. Use of steroids and immunosuppressant therapy also affected growth. However, the degree of disease severity was the strongest association with impaired growth, height and weight failure with odd ratios of 6.12 and 4.5, respectively. Other studies have also implicated *NOD2* mutations with growth retardation (381).

Thus, short stature and poor growth in Crohn's disease is known to be primarily the result of inadequate nutrition due to anorexia and postprandial pain leading to poor intake, compounded by malabsorption and by other factors such as steroid treatment. Improvement usually follows nutritional rehabilitation. The genotype may affect growth through *NOD2* mutations which determine the disease location and severity. Multifactorial nutritional alterations include decreased nutrient intake, impaired nutrient absorption, specific nutrient deficiencies (especially fat-soluble vitamins), and enhanced protein loss through the gastrointestinal tract (382). Anorexia and the early satiety and discomfort that accompany eating contribute to the decreased energy intake in children with IBD. Total daily calorie intake of IBD patients does not usually exceed the recommended dietary allowance (RDA) for height age and multiple studies have documented catch-up growth when adequate energy is provided (383,384). Various forms of nutritional support (parenteral, elemental, or complex diet) have been employed, often resulting in decreased disease activity and improved growth (385–387). In addition to energy and protein deficits, specific micronutrient deficiencies may affect growth in patients with IBD. Iron deficiency, particularly when there is blood loss through the stools, may compound anorexia and poor growth (382,385,387). These patients also develop deficiencies of magnesium (388) and zinc (389). Large enteric losses of zinc occur in the setting of diarrhea and small bowel disease, and zinc absorption may be reduced. Stable zinc isotope studies revealed significantly reduced zinc absorption in adolescents with Crohn's disease compounded by a failure to reduce endogenous fecal zinc excretion as normally occurs to restore zinc balance (390). Nutritional rehabilitation is therefore essential for the treatment of IBD patients, as it may ameliorate the growth retardation and even improve the disease activity itself (391,392). Low circulating IGF-I levels are common in Crohn's disease patients, frequently without GH deficiency (383). A trial of GH therapy in adults lowered their Crohn's disease activity (393), and in children, improved growth velocity despite concomitant glucocorticoid treatment (394).

Growth failure is also common among children with celiac disease, and like Crohn's disease, can occur with or without gastrointestinal symptoms (25). Up to 55% of patients with celiac disease were below the third percentile for height and up to 60% were below the same percentile for weight at the time

of diagnosis. FTT was present in 25% of children with celiac disease (395). Several investigators have reported short stature as the sole manifestation of celiac disease (25,395–397). These asymptomatic patients, considered to have so-called occult celiac disease, are of variable prevalence; some studies cited prevalence up to 8% (396,398), while others found it as high as 24% and 48% (395,397). This disparity may reflect geographic and genetic differences in the prevalence of the disease, the level of suspicion in recognizing these patients, and/or the tests employed for the assessment of celiac disease. The incidence of celiac disease in the western New York area, estimated by serum IgA–endomysial antibody, was reported to be 1:3752 (399). The prevalence of celiac disease assessed by specific antibody assays in blood of normal children ranges between 0.4% and1.0% (400,401); whereas, the prevalence of celiac disease autoimmunity was 3% in a genetically susceptible population (402). Thirty-three of 1234 children presented positive IgA tissue tranglutaminase antibodies in a cohort of HLA-DRB1*03 children followed since birth, first becoming apparent around four years of age. Most often they had no clinical findings, though some presented with lower BMI's, reduced mid-arm circumference and mild GI complaints that were associated with decreased weight gain. Thirteen of these 18 children had intestinal mucosal evidence of celiac disease.

Diagnosis of occult celiac disease is dependent on the clinician's alertness in considering this entity as a cause of short stature. Many asymptomatic celiac disease patients have a history of diarrhea at an early age or have iron deficiency. Increased stool fat and low serum folate and ferritin levels may point to the possibility of celiac disease as the cause of short stature. Tissue transglutaminase antibodies and endomyseal antibodies offer higher sensitivity and specificity than antigliadin antibodies in screening for celiac disease (403). Although various methods may be used for screening purposes, these are not diagnostic of celiac disease, and small bowel biopsy remains the ''gold standard'' for the diagnosis (404–406). Small bowel biopsy should be performed in every short child showing NGR from unidentified causes, as well as those with a history of chronic diarrhea during the first year of life and/or the presence of iron deficiency. The diagnosis of celiac disease should be confirmed by documentation of catch-up growth with weight and height gain after institution of a gluten-free diet, which is the only available treatment. Children with celiac disease who do not adhere to the diet have significantly lower mean heights and weights and a greater abnormality of the intestinal mucosa than those who are compliant. Long-term untreated celiac disease also increases one's risk of other autoimmune conditions, like thyroiditis (407), osteoporosis and lymphoma (408,409).

However it should be kept in mind that tissue transglutaminase and endomyseal antibodies have a low predictive value of detecting alterations in the small bowel (410). The presence of such antibodies may indicate celiac disease autoimmunity, but not active celiac disease. There are no data available regarding the risks of asymptomatic patients with positive antibodies. Nontheless, celiac disease screening of at-risk patients is increasingly performed by pediatric endocrinologists, especially for patients with short stature, Turner syndrome, Down syndrome and DM, detecting celiac disease autoimmunity in up to 15%.

Such case finding efforts need to be tempered with the cost of labeling children with celiac disease, realizing that this disease is difficult to prove and that only half of patients with symptomatic celiac disease follow a strict gluten-free diet (411). The natural history of celiac disease autoimmunity is not fully known, and the benefits of early diagnosis and treatment of silent patients have not been demonstrated. Thus more research is warranted together with careful assessment and monitoring of height and weight progression of short children, particularly in those with celiac disease autoimmunity.

Nonorganic Causes

The prevalence of nonorganic NGR leading to malnutrition and poor growth in affluent communities is unknown. Only those patients whose height is markedly impaired have been recognized thus far. However, suboptimal nutritional intake may result in a fall in height within the normal percentiles that may elude medical attention. A 1983 survey of 1017 high school students from a middle-class parochial school found a high incidence of low-weight students; more than 25% of the students weighed less than 90% of their ideal body weight for height, but only 1.8% of them had growth patterns suggestive of NGR (329). In the same geographic area, we detected more than 300 patients with NGR in a referral center pediatric endocrine clinic.

Among the referred adolescents, the most common causes (73%) of nutritional alterations resulting in NGR and delayed sexual development were nonorganic. Some patients had an identifiable specific fear or health belief that caused their poor nutritional intake (261,324,327). Fear of obesity and fear of hypercholesterolemia were specifically verbalized by some. However, most patients with nonorganic NGR expressed preoccupations that involved similar issues of body weight and cholesterol and concern with a so-called healthy dietary intake. They avoided excess dietary fat and cholesterol and what they termed junk food (327). Regardless of their reason for the inadequate nutritional intake, the common result was NGR.

Notably, severe psychopathology was absent in these NGR patients. They did not meet the diagnostic criteria for severe eating disorders, such as anorexia nervosa or bulimia nervosa. Moreover, a controlled,

double-blinded, prospective study demonstrated that these children did not have behavioral or psychosocial deviations and did not differ from a group of normal or short-statured children (412). Thus, we concluded that the dietary habits that led to NGR were a result of the prevalence of current health beliefs and preoccupation with slimness, weight control and the search for longevity through the intake of idealized diets (259,261,413,414).

The American Academy of Pediatrics Diagnostic and Statistical Manual for Primary Care (DSM-PC) distinguishes dieting and body image behaviors that were in the past difficult to categorize as eating disorders. Children and adolescents may exhibit behaviors that do not meet full DSM-IV criteria for an eating disorder, yet deserve attention as they diet and or engage in inappropriate eating behaviors and may have NGR. The two specific complexes in the DSM-PC related diagnostic categories include dieting/body image behaviors and purging/binge-eating behaviors (415). These variations constitute minor deviations from normal that may lead to nutritional or psychological alterations and result in inappropriate weight and height gain (261,412–414). An adolescent with a dieting/body image problem exhibits voluntary food limitation in pursuit of thinness and of an idealized diet. If he or she experiences a systematic fear of gaining weight that extends beyond a simple dieting/body image variation with a more severe intensity and purging/binge-eating behaviors, then bulimia or anorexia nervosa need be considered.

The dieting/body image phenomenon starts at a young age. A 2000 national survey found that 31% of fifth grade girls dieted (416). Abramovitz and Birch explored five-year-old girls' ideas, concepts and beliefs about dieting (417). They found that 34% to 64% of the girls had ideas about dieting and weight loss and understood the link with body shape, though the girls' knowledge about how people diet was inappropriate. They modified their eating behaviors through drinking diet shakes and sodas, eating special foods, and had restrictive eating behaviors. Their mothers modeled both health-promoting and health-compromising eating behaviors. Girls whose mothers reported food restrictions were more than twice as likely to have ideas about dieting (417). Maternal perceptions of their child's weight status were also important, but often not accurate (418). Other factors found to influence girls' perceptions were family history of overweight and the media, which was mentioned by 55% of the children as a source of dieting ideas (419).

National surveys examining weight-related behaviors among American children (grades 5–12) showed that female adolescents were significantly more likely to diet than younger children; dieting was reported by 31% of fifth graders and increased consistently up to 62% among 12th graders. Thirteen percent of the girls and 7% of the boys reported disordered eating behaviors (416). A high prevalence of reported weight control behaviors was also found among adults and adolescents from four regions of the United States (420).

Although the current obesity epidemic makes dieting a seemingly timely and appropriate response, Moses et al. found a high rate of dieting among high school adolescents in an affluent suburban location in the 1980s (421). Of concern, dieting efforts did not relate to body weight. About 30% of dieters were underweight and normal weight for height, while the proportion of overweight students who were dieting was relatively low (50–60%). The relation between dieting and weight change in preadolescents and adolescents is often counterproductive (422).

Children not only diet but also worry about their body appearance and weight proportions, which are frequently distorted. More and more children are concerned and dissatisfied with their body image. Fifty-five percent of girls and 35% of boys in grades 3 to 6 reported a desire to be thinner (423). Stice et al. found that eating disturbances that emerged during childhood led to inhibited and secretive eating, overeating and vomiting. Maternal body dissatisfaction, internalization of the thin ideal, dieting, bulimic symptoms, and maternal and paternal body mass prospectively predicted the emergence of childhood eating disturbances (424).

Parents who worry about their children becoming overweight may set the stage for a vicious cycle. Parents who control what and how much their children eat may impede energy self-regulation and put their children at a higher risk for being overweight (425). Furthermore, children whose parents had high degrees of dietary control had greater increases in body fatness than did children whose parents had the lowest levels of dietary restraint and noninhibition (426).

Distorted perceptions of ideal body weight are very prevalent. Adolescents often know what their ideal weight should be, but some prefer to be 10% less than their ideal weight-for-height (421). Fear of obesity may further lead adolescents to not only diet, but also develop inappropriate eating habits and purging behaviors (421,427,428). These data indicate just how powerful and important it is for adolescents to achieve an ideal slim and trim figure. Young persons, even when they are not overweight, diet to avoid obesity at a time when they are still growing and developing (413,421,427,428). Regardless of their physical needs, they strive to reach a thin ideal, consequently developing NDR (413,421). In addition to growth retardation, other potential medical complications may be associated with excessive dieting, binging, and purging: electrolyte disturbances, dental enamel erosion, acute gastric dilation, esophagitis, and enlargement of the parotid glands, aspiration pneumonitis, and Pancreatitis (428).

Avoiding obesity is not the only dieting motivator; the population at large is also quite concerned about cholesterol levels and preoccupied with healthy

diets and junk food (413). These concerns are also prevalent among children (429). The medical community and various national committees have recommended a low-fat/low-cholesterol diet for the population at large in an effort to prevent adult-onset diseases (430–433). However, there are potential harmful consequences of feeding children with adult diets (413). A low-fat/low-cholesterol intake may lead to nutritional short stature (413,429) and nutrient deficits (324). A recent study confirmed our observations, namely that children on low-fat/low-cholesterol diets can easily ingest an inadequate nutrient intake (434). Even in well controlled clinical trials designed to assess the efficacy and safety of cholesterol reducing diets, such as the National Institutes of Health (NIH)-funded Dietary Intervention Study (DISC), there was a potential for inadequate nutrient intake and limited efficacy in reducing cholesterol levels over a seven year follow-up period (435). Despite providing intensive nutritional counseling, the dietary intake often failed to provide the requirements of some of the essential nutrients for growth, including zinc, magnesium, phosphate, and several vitamins; zinc, calcium and vitamin E intakes were less than two thirds of the recommended allowances (436). Although the experts who participated in the study concluded that the diets were safe and effective and did not lead to NGR, multiple questions remain to be elucidaded in this regard.

Careful assessment of weight and height progression will clearly identify children who are not gaining weight and growing appropriately (258,324,373,421). Awareness by health care providers and pediatric endocrinologists of the prevailing eating attitudes and behaviors and nutrient intake among adolescents in the population of their practice's area may help detect the adolescent at risk for NGR and other more serious disorders. Simple tests and questionnaires may help identify the patient with eating disorders, EAT score questionnaire in Vol. 1; Chap. 20 (437). A 24-hour dietary recall may reveal which short-stature patients have inappropriate dietary intakes.

Obese patients constitute another group of children who often diet. Although these children usually do not present to the pediatric endocrinologist because of poor growth, when obesity occurs in association with short stature, concern is raised for a variety of endocrine disorders that increase weight gain yet impair linear growth (Vol. 1; Chap. 1). Diet-related growth failure may be revealed by a careful history, thereby eliminating other concerns. Weight loss is associated with a negative balance that does not allow linear growth, even if the child is obese (438). Therefore, treating obesity in children must always make allowances to maintain a balance between the need of a patient to lose weight and the nutritional requirements that sustain growth in height.

Nutritional rehabilitation for NGR of nonorganic origin requires providing the patient with adequate caloric and nutrient intake for the restoration of previous growth patterns. Initially, estimation of energy requirements should be based on the age-and gender-specific RDA using the patient's theoretical weight. Adequate intake of protein usually accompanies sufficient caloric intake, but care should be taken that micronutrient intakes meet the RDA and specific deficiencies, such as iron or zinc, should be treated. Some patients may not be willing or able to consume a completely balanced diet and may require a multivitamin and mineral supplement. A careful diet history can elucidate food preferences and eating patterns that need to be used in devising an appropriate dietary plan. Our experience has been to offer general dietary suggestions rather than to prescribe a specific diet. Frequent follow-up visits provide an opportunity to revise and update dietary recommendations and to assess weight and height improvements.

Although the appropriate diet can be easily determined, successful intervention requires a change in dietary behaviors and possibly health beliefs as well. Increasing the caloric density of the child's diet often involves raising the dietary fat and providing nutrient dense foods that the patient and the family may not accept. The assurance that an appropriate nutritional intake will result in normal growth, without producing obesity, is necessary supportive therapy. This is of particular concern in the initial stages of the treatment, when weight increases rapidly before any noticeable effect on height is observed.

The USDA guidelines for dietary intake were released September 24, 2004 (439). The DRIs are evidence-based recommendations for planning and assessing dietary intake of apparently healthy people and the reader is encouraged to refer to the DRI book (440) or website (441) when evaluating the dietary intake of a given patient. However, the USDA food guide is presented in Table 2 as this is a simpler guideline which should serve the needs of Pediatric Endocrinologists when evaluating the quality of the diatary intake of a short child and to provide guidelines for intake to the patients. The consumption of the recommended foods from each group is a fairly good indicator of the adequacy of the dietary intake, if maintained over time.

Vitamin and Mineral Deficiencies

NGR patients may present with multiple vitamin and mineral deficiencies that contribute to growth failure regardless of the cause (327,413). There may be generalized malnutrition with multiple macro- and micronutrient deficits, or there may be more specific nutritional alterations, as discussed below. In the section below we review some aspects of micronutrient intake and deficiencies that impact growth.

Vitamin A is an important factor for GH gene expression. Studies have shown improved linear growth in some subsets of Vitamin A-supplemented children (442). A study in Java found that children who consumed small frequent amounts of vitamin

A in fortified monosodium glutamate experienced greater height gain but similar weight gain as compared with control children (443). In the Sudan, dietary vitamin A intake, but not vitamin A supplements given once every six months, was positively associated with greater weight gain and linear growth (444,445), suggesting that small daily supplements of vitamin A may be more beneficial than periodic doses. However, in other intervention studies, vitamin A supplements had no effect on either linear growth or weight gain even when other vitamins and nutrients were supplemented in addition to vitamin A. In a large placebo-controlled study in Tamil Nadu, India, children were visited and dosed weekly. In spite of a large protective effect against mortality, the Vitamin A supplements had no effect on growth (446). Multiple postulated mechanisms link other vitamin deficiencies to poor growth. These include folate, and/or B12, whose deficiencies impair tissue oxygen delivery and lead to multiple metabolic pathways that alter protein synthesis and stunt growth. Vitamin D-deficiency of course, present with growth failure and osteomalacia (447).

In several prospective cohort studies in developing countries, the onset of stunting coincided with dietary deficiencies of several micronutrients, including iron, zinc and iodine (448). Iron deficiency is often compounded by anorexia and inappropriate dietary intake. Iron supplementation in school children demonstrated beneficial effects on appetite, growth and anemia (304,449).

Height deficits were associated with chronic deficits in energy and protein, as well as suboptimal zinc levels. Multiple micronutrient deficiencies may explain why supplementary feeding programs aimed at increasing only energy and protein intake resulted in limited physical growth (450). However we showed that energy intake appears to be the determining factor for appropriate growth in rats subjected to suboptimal calorie and zinc intake (358).

Human growth retardation from zinc deficiency was first reported by Prasad et al. in 1963. The patients had short stature, hypogonadism and zinc deficiency was documented by decreased zinc concentrations in plasma, erythrocytes and hair. Studies with ^{65}Zinc revealed that plasma zinc turnover was greater, the 24-hour exchangeable pool was smaller, and the excretion of ^{65}Zinc in stool and urine was less in the growth-retarded subjects than in the controls (451). Growth rates were greater in patients who received supplemental zinc than in those receiving only an adequate animal protein diet (452). Since then, many cases of marginal or moderate growth impairments in children with zinc deficiency as a consequence of inadequate zinc intake have been reported from various parts of the world (453–455). Marginal zinc deficiency is prevalent throughout the world in both developed and developing countries. Favier indicated that, depending on the country, 5% to 30% of children had moderate zinc deficiency, responsible for small-for-age height (456). Oral zinc supplementation showed positive effects on growth velocity in the zinc deficient children (452,453,456).

It is also well known that zinc deficiency in pregnant women causes fetal growth retardation. Women with serum zinc concentrations in the lowest quartile during early pregnancy had an eight-fold higher prevalence of LBW infants, independent of other risk factors (457). Maternal plasma zinc concentrations measured at midpregnancy also correlated significantly with birth weight (458). However, the effects of zinc supplementation during pregnancy are not clear, and it is curently speculated that such supplementation may be beneficial only in populations that are zinc deficient and at high risk of poor fetal growth (459).

Marginal zinc deficiency seems to be prevalent in infancy and in short statured children. A study of healthy, term, Danish infants from birth to 12 months of age found suboptimal zinc status in many subjects during late infancy, and a positive association between serum zinc levels at nine months with growth velocity during the period of six to nine months (460). Preterm babies given zinc supplementation had greater growth velocity, though IGF-I and IGFBP-3 levels were unchanged (461). Body zinc clearance studies in short Japanese children with normal GH secretion found marginal zinc deficiency in about 60% of the short children (462). Oral zinc supplementation effectively increased height gains in the short boys, but not girls, with marginal zinc deficiency. There was also a significant correlation between the body zinc clearance values and percent increases in growth velocity after oral zinc supplementation (462). The reason for such a high incidence of marginal zinc deficiency in Japanese short children may be the recently increased intake of precooked food, snacks and convenience foods. An age-matched controlled study conducted five years earlier also showed improved growth velocity by oral zinc supplementation in short Japanese children with marginal zinc deficiency; supplementation in this study was further reported to increase serum IGF-I, osetocalcin and alkaline phosphatase activity (463). In a meta-analysis of the growth effects of zinc supplementation, 26 studies showed increased growth while seven failed to show improvement (464). Thus, interventions to improve growth with zinc appear to be successful if there is NGR.

The mechanism(s) by which zinc deficiency impairs growth remains controversial. Zinc is required for the activity of over 300 enzymes, called zinc metalloenzymes, whose active sites contain a zinc ion. Zinc metalloenzymes include DNA polymerase, RNA polymerase and thymidine kinase, which are important for nucleic acid and protein synthesis and cell division. Furthermore, several hundred zinc-containing nucleoproteins are probably involved in gene expression of various proteins (452,465).

Zinc has also been reported to impact function at several steps of the GH/IGF axis. Partial GH

deficiency due to chronic mild zinc deficiency was described in a case report of a 13-year-old Japanese boy with short stature whose growth velocity improved with oral zinc supplementation; his GH responses to provocative tests also normalized with zinc supplementation (454). Zinc-deprived rats had low circulating IGF-I levels, decreased hepatic IGF-I gene expression, and a reduction in hepatic GH receptors and circulating GH-Binding protein (GHBP) (466). Tumor necrosis factor-α converting enzyme, the protease that cleaves GHBP from surface GH receptors, is a zinc-dependent metalloprotein (467). Zinc was also found to inhibit protein tyrosine phosphatases, such that experimentally lowered intracellular zinc concentrations suppressed insulin and IGF-I-stimulated phosphorylation of the insulin/IGF receptors (468). Thus, zinc deficiency not only reduces IGF-I production, but also decreases cellular IGF-responsiveness. This may explain why a study of GH deficient children found zinc status to significantly affect their response to GH treatment; growth velocity on GH therapy was lower in the zinc deficient than in zinc sufficient children, and it improved with oral zinc supplementation (469).

The presence of large amounts of zinc in bone tissue suggests that zinc plays an important role in skeletal development (470). Retardation of bone growth is a common finding in various conditions associated with zinc deficiency. Zinc has a stimulatory effect on bone formation and mineralization (471). Zinc is a required cofactor for alkaline phosphatase activity, an enzyme mainly produced by osteoblasts whose major function is to provide calcium deposition in bone diaphyses. Bone alkaline phosphatase activity was significantly increased by the administration of vitamin D3 or zinc, and DNA content was synergistically enhanced by the simultaneous treatment with zinc (471). The receptors for 1,25-dihydroxy-Vitamin D3 were shown to have two zinc fingers at the site of interaction with DNA, a common motif in DNA-binding proteins (472). Zinc also directly activates aminoacyl-tRNA synthetase in osteoblasts, and it stimulates cellular protein synthesis. Moreover, zinc has an inhibitory effect on osteoclastic bone resorption by suppressing osteoclast-like cell formation from marrow cells (470).

Thus, zinc deficiency should be considered as a causal factor in children with unexplained short stature who are growing poorly. However, serum zinc concentration, the most readily available clinical measure, is not a good indicator of zinc deficiency. Circulating zinc levels do not always reflect tissue zinc stores, but going after tissue zinc concentrations usually involves methods, such as liver biopsy, that are too invasive to be clinically feasible. A zinc clearance test has been recommended as a sensitive indicator of marginal zinc deficiency for clinical purposes (463). Serum zinc levels are measured every 30 minutes after intravenous zinc administration, and the decay curve provides valuable data to determine the zinc status of the patient.

Once the status of the child's zinc nutrition is established, oral zinc supplementation should be administered as a growth-promoting therapy. However it should be kept in mind that there may also be other nutrient deficits that may compound the problem and the response to therapy (473,474).

LABORATORY AIDS IN DIFFERENTIATING SHORT STATURE

Any patient who falls below the third percentile in height and/or has decreased growth velocity (falling across major percentiles) should receive a complete diagnostic evaluation. Because the possible causes of short stature and growth retardation are so numerous, laboratory investigations should be geared towards confirming or ruling out specific disorders. The differential diagnoses must be based on information obtained from the history and physical examination (136). It is important to assess, in addition to the growth rates: history of chronic illness and medications, mid-parental target height and parental timing of puberty, birth size, previous growth pattern, nutritional state, pubertal stage, body segment proportions, bone age and predicted adult height. The previous growth pattern, including height and weight measurements, cannot be overstated as a critical component of any growth evaluation, so carefully maintained growth charts are imperative. Regretably, retrospective chart reviews of children referred in 2001 to an academic tertiary care insitution for short stature evaluations found prior growth curves from the referring pediatricians in the charts of only 41% of the children (9).

Laboratory tests can be divided into several major categories to target the multiple conditions in the differential diagnosis (Table 1). General screening tests should be performed to assess overall electrolyte balance, hematologic, hepatic and renal functions. These include: general chemistries including BUN, creatinine and liver function tests; urinalysis; and complete blood count with differential. Ferritin levels are helpful to further investigate iron deficiency, and blood gas with simultaneous urine pH can identify renal tubular acidoses if the serum bicarbonate is unexpectedly low. Sedimentation rate and C-reactive protein are useful to screen for inflammatory conditions, such as IBD, and celiac autoimmune panel (total IgA, tissue transglutaminase antibody and/or antiendomyseal antibody), should also be considered in patients with NGR, even in the absence of gastrointestinal symptoms. Additionally the medical history and physical examination may suggest sweat testing for cystic fibrosis, and testing for infections like HIV or tuberculosis. A karyotype is imperative in every girl with short stature, even in the absence of any stigmata of Turner syndrome. Children who demonstrate skeletal abnormalities and alterations in body proportions deserve evaluation for metabolic bone disease, such as mucopolysaccharidosis, mucolipidosis,

and gangliosidosis, or the skeletal dysplasias (Vol. 2; Chap. 6). A thorough nutritional assessment should be made if there is a growth pattern of NGR. If zinc deficiency is suspected, serum zinc levels may be obtained, but they are usually not sufficient to establish the diagnosis, as discussed above.

Endocrine causes of short stature and/or poor growth are also multiple. Thyroid function tests should always be checked (Vol. 2; Chap. 33). If the physical exam reveals a Cushingoid phenotype in a child who is not growing well, a 24-hour urine collection for free cortisol-to-creatinine ratio should be performed, once a history of steroid medications frequently used for a variety of pediatric disorders has been considered and eliminated if possible. Conversely, hyperpigmentation, fatigue and poor weight gain indicative of primary adrenal insufficiency warrant measurement of Adrenocorticotropic hormone and cortisol levels (Vol. 2; Chap. 8). Examination of the eye grounds and visual fields should always be done, but a magnetic resonance imaging (MRI) scan may be necessary if hypopituitarism is considered (475).

Diagnosis of GH deficiency is not straightforward, and should be deferred to a pediatric endocrinologist. Due to its pulsatile secretion during stage IV sleep, random GH levels are useless except for the first few months of life before the sleep entrainment develops. Random IGF-I and IGFBP-3 measurement have become the first-line screening test for GH deficiency (476); their levels do not fluctuate significantly throughout the day, but are highly dependent on age and gender so appropriate normative reference ranges are required. Although normal IGF-I levels suggest normal hepatic stimulation by GH, low IGF-I levels may indicate GH deficiency, GH resistance, primary IGF deficiency, or states of undernutrition like anorexia nervosa, hepatic or gastrointestinal diseases (136). Thus, the current standard is to follow up low IGF-I levels with provocative GH testing to directly assess GH secretory capacity. A number of tests have been devised to stimulate GH secretion, each taking advantage of a different regulatory mechanism of endogenous GH secretion (477,478). These may be combined with stimulatory tests of other pituitary axes to simultaneously assess for multiple pituitary hormone deficiency. However, provocative GH tests are fraught with numerous technical limitations, such that, despite their widespread use in diagnosing GH deficiency, they are acknowledged as inferior to any ideal "gold standard" test (479). The controversy has reached the point where some advocate eliminating provocative GH testing altogether, arguing they do not reliably contribute to the diagnostic algorithm beyond suspicious growth pattern, IGF measurement, bone age delay and pituitary MRI (480). One short-coming of the provocative GH tests is that they are pharmacologic rather than physiologic. Thus, they would fail to diagnose neurosecretory GH deficiency, in which the pituitary is normal and can synthesize GH normally, but fails to release GH normally due to disrupted signaling from the hypothalamus and higher brain centers (477). The pharmacologic pituitary stimulation of provocative GH testing would by-pass the defect and lead to falsely normal results. An overnight neurosecretory test, in which endogenous GH secretion is assessed by measuring GH levels every 20 minutes overnight, is the diagnostic test for neurosecretory GH deficiency, but is cumbersome, invasive and expensive. GH testing protocols are described in detail in Vol. 2; Chap. 33. In any case, serial growth measurements are better guidelines than many of the aforementioned tests, and without the clinical growth data, the test results are difficult to interpret.

FINAL CONSIDERATIONS

Short children and their parents face a number of specific psychosocial problems that are frequently associated with the child's developmental stage. For example, small size frequently leads to juvenalization during childhood and is further compounded by delayed sexual development in adolescence. Despite these developmental problems, short individuals usually adjust normally to life function as adults (14). General personality mechanisms of the small child and his or her parents play an important role in this adjustment. These children's school achievements are important, as are the specific techniques they develop in coping more effectively with their environment. One of the most important problems of short children is being treated in an infantile manner, appropriate for their size, but not for their age. Some children respond to this phenomenon being by behaving immaturely (Peter Pan reaction), while others rebel against being pampered and sometimes develop various neurotic and psychosomatic symptoms, including denial, withdrawal, phobias and compensatory fantasies. Still other children find a more satisfactory solution in the reaction of "mascotism."

Frankness (diplomatic, not brutal) is desirable in counseling short and dwarfed people about their situation. Short stature patients are then able to plan the future realistically, to discuss the stresses that beset them, and to master techniques for dealing with silly yet potentially hurtful comments about their size and age. Usually they accomplish an adaptive equilibrium in adulthood. Should we be medically treating the height of healthy short children? If the problem is psychosocial stress, wouldn't counseling directed at self-esteem and coping mechanisms be a more appropriate therapy?

Short stature is not typically associated with intellectual deficit, so dwarfed people should be encouraged to pursue appropriate educational and career goals. Likewise, short children may legitimately hope for romance, marriage and a successful sex life. They may take advantage of simple interventions like height-augmenting footwear (Elevators, Richlee Shoe Co., Frederick, MD), or available support groups, including Little People of America, San

Bruno, CA; Human Growth Foundation, Inc., Cherry Lane, MD; or Magic Foundation, Oak Park, IL.

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2

Idiopathic Short Stature

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INTRODUCTION

If stature follows a Gaussian distribution, then the height between the 2 SD will constitute normal height (Fig. 1A). The individuals below −2 SD (or −2.25, −2.5, or −3 SD, all are arbitrary points) will be considered having short stature (Vol. 2; Chap. 1). if the reasons of the short stature in these children are unknown or the investigation does not reveal any pathology, these children are then labeled as "normal short child" (Fig. 1A).

We have been tempted to use the term "normal" in describing "abnormal" stature, largely because of an inability to identify the etiology of short stature and to link it with significant health risks. In this chapter we plan on discussing the origins of the terms idiopathic short stature (ISS) as alternatives to normal variant short stature (NVSS), a term widely used previously to describe short, poorly growing children who have passed a growth hormone (GH) stimulation test.

The most common cause of referral to the pediatric endocrinologist remains short stature. At least one widely accepted definition of short stature in children is defined arbitrarily as a height less than −2.25 SD for age and sex. This definition has also been accepted by the U.S. Food and Drug Administration (USFDA) (Fig. 1B).

Investigation in the majority of these cases is initiated to rule out the presence of growth hormone deficiency (GHD) (Vol. 2; Chaps. 1 and 3). Although idiopathic GHD makes up the single largest portion of cases of GHD, the last decade has seen a reclassification of many of them as the molecular basis for GHD becomes more understood (2). Multiple laboratories have demonstrated and reported that isolated GHD results from mutations in the growth hormone releasing hormone receptor (GHRHR) gene (3,4), mutations or deletions of the GH gene (5,6), and also in combination with deficiencies of multiple pituitary hormones as the result of abnormalities of genes and transcription factors such as Pit-1, PROP1, HESX1, LHX3, etc.,(7–10).

The incidence of GHD is only in the magnitude of 1:4000–1:10,000 of short children (11). Therefore, most short children seen in pediatric clinics are non-GHD. However with the unlimited availability of recombinant human growth hormone (rhGH), the last few years have seen a dramatic increase in the utilization of GH therapy for a variety of non-GH-deficient states (Vol. 2; Chap. 5). This in general has brought the idea of treating "short stature" into the homes of most western societies and in part has shaped our expectations and biases about height. The desire to treat stems not only from the desire to correct height deficit, but rather an appreciation that children with short stature have been confronted with a number of psychological challenges including juvenilization, teasing, and bullying (12).

In simple terms, the GH provocative tests, as imperfect as it may be, are utilized by most pediatric endocrinologists to discriminate between GH sufficiency and GHD. Children who pass the provocative pharmacologic GH stimulation tests and still fail to grow are classified as non-GH-deficient disorder of growth, NVSS, or ISS. In recent years there have been multiple reports of patients, who were initially classified as ISS, and subsequently were found to have defects in the GH-insulin-like growth factor (IGF) -I-growth plate axis. Examples of such patients are those with relative insensitivity to GH (13–15), or who have bioinactive GH (16–18).

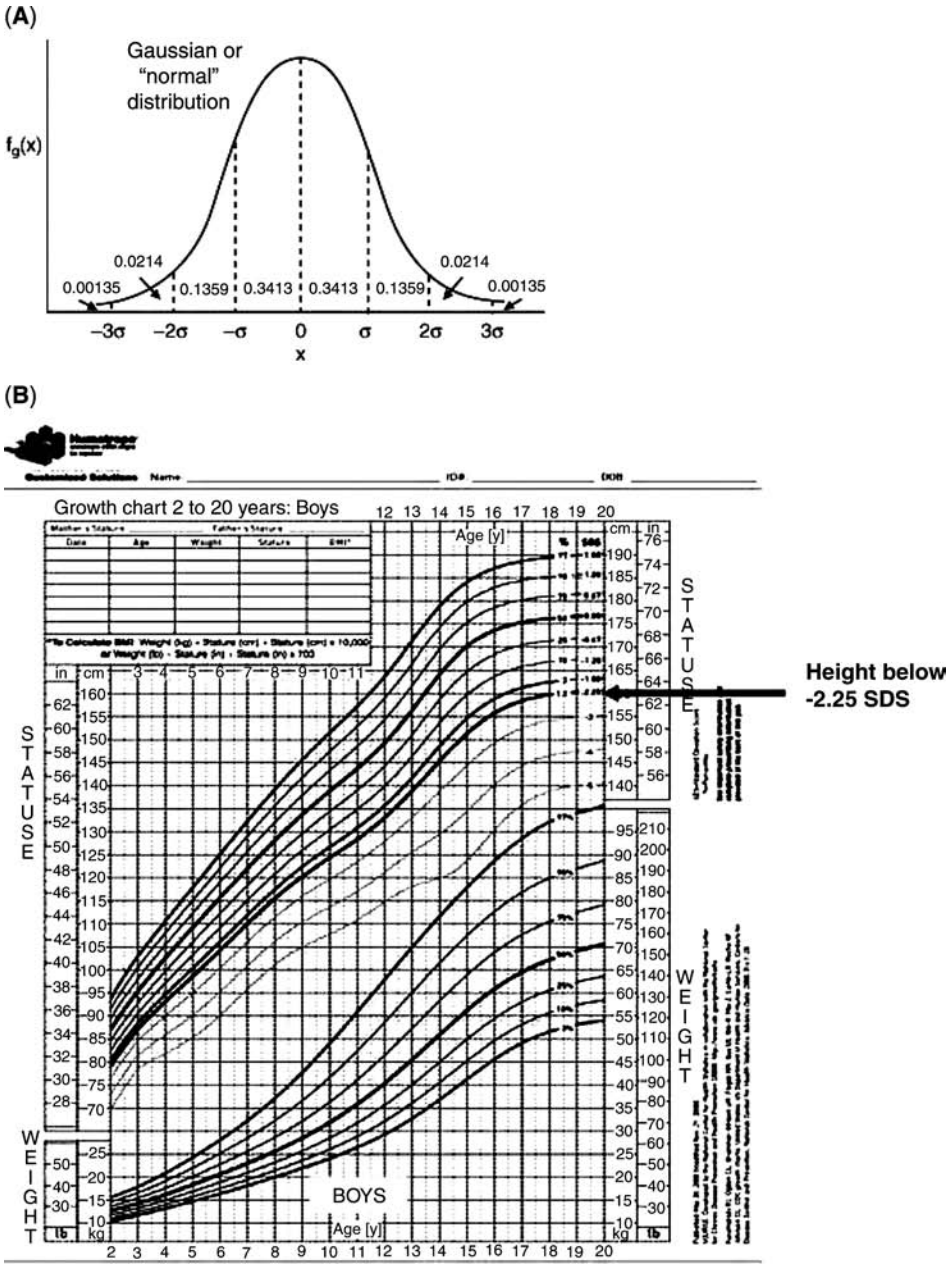

Figure 1 (**A**) The bell-shaped curve of Gaussian, or ''normal,'' distribution. Numbers indicate the fraction of the population outside of the standard deviation score indicated. (**B**) Growth curve for boys showing the −2.25 SD below the mean. *Source*: From Ref. 1.

Mutations at the post-receptor signal transduction system such as signal transducer and activator of transcription 5b (STAT5b) (19,20), in the IGF-1 receptor (21,22), and mutations in the gene for acid labile subunit (ALS) have also been described in patients with ISS (20,23). The cases above represent a few instances in which the molecular basis of ISS has been elucidated. With the latest pace of research we anticipate that over time a greater number of patients who are currently classified as ISS will ultimately be found to have a molecular basis for their growth disorder (2). We will cover the various aberrations in the GH-IGF-I axis that may underlie the cause of growth failure when a patient is classified as ISS (passed a GH stimulation test). And finally, we will also review results of clinical trials that address safety and efficacy of rhGH treatment of children with ISS.

HISTORICAL CONSIDERATIONS—THE BIRTH OF THE TERM IDIOPATHIC SHORT STATURE

In the early days of GH treatment, pituitary derived GH was very scarce and pharmacologic,

provocative, diagnostic tests were employed in the following decades with the hope that only truly GH deficient patients would qualify to receive it. Endocrinologists used these GH stimulation tests and integrated 24-hour GH values with arbitrary cut-offs (that changed as assays changed), as a way of classifying patients into broad, relatively non-descript categories of ''growth hormone insufficiency'' (GHI) (some define as having a peak GH level of 7.5–10 ng/mL), ''GHD'' (peak GH level of less than 7.5–10 ng/mL) and ''neuro-secretory dysfunction'' (low integrated overnight values). Those who failed the stimulation test were diagnosed to be GH deficient. Those who passed the stimulation test are deemed to be GH sufficient. The etiology of short stature in children who passed the provocative GH stimulation test and continued to grow poorly remained poorly understood, hence the birth of the term ''NVSS'' or as of late ''ISS.'' Classifications based purely on GH stimulation tests are arbitrary to some degree and certainly inadequate in elucidating pathogenic mechanisms. These non-physiologic tests have not withstood the test of time and have ceased to be employed by many pediatric endocrinologists due to their lack of reproducibility and predictive value of who will respond to GH treatment (24). Initial data showed no correlation between peak and baseline stimulated GH values and growth response except in those who scored the very lowest on provocative testing. On the other hand recent, yet unpublished data suggest that the magnitude of response to treatment may be correlated with level of GH response to provocative, pharmacologic stimuli (Cohen et al. unpublished data—The Novo–Nordisk trial).

ISS is a diagnosis that is controversial at best and includes all children with unexplained short stature (less than −2 SD as defined by the American Academy of Pediatrics or less than −2.25 SD as accepted by the USFDA)(Fig. 1A and B) for age and sex and who are felt to be GH sufficient (defined as a GH value of greater than 10 ng/mL in response to a pharmacologic challenge with a variety of provocative agents). Additionally, children must have the absence of an identifiable disease process, not intrauterine growth retarded (IUGR), normal body proportions, good caloric intake, and the absence of a psychiatric disorder (1). Other important parameters, which the pediatric endocrinologists routinely use in assessing growth of a child, predicted adult height, IGF-I, and IGFBP-3 levels are not included in the definition.

The controversy surrounding the term ISS, in general, is whether it is a medical disorder or a normal variation. Idiopathic is defined properly as ''arising spontaneously or from an obscure or unknown cause'' (25). In that sense, once an anatomical or molecular etiology is identified, the term idiopathic is no longer appropriate. Thus, as the molecular basis

Table 1 Genetic Alterations Identified in Cases of Idiopathic Short Stature

Patients	Genes	Mutations	Inheritance	Functional effect	Ref.
1	GH1	Arg77Cys	Heterozygous	Bioinactive GH	(16)
1	GH1	Asp112Gly	Heterozygous	Bioinactive GH	(17)
1	GH1	Ile179Met	Heterozygous	Reduction in ERK activation	(18)
1	GH1	C53S	Homozygous	Bioinactive GH	(26)
1	GHR	Glu44Lys,R161C	Compound heterozygous	Reduced GH binding	(13)
1	GHR	C122X	Heterozygous	Reduced number of GHRs	(13)
1	GHR	Glu244Asp	Heterozygous	Aberrant subcellular localization	(13)
2	GHR	R211H	Heterozygous	Reduced expression of the extracellular domain	(13,14)
2	GHR	Arg161Cys	Heterozygous		(14)
1	GHR	A478T	Heterozygous		(14)
1	GHR	V144I	Heterozygous	Not determined	(15)
4	GHR	Pseudoexon	Homozygous		(27)
1	STAT5b	Ala630Pro	Homozygous	Aberrant transcription of IGF-I/IGFBP3	(19)
1	IGF-1	Deletion: Exon 4&5	Homozygous	Deficiency of IGF-I	(28)
1	IGF-1	Met44Val		Bioinactive	(29)
1	IGFALS	1338delG		Deficiency of ALS	(23)
1	IGFALS	D440N		Deficiency of ALS	(20)
1	IGF-1R	Arg108Gln,Lys115Asn		Decreased IGF-IR function	(21)
2	IGF-1R	R709Q	Heterozygous	Failure to process the proreceptor	(22)
4	IGF-1R	R59X	Haploinsufficiency	Reduced number of IGF-I Rs	(21)
1	SHOX	R195X nonsense mutations			(30,31)
12	SHOX	Nonsense, missense mutations and deletions	Haploinsufficiency		(31)
16	NPR2		Heterozygous		(32)

Abbreviations: GH, growth hormone; GHR, growth hormone receptor; STAT, signal transducer and activator of transcription; IGF-I, insulin-like growth factor-I; IGFBP-3, IGF binding protein-3; ALS, acid labile subunit; IGFALS, ALS gene; IGF-IR, IGF-I receptor; SHOX, short stature homeobox gene; NPR2, NP receptor natriuretic peptide receptor-B (NPR-B) gene.
Source: From Ref. 33.

becomes known, many of these children who have been traditionally labeled as ISS will be reclassified.

GROWTH HORMONE-INSULIN-LIKE GROWTH FACTOR AXIS IN IDIOPATHIC SHORT STATURE

After secretion from the anterior pituitary, GH binds to the growth hormone receptor (GHR) in peripheral tissues. The binding leads to GHR dimerization, recruitment of Janus kinase 2 (JAK2) molecules, there by promoting their enzymic activity via cross-phosphorylation, as well as leading to the phosphorylation of critical tyrosines on the intracellular portion of the GHR. This provides docking sites for critical intermediary proteins known as STATs, mainly the STAT5b and possibly, other STATs. The dissociation of phosphorylated STAT5b from the intracellular domain of the GHR then occurs. The phosphorylated STAT5b dimerizes and enters the nucleus were it binds to the DNA and initiates of gene transcription. The transcription of the IGF-I gene leads to the production of IGF-I. The IGF binding protein (IGFBP) -3 and the ALS are also transcripted and produced of in a similar manner. These three molecules together form IGF ternary complex. In plasma, IGF-I binds to the soluble IGF-IR At target cells, this complex activates signal-transduction pathways that result in the mitogenic and anabolic responses that lead to growth.

Various disorders in the GH-IGF-I signaling were found in children who initially were classified as ISS (Table 1).

Different mutations in the GH1 gene can result in bioinactive GH, reduced extracellular signal-regulated kinase (ERK) activation leading to the phenotype of ISS (16–18). A relative insensitivity to GH secondary to mutations in the GHR gene can be one of the reasons of ISS (13–15). Post-receptor defects, for instance, STAT5b, IGF-I gene, and IGFALS (ALS) gene mutations have been identified in patients with IGF-I deficiency (IGFD) (19,20,23,28,34). Compound heterozygous and single heterozygous point have been identified in gene for IGF-IR resulting in decreased IGF-IR function and IGF-I resistance (Table 1) (Fig. 2) (21,22).

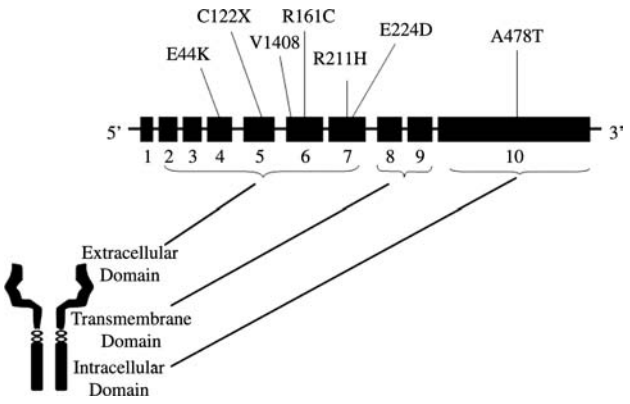

Figure 2 Mutations in the growth hormone receptor gene responsible for idiopathic short stature.

THE MOLECULAR BASIS OF IDIOPATHIC SHORT STATURE

Growth Hormone 1 Gene Mutations

The molecular basis for GHD was unknown when about 40 years ago GH first became widely available for the treatment of GHD. Similarly we are beginning to understand the molecular basis of ISS as some of the genetic defects are beginning uncovered (Table 2).

The first report of a bioinactive GH were two boys with dwarfism, low levels of IGF-I, delayed bone ages, and had normal GH responses after stimulation test. These patients responded with a substantial increase in the growth velocity after administration of hGH. The GH when measured was detectable but was biologically inactive (35). The genetic defect was described in the GH1 gene of a similar patient, a 4.9 years old boy with height −6.1 SD, low IGF-I, high basal serum GH, and normal peak GH concentrations after provocative pharmacological testing. A heterozygous single base substitution was identified in the GH1 gene of the proband, predicted to convert codon 77 from arginine to cysteine (R77C). The mutant GH bound to growth hormone binding protein (GHBP) approximately six times stronger than the wild-type GH and failed to stimulate tyrosine phosphorylation (16). A heterozygous single-base substitution (A→G) in exon 4 of the GH1 gene (Gly112Asp) was identified in a girl with short stature. The mutant GH was less potent than wild-type GH not only in phosphorylation of tyrosine residues in GHR, but also in the activation of JAK2 and STAT5 (17). Another case of a heterozygous mutation, Ile179Met substitution in the GH1 gene has also been described. This mutation retained the normal ability to activate STAT5 but resulted in reduction of ERK activation and subsequently to a decreased production of IGF-I (18). Another patient with height −3.6 SD, low IGF-I levels, high GH levels on provocative testing was reported with a mutation (C53S) which abolishes the disulfide bridge Cys-53–Cys-165 leading to the production of a bioinactive GH molecule (26).

Abnormalities of the Growth Hormone Receptor Resulting in Defective Signal Transduction

Patients previously classified as ISS were found were speculated to have partial insensitivity to GH as about 25% of such patients have abnormally low serum concentrations of IGF-I (36,37). To date, several distinct molecular defects in a few hundred of the ISS patients have already been identified at most of the steps involved in the GH regulation of IGF-I production. Accumulating evidence from the studies suggests that GHR gene mutations account for about 5% of all ISS patients (14,15). Few of these molecular defects are listed below (Fig. 2).

Table 2 Clinical and Biochemical Features of Molecular Defects Resulting in Idiopathic Short Stature

Molecular defect	Height	Birth size	GH	GHBP	IGF-I	IGFBP-3	ALS	Immune defects
GHR (extracellular domain)	↓↓↓	–	↑↑	N-↓↓	↓↓↓	↓↓↓	↓↓↓	–
GHR (dimerization defect)	↓↓↓	–	↑↑	N	↓↓↓	↓↓↓	↓↓↓	–
GHR (transmembrane defect)	↓↓↓	–	↑↑	N-↑	↓↓↓	↓↓↓	↓↓↓	–
GHR (intracellular domain)	↓↓↓	–	↑↑	N-↑	↓↓↓	↓↓↓	↓↓↓	–
STAT5	↓↓↓	–	↑↑	N	↓↓↓	↓↓↓	↓↓↓	Variable
ALS	↓	–	↑	N	↓↓	↓↓	Absent	–
IGF-I gene deletion	↓↓↓	↓↓	↑↑	N	Absent	↑	↑	–
IGF-I bioinactive	↓↓↓	↓↓	↑↑	N	↑↑	↑	↑	–

Abbreviations: GH, growth hormone; GHR, growth hormone receptor; STAT, signal transducer and activator of transcription; IGF-I, insulin-like growth factor-I; IGFBP-3, IGF binding protein-3; ALS, acid labile subunit.
Source: From Ref. 33.

ISS patients with low GHBP and IGF-I levels were supposed to represent a part in the spectrum of GH insensitivity. The findings were confirmed with genetic analysis of the GHR gene of such patients (13). One patient in this study was a compound heterozygote for the E44K and R161C substitutions. The R161C mutation in the homozygous state is associated with complete insensitivity to GH (13). The other three patients were heterozygous for C122X, R211H, and E224D mutations (13). In vitro expression studies revealed that E44K cause a 330-fold reduction in the receptor affinity for GH. The R211H mutation leads to a reduced expression of the GHR extracellular domain by approximately four orders and E224D mutant causes an aberrant subcellular localization of the GHR receptor (13). The authors extended the study and reported four more patients, two of them were heterozygous for R161C mutation; the other two were also heterozygous for R211H missense mutation and A478T mutation on exon 10 (14). In another study the GHR gene was analyzed in 17 subjects with ISS. A novel heterozygous mutation resulting in a Val144-to-Ile (V114I) substitution in exon 6 of the extracellular domain was found in the proband (height −1.8 SD). The proband's mother (height −2.5 SD) and brother (height −2.3 SD) also had the identical mutation (15).

Four consanguineous patients had marked short stature (height SD from −3.3 to −5.6), normal facial features, low IGF-Is, and normal GHBP levels. They were found to be homozygous for an A–G change at position-1 of the acceptor splice site at the 5-prime end of the pseudoexon point mutation in GHR that led to activation of an intronic pseudoexon resulting in inclusion of an additional 108 nucleotides between exons 6 and 7 in most GHR transcripts (Fig. 2) (27).

The GHR gene was sequenced in two patients a mother and a daughter with the phenotype of ISS. Analysis revealed a G–C transversion at position-1 of the splice acceptor site of intron 8 (IVS8as-1 G→C), suggesting that the mutation caused deletion of exon 9. Cotransfection of the mutant and wild-type GHR showed that the mutant GHR was unable to activate STAT5. The mutant truncated receptor exerted a marked dominant-negative effect on GHR and was transmitted in an autosomal dominant manner (38).

Only several hundred patients with mutations of the GHR gene have been identified, the molecular defects are elicited in only in the most severe cases that eventually will be followed by characterization of more subtle GHR abnormalities.

Post-Growth Hormone Receptor Signaling Defects

Recently, a patient with severe postnatal growth failure and IGFD was found to be homozygous for an autosomal recessive missense mutation in the DNA-binding domain of STAT5b (A630P) (19). This mutation resulted in a marked reduction of phosphorylated STAT5b, as well as an inability to raise serum concentrations of IGF-I, IGFBP-3, and ALS in response to administration of exogenous GH, consistent with severe GH insensitivity. Investigations in mice with partial inactivation of STAT5b resulted in significant growth retardation and viable animals (39). A second patient, with a novel STAT5b mutation, has also been identified with a similar growth and biochemical phenotype (34). In this case, the patient was homozygous for a single nucleotide insertion in exon 10 of the STAT5b gene, resulting in a frame shift and early protein termination, with no detectable STAT5b on immunoblot. It is of note that both reported patients with STAT5b mutations also were characterized by immune defects, with recurrent pulmonary infections and subsequent lymphocytic interstitial pneumonia or pulmonary fibrosis. As STAT5b is also part of the signaling pathway for a variety of cytokines, such as IL-2 and gamma interferon, it appears likely that both the growth failure and the immune defects are the consequences of the same underlying molecular defect. Most importantly, these patients have confirmed the hypothesis that STAT5b is the major (if not the sole) mediator of GH stimulation of IGF-I gene transcription. The molecular defects thus far described are summarized in Table 2 and in Fig. 3.

Insulin-Like Growth Factor-I Gene Deletions or Mutations

Animal knock out studies elegantly performed by Efstratiadis and coworkers as well as subsequent

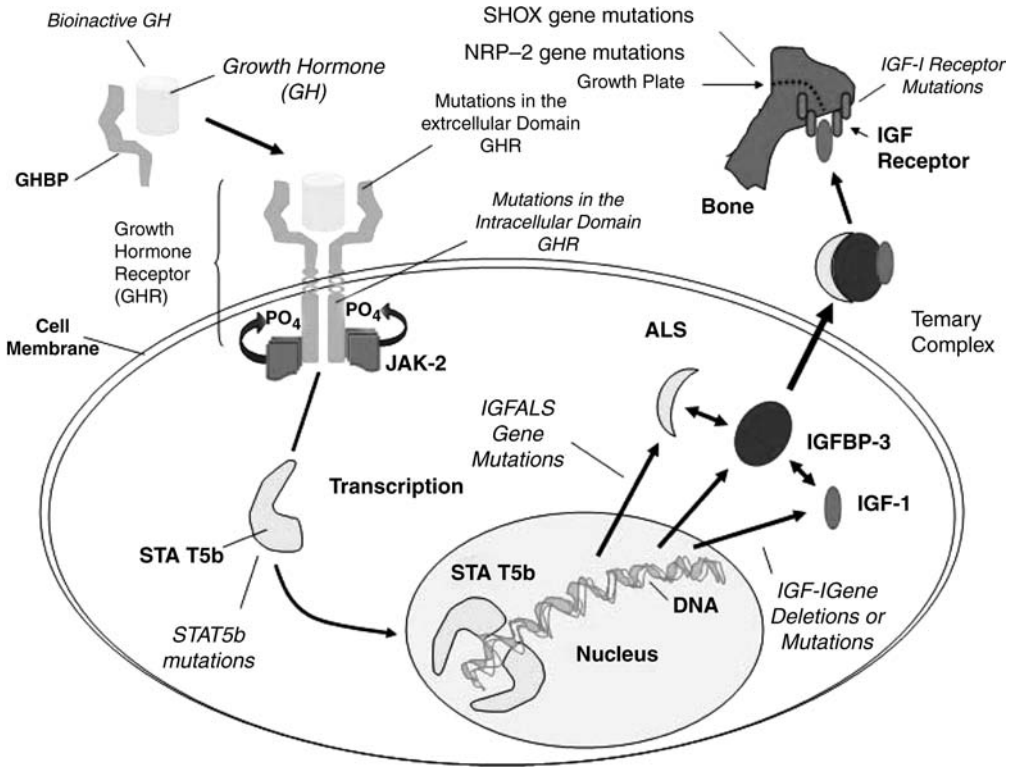

Figure 3 Growth hormone (GH) signaling pathways. GH associates with GHBP. The binding of a molecule of GH, through each of two specific sites on GH, to the extracellular domains of membrane-associated, dimerized GHR. Interaction of GHR with JAK2. Tyrosine phosphorylation of JAK2 and critical residues of the intracellular domain of the GHR provides docking sites for other cytoplasmic signal mediators. Recruitment of STAT5b to phosphorylated tyrosine residues on the intracellular domain of the GHR. Phosphorylation of STAT5b.Dissociation of phosphorylated STAT5b from the intracellular domain of the GHR and entry into the nucleus. Binding of STAT5b dimer to DNA and initiation of gene transcription. Transcription of the IGF-I gene and production of IGF-I. Transcription and production of IGF binding protein (IGFBP)-3 and the acid-labile subunit (ALS) and formation of the IGF ternary complex. It depicts our current understanding of GH regulation of IGF-I production, and illustrates the identified defects for known causes of ISS. The defects resulting in ISS are as follows Bioinactive GH, Mutations in the extracellular and intracellular domains of GHR, STAT5b mutations, IGFALS Gene Mutations, IGF-I Gene Deletions or Mutations, IGF-I Receptor Mutations, SHOX gene mutations and NRP-2 gene mutations. *Abbreviations*: GH, growth hormone; GHR, growth hormone receptor; JAK, janus-family tyrosine kinase; STAT, signal transducer and activator of transcription; IGF-I, insulin-like growth factor-I; IGFBP-3, IGF binding protein-3; ALS, acid labile subunit; IGFALS, ALS gene,; IGF-IR, IGF-I Receptor; SHOX, short stature homeobox gene; NPR2, CNP receptor natriuretic peptide receptor-B (NPR-B) gene.

reports of humans with IGF-I gene deletions further established growth dependency on normal induction of IGF-I (40–42). Only one case, to date, of an IGF-I gene deletion (involving exons 4–5) has been reported (28). As predicted by the mouse knockout model, this patient who was a product of a consanguineous relationship had significant IUGR (birth length −5.4 SD, birth weight −3.9 SD, head circumference −4.9 SD), followed by pronounced postnatal growth retardation (height at 15.75 years was −6.9 SD). Other clinical features included microcephaly, severe developmental delay, sensorineural deafness, and delayed puberty. This patient had high basal and stimulated GH levels and profoundly low IGF-I. The combination of prenatal and postnatal growth failure differentiates this defect from the prior ones described and supports the hypotheses that: (1) IGF-I is a major mediator of both prenatal and postnatal growth; (2) IGF-I production in utero is largely GH-independent; and (3) while IGF-I production postnatally is largely GH-dependent (Fig. 3).

Inactivating Mutations of the Insulin-Like Growth Factor-I Gene—Bioinactive Insulin-Like Growth Factor-I

These conclusions are further supported by the recent report of a patient with a virtually identical clinical phenotype, with striking prenatal, and postnatal growth failure, but with elevated serum concentrations of IGF-I (29). This 55-year-old male, with a birth length of −4.3 SD and an adult height of −8.5 SD, was found to have a missense mutation, resulting in the substitution of methionine for valine at position 44. The resultant IGF-I molecule was readily immunodetectable and was capable of binding to IGFBPs, but bound with markedly decreased affinity to the IGF-IR, and only poorly stimulated the receptor

phosphorylation and DNA synthesis. In this case, the IGFD was detectable only by bioassay, and not by conventional radio-assays.

Mutations of the Gene for Acid Labile Subunit

After IGF-I binds to IGFBP-3 or IGFBP-5, it further associates with the ALS, to form a ternary complex, which serves as the major carrier complex in serum for both IGF-I and IGF-II. Two unrelated adolescent boys with novel mutations of the IGFALS gene have been reported (20,23). Both were characterized by modest growth failure (height approximately −2.5 SD), micrognathia and truncal obesity, mild delay in bone age (two years), accompanied by low serum concentrations of IGF-I (−5.3 SD) and IGFBP-3 (−9.7 SD), which failed to rise upon administration of exogenous GH. They had normal GH stimulation test results. The first mutation, 1338delG, resulted in a substitution of lysine for glutamic acid at codon 35, and an early stop codon at position 120. The second mutation, D440N, replaced a negatively charged D-aspartate with asparagine in the leucine-rich repeat section of the ALS molecule. The inability of ALS to bind to binary complexes of IGF-IGFBP-3 is the presumed cause of the markedly reduced serum levels of IGF-I, although it remains unclear as to whether the amount of IGF-I (perhaps in ''free'' form) available to the growth plate is compromised. The degree of growth failure in these patients is considerably milder than that observed in the other forms of primary IGFD described in this chapter, and in that feature, resembles the mild growth failure observed in the mouse ALS knockout model (43).

Insulin-Like Growth Factor Receptor Type 1 Mutations

Mouse knockout models have demonstrated the critical importance of IGF-I and IGF receptor type 1 (IGF-IR) for in utero fetal growth as well as postnatal growth (40,41). The clinical phenotype of such patients, as predicted had IUGR and moderate progressive postnatal growth failure. Additionally, nonverbal learning disorders, mild motor developmental delay, delayed dentition, retarded bone age, brisk response to GH stimulation, and high IGF-I concentrations One patient had a compound heterozygous missense mutation (R108Q and K115N), leading to decreased binding affinity of IGF-IR to one-third of that of the controls. Another patient was reported with a heterozygous nonsense mutation (Arg59stop) resulting in reduced number of IGF-IR (21). In another report two patients were described with IUGR and short stature bearing a heterozygous missense mutation (R709Q) changing Arg–Lys–Arg–Arg to Arg–Lys–Gln–Arg at the cleavage site of IGF-IR, resulting in failure of processing of the proreceptor IGF-IR to mature IGF-IR (22). In summary the presence of growth failure and high IGF-I levels in these patients with IGF-IR mutations represent the phenomenon of IGF-I resistance in humans. The clinical phenotype is very similar to IGF-I gene deletions yet milder.

Mutations in the Short Stature Homeobox Gene

Short stature homeobox (SHOX) containing gene mutations are an entirely new system affecting growth. Mutations in the SHOX gene resulting in SHOX haploinsufficiency have been described in up to 1% to 2% of individuals classified as having ISS (30,31,44,45). The SHOX gene is located near the distal tip or pseudo-autosomal region of the X and Y chromosomes and encodes a transcription factor, which is expressed in bone marrow fibroblasts and in other tissues. It is not a classical X-linked gene because it does not undergo X-inactivation as two active copies of the gene are required for normal linear growth. Because it is located on both the X and the Y chromosomes, a defect can be inherited from either parent. Clinical features of SHOX haploinsufficiency in childhood can include short stature, short limbs, wrist changes, and tibial bowing (46). SHOX mutations have been found in about 60% of the cases of Leri–Weill syndrome of dyschondrosteosis although the specific action of this gene is not well known (47).

Natriuretic Peptide Receptor-B Mutations

C-type natriuretic peptide (CNP) is an important regulator of skeletal growth. Loss-of-function mutations affecting the CNP receptor natriuretic peptide receptor-B (NPR-B, gene NPR2) if inherited in an autosomal recessive fashion results in skeletal dysplasia, acromesomelic dysplasia, Maroteaux type. Height z-scores of the heterozygous carriers of NPR2 mutation were -1.8 ± 1.1 (mean $\pm$ SD), which was significantly less than the non-carriers (-0.4 ± 0.8, $p < 0.0005$) and the general population (32). Thereby the heterozygous mutations in NPR2 gene are associated with short stature. The authors estimated that 1 in 30 individuals with ISS could be carriers of NPR2 mutations (32).

THE SPECTRUM OF GROWTH HORMONE-INSULIN-LIKE GROWTH FACTOR DEFICIENCY IN IDIOPATHIC SHORT STATURE

Retrospective analyses of biochemical data on patients with ISS have indicated that 25% to 50% of such patients have abnormally low serum concentrations of IGF-I in the presence of normal GH secretion i.e., primary IGFD (36,37). To date, several distinct molecular defects in patients presenting with growth failure have already been identified at most of these steps involved in the GH regulation of IGF-I production, thereby providing a firm molecular basis for the diagnosis of primary IGFD. It is likely that these rare disorders represent the extreme of primary IGFD, and that many other patients will prove to

have more subtle abnormalities of the GHR, GH signaling cascade, or IGF-I gene expression, ALS deficiency (Fig. 3).

Growth Hormone Deficiency

Isolated GHD can be caused by the molecular defects of the GHRHR gene, GH gene. GH gene deletions and mutations are inherited as autosomal recessive, autosomal dominant, and X-linked manner (3–6). There have been at least four point mutations in the GH1 gene of patients described with short stature, low IGF-I, and normal or high GH levels on provocative testing. In these cases the GH was produced and detected but the mutant GH was bioinactive (16–18,26). So the defects of production of GH from the anterior pituitary can vary from complete deficiency of GH secretion to a few defects are associated with inactive GH presenting with the phenotype of ISS.

Growth Hormone Insensitivity

The hypothesis that Laron syndrome was the consequence of a defect in the GHR was proven in 1989, with the report of a large deletion of a portion of the GHR gene, which encodes for the extracellular domain of the receptor (48). Shortly thereafter, the first point mutations affecting the extracellular portion of the GHR gene were reported to date (49); over 60 distinct mutations have been described, with the overwhelming majority affecting the extracellular domain (50). Mutations were also identified in the transmembrane and intracellular domain present with normal and increased GHBP along with IGFD are inherited autosomal recessive and dominant manner, respectively (51,52).

Total loss of GH binding results in the classical phenotype of Laron syndrome characteristically, low serum concentrations of GHBP, IGF-I, IGFBP-3, and ALS are markedly reduced (53). The overwhelming majority of the molecular defects identified to date in severe GHI (Laron syndrome) have usually involved the extracellular domain of the GHR. Some studies were undertaken to characterize ISS according to the serum GHBP levels. It was found that approximately 90% of the ISS subjects had GHBP concentrations below the mean when adjusted for age and sex (54). The molecular defects described previously in this chapter underscore the importance of defects in GHR; postreceptor signaling pathway has in the understanding of etiology of short stature (13–15,19,34,38). It is possible that many cases, which have been categorized to ISS, actually will prove to have underlying GH insensitivity.

Partial Deficiencies/Insensitivities

GH neurosecretory dysfunction is characterized by short stature, poor growth rate, normal serum GH response to provocative testing, and reduced IGF-I levels. The diagnosis is usually made by collecting continuous or frequent serum samples over 12 to 24 hours time period (55,56). The serum GH levels are measured to low according to an arbitrary set criteria (55,56). The children with a history of prior cranial irradiation were included in the category of neurosecretory dysfunction. Previously the etiology of ISS was speculated to be caused by the GH neurosecretory dysfunction in the hypothalamic-pituitary axis. However, the studies concluded that patients with ISS appeared to have normal 24-hour GH production rates (57,58). The cost and difficulty in performing this test, does not support the measurement of spontaneous GH as a routine test in short children.

Given that approximately 75% of children identified as ISS have apparently normal serum concentrations of IGFs, which can apparently be IGF insensitivity, either at the receptor or at the postreceptor level. Only four patients are reported in the literature with identified defects in the IGF-IR; these cases give an insight in the molecular mechanisms of IGF-I resistance (21,22). The possible role of abnormalities of IGFBPs in patients with IGF-I resistance has been a subject of speculation but no molecular defect of genes for the IGFBPs have been identified in the context of growth failure. Also our understanding of the physiological roles of bound and free IGF-I in stimulating skeletal growth remains incomplete at this time. The combination of GH and IGF resistance can result from a downstream defect, involving end organ unresponsiveness to IGF-I. Our understanding of proteins that are active at the epiphyseal growth plate and interact with growth factors, such as IGF-I, to mediate normal skeletal growth, is also incomplete.

DIAGNOSIS OF IDIOPATHIC SHORT STATURE

Clinical Diagnosis

ISS is diagnoses of short stature in all children with their height less than −2.25 SD [as defined by food and drug administration (FDA)] for age and sex and who have passed the provocative pharmacologic test. Children with an identifiable disease process, IUGR or psychiatric disorders are excluded from the clinical diagnoses of ISS (1). Other important parameters, such as serum IGF-1 and IGFBP-3 levels, which are helpful in the diagnosis of GHD or GHI are not well defined in ISS (59). IGF-I may be low or normal in ISS due to partial insensitivity to GH (13–15). Patients with a GHD, GHI, or ISS are difficult to distinguish based upon serum levels of GH or IGF-I as the biochemical profiles frequently overlap. The measurement of serum IGF-I levels can be helpful to characterize this subgroup of ISS. Depending on the serum IGF-I levels ISS can be classified into IGFD or IGF resistance (Table 3). The IGF-I generation test can thus be a useful tool to differentiate the various etiologies.

The first large study that provided normative IGF-I generation data enrolled 189 subjects with GHI, GHD, ISS, and normal subjects. These subjects were randomized to self-administration of either a

Table 3 Molecular Basis of Idiopathic Short Stature on the Basis of IGF-I Levels

IGF-I serum concentration	Etiologies
Low (IGF deficiency)	Polymorphisms of the IGF gene resulting in altered transcription and/or translational efficiency
	Abnormalities of IGF-binding proteins
	Mild abnormalities of the GH receptor
	Heterozygosity for mutations or deletions of the GH receptor gene
	Mild defects of post-GH receptor signaling (JAK/STAT system)
Normal or elevated (IGF resistance)	Bioinactive IGF-I, resulting from inactivating mutations of the IGF-I gene
	Abnormalities of IGF-binding proteins
	Mild abnormalities of the IGF receptor
	Mild defects of post-IGF receptor signaling
	End-organ resistance to IGF action at the epiphyseal growth plate

Abbreviations: GH, growth hormone; JAK, Janus-family tyrosine kinase; STAT, signal transducer and activator of transcription; IGF-I, insulin-like growth factor-I.

high (0.05 mg/kgd) or a low (0.025 mg/kgd) dose of GH for 7 d. After a two weeks washout period, they received the alternate dose. Fasting morning samples were collected on d 1, 5, and 8 of each treatment week. It concluded that the ISS patients had low-normal IGF-I levels that did not stimulate beyond the baseline normative ranges for age (37). The ISS subjects show some degree of GH insensitivity as they failed to raise their serum IGF-I concentrations in response to GH; many did not even attain levels within the baseline normal range. The clinical features of various defects described in ISS are summarized in Table 2 .

Undoubtedly there is bound to be an overlap of children with constitutional delay of growth and puberty, nutritional growth retardation as well as children with familial short stature. ISS can be similar to the presentation as short stature caused by nutritional deprivation. Nutritional status, energy and protein intake are critical regulators of IGF1 and IGFBP-3 synthesis. In a study serum IGF-1 and IGFBP-3 levels, alterations on body growth velocity and energy metabolism were measured in underfed rats to investigate an experimental model of nutritional dwarfing (ND). Decreased serum concentrations of IGF-1 and IGFBP-3 were observed with growth deceleration in ND rats (60). The effects of mild to moderate energy restriction can be reversed by the administration of rhGH (61). Nutritional growth retardation has been identified as a frequent cause of short stature and delayed sexual development in children with suboptimal nutrition. Altered behavior pattern in children can contribute to their short stature. In 214 children with ISS data on body mass index (BMI), leptin, and ghrelin was collected. The pattern of eating behavior was assessed by using the child eating behavior questionnaire, the percent energy intake as fat was assessed by using the Leeds food frequency questionnaire. The data was compared with normal population norms. The BMI was significantly lower (−0.33 SD), mean child eating behavior questionnaire score(1.9 vs. 2.4), reduced enjoyment of food (3.2 vs. 3.9), emotional under eating (2.6 vs. 3.0), lower desire to drink (2.0 vs. 2.8), and increased fussiness over food (3.2 vs. 2.9) were consistent with a decreased food responsiveness in patients with ISS (62).

Molecular Diagnosis

The heterozygous mutations in NPR2 gene are associated with short stature. The authors estimated that one in 30 individuals with ''idiopathic'' short stature could be carriers of NPR2 mutations (32). NPR2 gene is a potential gene that can be identified in patients with ISS. The mutations GHR gene account for up to 5% of all ISS patients (14,15). A recent study, investigating the impact of GHR polymorphisms, has suggested that short children with a deletion of exon 3 (d3-GHR), in either homozygous or heterozygous form, were more responsive to therapy with GH (63). Now patients have been identified with mutation in STAT5b, IGF-I gene deletion (28). IGFALS gene mutation was identified in two cases with what could be typical picture of ISS (20,23). Subtle defects in the IGF-1 gene can presumably present similarly with low-normal IGF-I levels. The molecular basis of other forms of IGFD deficiency should be looked into when investigating a patient with ISS. The molecular defects in ISS patients with normal or increased IGF-I levels are beginning to be understood. The patients with mutations in the IGF-I receptor (IGF-IR) have elevated IGF-I levels (21,22). These patients with IGF-IR mutations represent the phenomenon of IGF-I resistance in humans. The IGF-I produced can be bioinactive IGF-1, yet detectable with immunoassays making the diagnosis of ISS more complex (29). Thus molecular studies of the IGF-I and the IGF-IR gene can also be helpful to find the exact etiology of ISS. Mutations in the SHOX containing gene also have been described in up to 1% to 2% of individuals classified as having ISS (30,31,44,45). SHOX gene analysis is commercially available and can certainly be carried out in suspected cases of ISS (Fig. 3; Table 3).

RATIONALE AND MANAGEMENT OF IDIOPATHIC SHORT STATURE

Growth Hormone Treatment and Response

In 1985, the FDA of the United States approved the use of rhGH in children with GHD. However, over the last 5 to 10 years, non-GH deficient states Turner syndrome, Prader–Willi syndrome, small for gestation age infants who fail to experience catch up growth, and growth failure associated with chronic renal failure are now eligible for treatment in the United States (Vol. 2; Chap. 5). In a paper by Wit et al. the natural

growth of children with ISS were studied until adulthood (64). Many children's height SD scores improved spontaneously (−2.1 to −3.1 SD during childhood and −7 to −2.7 SD at adulthood). This is most certainly due at least in part to the fact that there were children included had constitutional delay of growth and puberty. So looking into the affects of GH treatment in patients with ISS can be misleading because ISS is a heterogeneous group. Patients with partial GH insensitivity and IGF-1 resistance could have been included into category of ISS patients. In 2003 the FDA approved the use of rhGH to treat children with ISS. There have been a number of studies that have demonstrated short term increases in height but final adult height data is still lacking. A recent meta-analysis demonstrated a net gain of approximately 4 to 6 cm after a mean of 5.3 years of treatment (65). In a paper by Leschek et al. reporting on the results of a randomized, double blinded placebo controlled trial in peripubertal children treated with GH for a mean treatment duration of 4.4 years the mean SD change was 0.51 or about 3.7 cm in the treated versus the placebo (66). This contrasts with a paper published by Wit in the Netherlands where the height SD scores increased by 1.52 to 1.85 in the treated versus the placebo arm. The differences between the two trials included a younger age population and a longer duration of treatment in the Netherlands study. In the Leschek study injections were administered three times weekly versus daily injections in the Netherlands (66). A weekly dosage of up to 0.37 mg/kg of body weight administered by subcutaneous injection is recommended. It should be divided into equal doses given six to seven times per week (FDA package insert). Acceleration of pubertal onset and bone maturation by approximately one year was reported when children (mean eight years) with ISS were treated with high-dose of 0.5 mg/kg/day (67). Although dose dependent improvements in growth have been demonstrated in rhGH treated children with ISS, no real benefits have been demonstrated in the psychological well being of these children. There seems to be no serious adverse effects in both the short term and the long term.

Gonadotropin Releasing Hormone Agonists

Gonadotropin releasing hormone (GnRH) agonists have been used to some degree in children with ISS; the goal is to prolong growth by delaying the epiphyseal closure in the bones. In one study, 50 short adolescents with low predicted adult height were randomized to receive either placebo or luteinizing hormone–releasing hormone (LHRH) agonist. Final adult height was measured when bone age exceeded 16 years in girls and 17 years in boys and when the rate of growth was less than 1.5 cm/yr. An increment in height of the LHRH agonist treated group (mean of 3.5 years) was 0.6 SD (4.2 cm) gain significantly greater adult height than the placebo group. Treatment, however, also resulted in decrease in the L2 to L4 vertebral bone mineral density (68). A randomized controlled trial of GH and GnRH agonists treatment for three years in children with ISS. The height SDS, bone age, and predicted adult height increased significantly after treatment with GnRH agonists (69). Final data on height, bone mineral density, cost-effectiveness, and potential adverse effects of combined GnRH agonist–GH therapy in this study is not yet available.

Aromatase Inhibitors

The P450-aromatase inhibitors block the conversion of estrogen from testosterone and can potentially delay maturation of the growth plates and ultimately result in increased adult height. A randomized, double-blinded, placebo-controlled study was conducted testing this hypothesis in which boys with constitutional delay of puberty were treated with testosterone and placebo, or testosterone and letrozole (P450-aromatase inhibitor). Administration of testosterone and letrozole, delayed bone maturation, and increased predicted adult height by 5.1 cm over an 18 months period as compared with controls treated with testosterone alone (70).

Therapeutic Indications and Potential Use of Recombinant Human Insulin-Like Growth Factor-I

Recombinant human insulin-like growth factor-I (rhIGF-I) therapy was tried in the patient mentioned in the text with IGF-I gene deletion (28). At the time of initiation of the rhIGF-I treatment he was in puberty. He was treated with rhIGF-I at a dose of 40 to 80 μg/kg/day and his growth rate increased from 3.8 to 7.3 cm/yr with normalization of IGFBP-3, IGFBP-1 levels post treatment (71). RhIGF-I therapy also suppressed elevated GH secretion and dramatically improved insulin sensitivity (72). RhIGF-I therapy promoted linear growth and normalized insulin sensitivity in this patient.

RhIGF-I was administered to 27 patients (14 female, 13 male, and three pubertal) with GH receptor deficiency (Laron syndrome) dosed between 40 and 120 mg/kg body weight twice a day for up to 12 months. The growth rate increased by more than 2 cm/yr as compared with that compared to before treatment (except in all two oldest patients). Asymptomatic hypoglycemia was recorded in 10 patients whereas four experienced symptomatic hypoglycemia. A transient asymptomatic decrease in serum potassium occurred in most patients after injections (73). Other adverse effects of RhIGF-I, which have been reported lipohypertrophy at the injection site, papilledema, benign intra-cranial hypertension, facial nerve paralysis, increase in fat mass and BMI, growth of lymphoid tissue, and development of acromegalic features (74). Most recently the identification of IGFD has allowed endocrinologists to pursue RhIGF-I replacement/treatment of children with ISS. Preliminary data on

the success of a phase 3 randomized, open-label, observation-controlled, multicenter, and parallel-dose comparison trial has not been completed at the time of this chapter's submission. ISS patients are a heterogeneous group; it represents the other end of the spectrum of GH insensitivity. The data from the use of rhIGF-I in growth hormone insensitivity syndrome (GHIS) can set some underlying guidelines to treat ISS.

Therapeutic Indications and Potential Use of Recombinant Human Insulin-Like Growth Factor-I/ Recombinant Human Insulin-Like Growth Factor Binding Protein-3

Recently published study investigated the effect of a newly developed drug rhIGF-I/rhIGFBP-3, a 1:1 molar complex of rhIGF-I and rhIGFBP-3 in adolescents with GHIS. RhIGF-I/rhIGFBP-3 was administered in a single sc. Injection at 0.5 and 1.0 mg/kg/dose (equivalent to 100 mg/kg and 200 mg/kg of rhIGF I) after breakfast with a two days interval between doses. Circulating total and free IGF-I levels increased into the normal range for a 24-hour period after a single sc administration. There were no acute adverse events reported and all blood glucose measurements were normal. The data for this study demonstrates that rhIGF-I/rhIGFBP-3 complex was effective, safe, and well tolerated. The use of rhIGF-I/rhIGFBP-3 therapy can potentially be beneficial especially in the IGFD subgroup of ISS (75).

PSYCHOSOCIAL AND ETHICAL CONSIDERATIONS

Reports of the success and safety utilizing GH for growth promoting therapy in the popular media has brought the idea of treating ''height'' into the living room and bedrooms of most western societies. This in part has shaped expectations and biases in the general culture about height. It is important to appreciate that children with short stature have been confronted with a number of psychological challenges including juvenilization, teasing and bullying, exclusion from peer activities, loss of independence or overprotection from parents, and sometimes siblings. The heartfelt concerns commonly expressed by parents and children alike should be taken into consideration and not be ignored (12,76–78), even in adulthood challenges may persist. Social isolation and reduced marriage rates seem to occur more commonly amongst short statured individuals, and perceptions by others of lower competence in those who are short than those of average stature. In addition, there are height limits for certain jobs, such as those in the aviation industry, military, law-enforcement as well as firefighters, and various other jobs that require a certain physical size to operate some machinery and tools. In addition, short stature may have significant impact on activities of daily living, such as the ability to ride a bicycle, or to safely drive an average-sized car especially in the era of air bags, and physical challenges in the home and workplace, such as reaching standard kitchen cabinets, shelves, or working comfortably with a standard-sized desk and chair. The National Insurance Institute for highway safety has cautioned that short adults may be at risk for serious injury from air bags as they deploy (79,80). Parental projection of their perception of their short child's vulnerability must also be appreciated and appropriate counseling needs to be offered. A paper from a joint Swedish and British research team reported a strong inverse association between height and suicide. They found that a 5 cm increase in height was associated with a 9% decrease in suicide risk (81). Despite the psychological data suggesting the potential difficulties experienced by short individuals, there is no guarantee that any short individual will be adversely affected by any of the above and that treatment will make any meaningful difference in any parameter except for height SD score. It is important to remember the rubric that short stature is not a disease but rather a symptom. Therefore, one shouldn't rush to treat simply based on the absolute height of a child without at least considering the potential molecular causes and how it impacts on this particular child's life now and in the future.

FUTURE DIRECTIONS

The pediatric endocrinologist has relatively poor tools to tease apart the etiology of growth failure in the majority of the cases. We are beginning to understand the molecular defects as described in this chapter provide a foundation for the reclassification of ISS. Although each of these, in its classic form, appears rare, with only one or two cases of each reported thus far, each defect serves to underscore the critical role of IGF-I in both intrauterine and postnatal growth. The major clinical and biochemical features of each condition are summarized in Table 2.

It seems reasonable to speculate that the identified cases of ISS represent the tip of the iceberg, and that our understanding of less severe cases will expand over ensuing years. In addition to the established molecular defects described above, future areas of investigation for molecular etiologies might include defects of JAK2, IGFBP-3, and various proteases and phosphatases that might impact GH signal transduction. As studies suggest that as many as 25% to 50% of children labeled as ''ISS'' have reduced serum concentrations of IGF-I in the face of normal or elevated GH, such cases will warrant careful biochemical and molecular investigations, in an effort to identify more subtle defects of the GHR, GH signaling cascade, or IGF-I gene expression.

In the absence of clear ability to define the exact nature of an individual's cause of growth failure it is necessary to adopt an approach that ensures an ethical and defensible position. Reliance on GH stimulation testing alone to discriminate between GHD and ISS is no longer adequate and must be

challenged by all reasonable thinkers. Until such time that we have widespread access to molecular techniques that may highlight a case of abnormality in the STAT system, we must treat growth failure equally. Therefore objective criteria for growth failure should be established regardless of the etiology and treatment needs to be offered equally. We must not approach the issue of short stature with the provision that if we can name it we should treat it (as in Turner syndrome) and if we cannot name it, that child is ineligible for treatment even though growth failure may be even more severe. Also one must not be complacent when a GH stimulation test reveals a ''pass'' rather it should stimulate the investigator to do a thorough search for other abnormalities that may exist in the GH-IGF axis. Finally, IGF-I can be considered in those children who fail a trial of rhGH therapy or who fail an IGF-I generation test. It is safe to say, that in the absence of molecular tools to diagnose the etiology of growth failure in most cases often remains obscure. Indeed, the greatest challenge facing growth research over the next decade will be the elucidation of the molecular basis of ISS.

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3

Hypopituitarism and Other Disorders of the Growth Hormone–Insulin-Like Growth Factor-I Axis

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INTRODUCTION

Disorders involving the growth hormone (GH) pathway that result in insulin-like growth factor-I (IGF-I) deficiency may be congenital or acquired. Congenital GH deficiency (GHD) is associated with structural malformations of the central nervous system, hypothalamus, or pituitary. IGF-I deficiency/resistance may result from genetic defects involving critical factors in the embryologic development of the pituitary or in the cascade from hypothalamic stimulation of GH release to completion of IGF effects on growth. Acquired abnormalities affecting the GH/IGF axis range from damage to the hypothalamic–pituitary region from trauma, tumors, infection, autoimmune disease, or radiation, to a broad spectrum of chronic conditions characterized by catabolism.

The frequency of GHD has been estimated in various studies to range from 1:4000 to 1:10,000 (1). Estimates based on clinic referral populations are inherently biased. A population-based study of 80,000 schoolchildren in Salt Lake City documented growth rates over one year. Of the 555 children who were below the third percentile in height and had growth rates less than 5 cm/year, 16 had previously undiagnosed GHD. Combined with known instances in the population of children being treated for GHD, this gave a prevalence estimate of 1:3500 in the school-age population (2) An estimate of relative contributions of organic and idiopathic or genetic causes can be gleaned from the large post-marketing databases of manufacturers of recombinant human growth hormone (rhGH). Among more than 20,000 children being treated with rhGH registered in the National Cooperative Growth Study, approximately 25% of those with proven GHD had an organic etiology. Nearly half of these (47%) were central nervous system (CNS) tumors, including craniopharyngioma, 15% were CNS malformations, 14% septo-optic dysplasia (SOD), 9% leukemia, 9% CNS radiation, 3% trauma, 2% histiocytosis, and 1% CNS infection (3). Comparable findings were obtained in the European post-marketing surveillance study (Kabi International Growth Study, KIGS). Twenty-two percent of approximately 15,500 children with GHD had an organic cause, congenital in 24% of this subgroup. The most common central malformation was empty sella, accounting for 37%, followed by SOD in 24% of those with congenital organic GHD. Among the 76% of organic GHD considered acquired, craniopharyngioma accounted for 24% and other CNS tumors 30%, leukemia 16%, histiocytosis 3.5%, trauma 3%, and CNS infection 1% (4).

PITUITARY GLAND

Embryology of the Pituitary Gland

The pituitary appears early in embryonic life in response to an orchestration of transcription factors appearing and disappearing in precise sequence. At three weeks gestation, the ectodermal stomodeum of the embryo develops an outpouching anterior to the buccopharyngeal membrane. This outpocketing is Rathke's pouch, which usually separates from the oral cavity and will give rise to the adenohypophysis (anterior lobe) of the pituitary gland. An evagination of the diencephalon then gives rise to the neurohypophysis of the pituitary gland. Rarely, the primitive oral cavity origin of the pituitary results in a functional pharyngeal adenohypophysis (5). The fetal pituitary gland consists of the pars distalis (anterior lobe), pars nervosa (posterior lobe), and the pars intermedia (6). Secretion of pituitary hormones can be detected as early as week 12 in the fetus, and some of these hormones are found within the pituitary by eight weeks gestation (7). Average newborn pituitary weight is 100 mg.

Differentiation of the primordial pituitary gland requires a cascade of factors to be expressed in critical temporal and spatial relationships. These include

extracellular signaling factors from the adjacent diencephalon that initiate anterior pituitary gland development from the oral ectoderm, and transcription factors that control pituitary cell differentiation and specification. Several homeodomain transcription factors directing embryologic development of the anterior pituitary have been found to have mutations that result in congenital defects affecting the synthesis of GH and additional pituitary hormones (8,9). The human mutations that cause isolated growth hormone deficiency (IGHD) or multiple pituitary hormone deficiency (MPHD) and the associated features are summarized in Table 1.

The homeobox gene expressed in embryonic stem cells (HESX1) gene is important in development of the optic nerve, as well as of the anterior pituitary. HESX1 inhibits PROphet of Pit1 (PROP1) -mediated gene effects and mediates forebrain development (10). HESX1 has also been referred to as the Rpx or Rathke's pouch homeobox gene. Some mutations that have been described account for a small subset of the cases of SOD with variable GH and other pituitary deficiencies (11).

PITX2 is a paired-like homeobox gene expressed in the fetal pituitary and adult gland, thought to be required for pituitary development shortly after formation of the committed Rathke's pouch. There have been at least eight mutations in PITX2 resulting in Rieger syndrome, which includes anomalies of the anterior chamber of the eye, dental hypoplasia, protuberant umbilicus, and mental retardation, but it is uncertain whether pituitary hormone deficiencies are associated (12).

LHX3 accumulates in the Rathke pouch and the primordium of the pituitary and is thought to be involved in the establishment and maintenance of the differentiated cell types (13). Mutations of this transcription factor result in deficiencies of all pituitary hormones except adrenocorticotropin (ACTH), and cervical spine rigidity indicating extrapituitary function for this factor in some families (14). LHX3 and LHX4 belong to the LIM family of homeobox genes expressed early in Rathke's pouch, with expression persisting into adulthood. This has suggested a maintenance function for anterior pituitary cells. Four patients in two unrelated families have been identified with LHX3 mutations with a hormonal phenotype similar to PROP1 deficiency (Table 1), including marked pituitary enlargement in one patient (14). There has been only one report of a mutation within LHX4 (15).

The sonic hedgehog signaling pathway, mediated by three GLI genes, has been identified in a variety of tissues and has been implicated in complex disorders of pituitary development. Mutations of GLI2 are associated with holoprosencephaly (16). Penetrance is variable, with all affected patients having pituitary gland dysfunction.

X-linked hypopituitarism results from duplications of Xq26–27, a region that includes the SOX3 gene, for which a polyalanine expansion has been described in a pedigree with X-linked mental retardation and GHD (17–19). Mutations that result in either overdosage or underdosage of SOX3 are associated with infundibular hypoplasia and variable hypopituitarism (20).

PROP1 represses HESX1 expression at the appropriate time and is required for initial determination of pituitary cell lineages, including gonadotropes and those of PIT1 [GH, thyrotropin (TSH), prolactin (PRL)]. Ten recessive mutations have been described in PROP1 that result in GH, PRL, TSH, gonadotropin and, as the patient's age, ACTH deficiency (21,22). Patients with PROP1 gene mutations may have pituitary gland enlargement originating from the intermediate lobe (23). Eleven recessive and four dominant mutations have been reported affecting the Pit1 gene (currently referred to as POU1F1), with resultant GH, PRL, and TSH deficiency (9,21).

Table 1 Mutations Resulting in IGHD or MPHD

Gene	Hormone deficiency	Pituitary anatomy	Other abnormalities	Inheritance
HESX1	IGHD to MPHD	AP hypoplasia, ectopic PP, absent infundibulum	SOD; absent corpus callosum	Recessive; dominant
LHX3	GH, TSH, LH, FSH, PRL	Small, normal, or enlarged AP	Short neck and cervical spine with limited rotation in some families	Recessive
LHX4	GH, TSH, ACTH	Small AP, ectopic PP	Cerebellar abnormalities	Dominant
SOX3	IGHD to MPHD	AP hypoplasia, ectopic PP, absent infundibulum	Mental retardation	X-linked
GLI2	MPHD	AP hypoplasia	Holoprosencephaly; multiple midline defects	Dominant
PITX2	Unknown in human	(Hypoplasia and MPHD in mice)	Rieger syndrome (see text)	Dominant
PROP1	GH, PRL, TSH, LH, FSH, ±ACTH	Small, normal, or enlarged AP	—	Recessive
PIT1 (POU1F1)	GH, PRL, TSH	Normal or hypoplastic AP	—	Recessive, dominant
GHRH receptor	IGHD	Hypoplastic AP	Small head size in one population	Recessive
GH1	IGHD; some mutations with MPHD	Hypoplastic or normal AP	Most IGHD-IA develop antibodies with GH treatment; agammaglobulinemia in some IGHD III	Recessive, dominant, X-linked

Abbreviations: ACTH, adrenocorticotropin; AP, anterior pituitary; FSH, follicle-stimulating hormone; GH, growth hormone; IGHD, isolated growth hormone deficiency; LH, luteinizing hormone; MPHD, multiple pituitary hormone deficiency; PP, posterior pituitary; SOD, septo-optic dysplasia; TSH, thyrotropin; PRL, prolactin; IGHD-IA, gene deletion with complete absence of GH.

POU1F1 gene defects are associated with variable pituitary hypoplasia (24).

Somatotroph development is also dependent on hypothalamic growth hormone -releasing hormone (GHRH). Mutation in the gene encoding the GHRH receptor results in severe GHD (25–28).

Functional Anatomy of the Pituitary Gland

The adult pituitary weighs 0.5 g and has an average dimension of $10 \times 13 \times 6$ mm. The pars intermedia is vestigial in the adult, except in pregnancy. The adenohypophysis receives hormonal modulating signals from the hypothalamus, transmitted from ventromedial and infundibular nuclei axons which terminate in the hypophyseal portal system. These signals result in production of ACTH by eight weeks gestation, TSH by 15 weeks gestation, GH by 10 to 11 weeks gestation, prolactin by 12 weeks, and the gonadotropes, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), by 11 weeks in specialized cells of the pars distalis. The pars distalis has at least three distinct hormone-producing cell populations classified by staining characteristics (29). Fifty percent of the cells are chromophobes, 40% are characterized as acidophils, and the remainder as basophils. Acidophils secrete GH or prolactin. Basophils secrete TSH, LH, FSH, or ACTH. Some basophils have a positive periodic acid-Schiff base reaction: these are the cells which secrete the glycoproteins LH, FSH, or TSH. While chromophobe cells are known to produce ACTH in the rat pituitary, the role of these cells in the human pituitary remains unclear.

Anterior pituitary hormones enter the portal venous system to drain into the cavernous sinus, enter the general circulation, and ultimately exert long-distance influence over their respective target organs. PRL has an effect on lactation through direct effects upon breast ductal tissue. ACTH stimulates the adrenal cortical production of cortisol and affects renal reabsorption of water. TSH promotes growth of the thyroid and production of thyroxine. LH and FSH stimulate gonadal maturation and hormonal cycling. GH exerts indirect growth effects through the elaboration of IGF-I in the liver and epiphyses, and direct metabolic effects primarily in adipose tissue. Although the pars intermedia had been identified as a site of possible melanocyte-stimulating hormone (MSH) production, more recent studies suggest that MSH is actually being produced in the pars distalis and enters the portal venous system to exert a distant effect on skin pigmentation (30).

The pars nervosa, or infundibular process, and the infundibulum, or neural stalk, comprise the neurohypophysis. The infundibulum consists of the pituitary stalk and median eminence and is the direct connection to the hypothalamus. The neurohypophysis receives, stores, and releases two important hormones produced in the hypothalamus. These hormones, oxytocin and arginine vasopressin, originate in the supraoptic and paraventricular nuclei of the hypothalamus. The 100,000 axons of these nuclei are unmyelinated and form the supraopticohypophyseal tract, which transports oxytocin and vasopressin to be stored in the posterior lobe of the pituitary (7,31).

The rich blood supply of the pituitary gland is subject to interruption during periods of severe hypotensive stress and hypoxia, resulting in the Sheehan syndrome of hypopituitarism, classically described after intrapartum hypotension, but possible in any hypovolemic crisis or increased intracranial pressure episode, as in hypopituitarism following recovery from cerebral edema complicating diabetic ketoacidosis (32). The internal carotid arteries supply the vascular branches, which bathe the pituitary. The right and left superior hypophyseal arteries, which branch into anterior and posterior divisions, supply the median eminence and infundibulum. The neurohypophysis and stalk are supplied by the right and left inferior hypophyseal arteries. The hypophyseal portal vessels, which originate from capillary beds in the median eminence and infundibular stem, supply the pars distalis (31,33).

GH-IGH-I AXIS

Biochemistry and Physiology of the GH–IGF-I–IGF-Binding Protein Axis

Growth Hormone

Human GH is a single-chain, 191 amino acid, 22-kDa protein, containing two intramolecular disulfide bonds (34). Release of GH from the anterior pituitary somatotrophs is controlled by the balance between stimulatory GHRH and inhibitory somatostatin (SS) from the hypothalamus (Fig. 1). This balance is regulated by neurologic, metabolic, and hormonal influences; numerous neurotransmitters and neuropeptides are involved. These include vasopressin, ACTH-releasing hormone, TSH-releasing hormone, neuropeptide Y, dopamine, serotonin, histamine, norepinephrine, and acetylcholine, which respond to various circumstances that affect GH secretion such as sleep, nutritional state, stress, and exercise. Other hormones including glucocorticoids, sex steroids, and thyroxin also influence secretion of GH. These various influences are important in the evaluation of GH secretion, which may be abnormal despite normal somatotroph function. Stimulation of GH release by GHRH is via specific GHRH receptors.

A number of synthetic hexapeptides, referred to as growth hormone-releasing peptides (GHRPs), have been developed that act on other receptors to stimulate GH release (36,37). The naturally occurring ligand for the GHRP receptor, ghrelin, has been isolated and cloned (38). Ghrelin is unique among mammalian peptides in its requirement of a post-translational modification for activation. This involves addition of a straight chain octanyl group conferring a hydrophobic property to the N terminus, which may permit entry of the molecule into the brain. Similarly to synthetic GHRPs, ghrelin binds with high affinity and specificity to a distinct G protein coupled

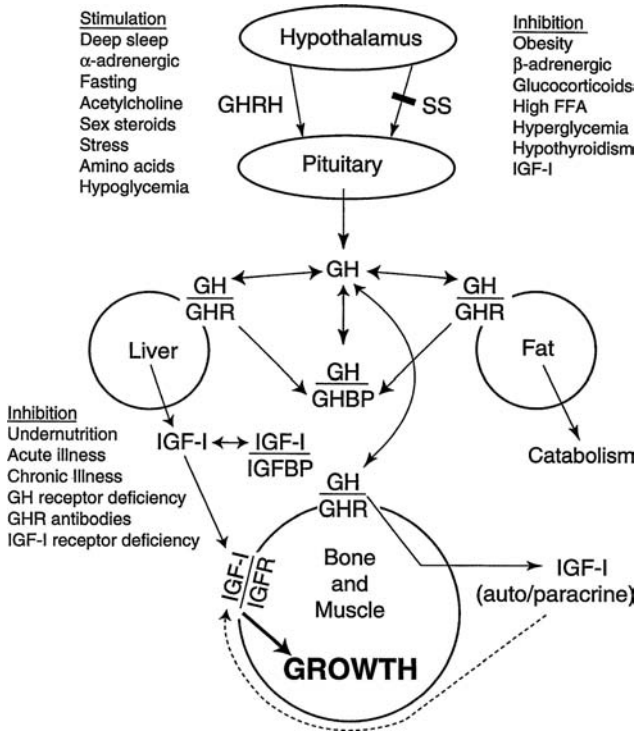

Figure 1 Simplified diagram of the growth hormone (GH)–insulin-like growth factor-I (IGF-I) axis involving hypophysiotropic hormones controlling pituitary GH release, circulating growth hormone-binding protein (GHBP) and its GH receptor source, IGF-I and its largely GH-dependent binding proteins, and cellular responsiveness to GH and IGF-I interacting with their specific receptors. *Source*: Adapted from Ref. 35.

receptor (39). Unlike GHRH, ghrelin is synthesized primarily in the fundus of the stomach (38), as well as in the hypothalamus (40), heart, lung, and adipose tissue and its receptor is more widely distributed than that of GHRH. Ghrelin has widespread metabolic effects in addition to inducing GHRH release and being synergistic with GHRH in the stimulation of GH release through the serine 3 residue of ghrelin. Ghrelin increases PRL, ACTH, cortisol, and aldosterone release and increases food intake and weight gain. The hunger-related effects of ghrelin appear to be mediated through receptors on arcuate neuropeptide Y neurons (41).

Some 75% of the circulating GH is in the 22-kDa form. Alternative splicing of codon 2 results in a deletion of 11 amino acids and formation of a 20-kDa fragment accounting for 5% to 10% of secreted GH. Other circulating forms include deamidated, *N*-acetylated, and oligomeric GH.

About 50% of GH circulates in the free state, the rest bound principally to GHBP. Because the binding sites for the radioimmunoassay of GH are not affected by the GHBP, both bound and unbound GH are measured (42).

Growth Hormone-Binding Protein

A high affinity GHBP was identified in rabbit and human serum in the mid-1980s (43), and separate reports in 1987 found this binding protein to be absent in the sera of patients with GH resistance (44,45), who were identified by high circulating GH concentration with a phenotype of severe GHD. The recognition that circulating GHBP in rabbit serum corresponded to liver cytosolic GHBP was followed by the purification, cloning, and sequencing of human GHBP (46). The human GHBP was found to be structurally identical to the extracellular hormone-binding domain of the membrane bound growth hormone receptor (GHR). The entire human GHR gene, localized to the proximal short arm of chromosome 5, was subsequently characterized (47). The GHR was the first to be cloned of a family of receptors that includes the receptor for prolactin and numerous cytokine receptors. Members of this family share ligand and receptor structure similarities, in particular the requirement that the ligand bind to two or more receptors or receptor subunits and interact with signal transducer proteins to activate tyrosine kinases (48).

In humans, GHBP is the proteolytic product of the extracellular domain of the GHR. This characteristic permits assaying circulating GHBP as a measure of cellular bound GHR, which usually correlates with GHR function. The GH molecule binds to cell surface GHR, which dimerizes with another GHR so that a single GH molecule is enveloped by two GHR molecules (49). The intact receptor lacks tyrosine kinase activity, but is closely associated with JAK2, a member of the Janus kinase family. JAK2 is activated by binding of GH with the GHR dimer, which results in self phosphorylation of the JAK2 and a cascade of phosphorylation of cellular proteins. Included in this cascade are signal transducers and activators of transcription, which couple ligand binding to the activation of gene expression, and mitogen-activated protein kinases. Other effector proteins have also been examined in various systems. This is a mechanism typical of the GH/PRL/cytokine receptor family (48,50).

The GHR in humans is also synthesized in a truncated form (GHRtr) lacking most of the intracellular domain. Although the quantity of this GHRtr is small relative to the full-length GHR, release of GHBP from this isoform is increased (51). Some of the changes in body composition that occur with GH treatment in GHD may be related to changes in the relative expression of GHR and GHRtr (52).

Insulin-like Growth Factor-I

Most of the growth effect that gives GH its name is actually an effect of IGF-I production in the liver and growing bone (53). IGF-I is a 70 residue single-chain basic peptide and IGF-II a slightly acidic 67 residue peptide. Their structure is similar to proinsulin, A

and B chains connected by disulfide bonds and a connecting C-peptide, but unlike the post-translational processing of insulin, there is no cleavage of the C-peptide. The two IGFs share approximately two-thirds of their possible amino acid positions and are 50% homologous to insulin (54,55). The connecting C-peptide is 12 amino acids long in the IGF-I molecule and eight amino acids long in IGF-II, and has no homology with the comparable region in the proinsulin molecule. The IGFs also differ from proinsulin in having carboxy terminal extensions. These similarities and differences from insulin explain the ability of IGFs to bind to the insulin receptor and insulin's ability to bind to the type 1 IGF receptor, as well as the specificity of IGF binding to the IGF-binding proteins (IGFBPs).

Insulin-like Growth Factor Binding Proteins

Hepatic IGF-I circulates almost entirely bound to IGFBPs, with less than 1% being free. The IGFBPs are a family of six structurally related proteins with a high affinity for binding IGF (Fig. 2). At least four other related proteins with lower affinity for IGF peptides have been identified and are referred to as IGFBP-related proteins (56). The principal BP, IGFBP-3, binds 75% to 90% of circulating IGF-I in a large (150–200 kD) ternary complex consisting of IGFBP-3, an acid labile subunit (ALS), and the IGF molecule. ALS and IGFBP-3 are produced in the liver as a direct effect of GH. The ALS stabilizes the IGF–IGFBP-3 complex, reduces the passage of IGF-I to the extravascular compartment, and extends its half-life (57). The remainder of bound IGF is in a 50 kD complex with mostly IGFBP-1 and IGFBP-2. IGFBP-1 concentrations are controlled by nutritional status as reflected in insulin levels, with the highest IGFBP-1 concentrations found in the fasting, hypoinsulinemic state. The circulating concentration of IGFBP-2 is less fluctuant and is partly under the control of IGF-I; levels are increased in IGF-I deficiency due to growth hormone insensitivity (GHI), but increase further with IGF-I therapy of such patients (58).

The actions of IGFBPs are under intense investigation (53,59,60). The IGFBPs modulate IGF action by controlling storage and release of IGF-I in the circulation and influencing its binding to its receptor, facilitate storage of IGFs in extracellular matrices, and exert independent actions. IGFBPs 1, 2, 4, and 6 inhibit IGF action by preventing binding of IGF-I with its specific receptor. The binding of IGFBP-3 to cell surfaces is thought to decrease its affinity, effectively delivering the IGF-I to the type 1 IGF receptor. IGFBP-5 potentiates the effects of IGF-I in a variety of cells. Its binding to extracellular matrix proteins allows fixation of IGFs and enhances binding to hydroxyapatite. IGFs stored in such a manner in soft tissue may enhance wound healing. IGF independent mechanisms for IGFBP-1 and IGFBP-3 proliferative effects have been demonstrated in vitro and nuclear localization of IGFBP-3 has been reported. In addition to IGFBP phosphorylation and cell surface association determining the influence of IGFBPs, specific protease activity, particularly affecting IGFBP-3, is also important in the modulation of IGF action in target tissues.

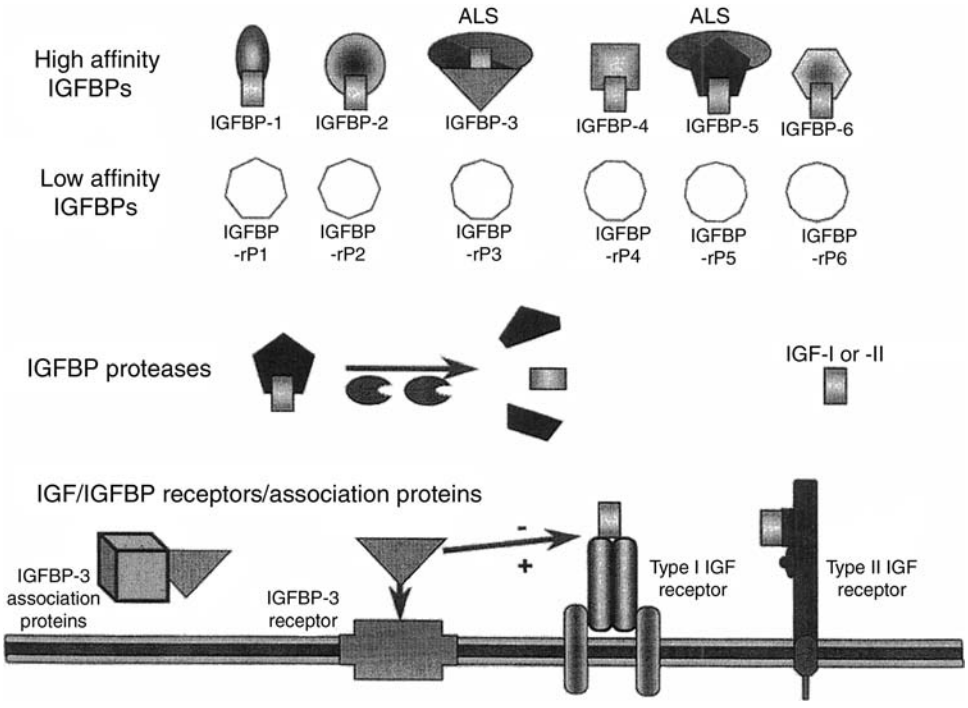

Figure 2 Components of the IGF–IGFBP–IGF/IGFBP receptor axis. *Abbreviations*: IGF, insulin-like growth factor; IGFBP, IGF-binding proteins. *Source*: Adapted from Ref. 59.

The proteolytic activity may alter the affinity of the binding protein for IGF-I, resulting in release of free IGF-I for binding to the IGF-I receptor (53,59,60).

Insulin-like Growth Factor Receptors

IGF binding involves three types of receptors, the structurally homologous insulin receptor and type 1 IGF receptor and the distinctive type 2 IGF-II/mannose-6-phosphate receptor (Fig. 2). Splice variants and atypical forms occur but have not been found to have physiologic significance, insulin/IGF-I hybrid receptors, however, are ubiquitous and may be the most important receptor for IGF-I in some tissues (59).

The type 1 IGF-I receptor and insulin receptor are heterotetramers consisting of two alpha subunits which contain the binding sites and two beta subunits containing a transmembrane domain, an ATP-binding site, and a tyrosine kinase domain comprising the signal transduction system (59). The IGF-I receptor is able to bind IGF-I and IGF-II with high affinity but the affinity for insulin is approximately 100-fold less. Although the insulin receptor has a low affinity for IGF-I, IGF-I is present in the circulation at molar concentrations that are 1000 times those of insulin. Thus, even a small insulin-like effect of IGF-I could be more important than that of insulin itself, were it not for the IGFBPs that control the availability and activity of IGF-I. In fact, intravenous infusion of recombinant human insulin-like growth factor-I (rhIGF-I) can induce hypoglycemia, especially in the IGFBP-3 deficient state (61). It is not known why IGF-II and M6P share a receptor. This receptor differs from the type 1 receptor in binding only IGF-II with high affinity, IGF-I with low affinity, and insulin not at all (59).

The Role of IGF-I in Growth

The importance of IGF-I in normal intrauterine growth in humans has been demonstrated in a single patient with a homozygous partial deletion of the IGF-I gene (62), a patient with mutation of the IGF-I gene resulting in high circulating levels of an ineffective IGF-I (63), and in two patients with mutations of the IGF-I receptor (64), all having severe intrauterine growth retardation. Cord serum IGF-I and IGF-II concentrations correlate with birthweight and are significantly increased in large for gestational age infants compared with appropriate for gestational age newborns (65). Intrauterine IGF-I synthesis, however, does not appear to be GH dependent, because most patients with genetically determined severe IGF-I deficiency, due to GHRH defects, growth hormone receptor deficiency (GHRD)/Laron syndrome, or GH gene mutations, have normal or only minimally reduced intrauterine growth. Standard deviations (SDs) for length decline rapidly after birth, however, in these conditions, demonstrating the immediate need for GH stimulated IGF-I synthesis for postnatal growth (35). Growth velocity in the absence of GH is typically half normal (35), but has been reported to be normal or supranormal despite absence of GH in some hypothalamic conditions (66). This apparent growth without GH has been described in patients after craniopharyngioma resection, in SOD, in obese children with GHD, in GH deficient infants, and in patients who had undergone resection of a variety of CNS tumors (67). Normal or supranormal growth velocity has been attributed to hyperinsulinemia, increased leptin levels, or hyperprolactinemia (67). PRL levels are not consistently increased, however. Obesity or rapid weight gain is a common denominator among these patients, who demonstrate low GH levels to provocative stimuli, low IGF-1 and IGFBP-1, and low IGFBP-3 levels. A similar phenomenon was described in a placebo-controlled study of rhIGF-I treatment of severely IGF-I deficient patients with GHRD/Laron syndrome, in which three placebo-treated subjects grew over the six-month study period at an accelerated rate comparable to that of rhIGF-I-treated subjects, attributed to improved nutrition (61). Sex hormones can also influence epiphyseal bone growth in the child entering spontaneous or induced puberty.

The metabolic and growth effects of GH and IGF-I are compared in Table 2. In addition to direct protein sparing effects and synthesis and release of IGF-I from the liver, GH stimulates autocrine and paracrine production of IGF-I in other tissues, primarily bone and muscle (Fig. 1). GH has a direct effect on the differentiation of prechondrocytes into early chondrocytes, which in turn secrete IGF-I. This local IGF-I stimulates clonal expansion and maturation of the chondrocytes, resulting in growth (68). It is estimated that 20% of normal growth is the result of the direct effect of GH on maturing bone and the autocrine/paracrine production of IGF-I in this tissue (69). Treatment studies of children with GHRD compared to GHD patients support this hypothesis (70,71). IGF-II is considered an important growth factor in utero, but its role in extra uterine life is unclear; concentrations of IGF-II in serum parallel those of IGF-I.

CLASSIFICATION OF DISORDERS INVOLVING THE GH–IGF-I AXIS

The approval by the US Food and Drug Administration of rhIGF-I and a complex of rhIGF-I and rhIGFBP-3 for what was termed ''severe primary IGF-I deficiency'' emphasized the need for a rational and unambiguous classification for disorders associated with IGF-I deficiency (72,73). While only mutations in the IGF-I gene can rationally be considered as 'primary' IGF-I deficiency (74), these approvals were based on data derived from treatment of approximately 100 children with GHRD, which causes *secondary* IGF-I deficiency, or the even more

Table 2 Metabolic effects of GH and IGF-I

	GH	IGF-I Deficiency Due to Congenital GHD
GH secretion	–	Decreased
IGF-I production	Increased	–
IGFBP-1	Decreased	Decreased
IGFBP-2	Decreased	Increased
IGFBP-3	Increased	No effect
Insulin: secretion	Increased	Decreased
Sensitivity	Decreased	Increased
Hepatic glucose output	Increased	Decreased
Muscle glucose uptake	Decreased	Increased
Lipolysis	Increased	No effect[a]
Nitrogen balance	Increased	Increased
Protein synthesis	Increased	Increased

[a]Decreased with very high doses.
Abbreviations: GH, growth hormone; IGF-I, insulin-like growth factor-I; IGFBP, IGF binding protein.

rare secondary failure of GH response to rhGH as a result of blocking antibody formation in patients with GH gene deletion (75). The accuracy of the nomenclature is important for the perspective clinicians will have when considering diagnosis and therapy of growth problems.

In the classification put forth in 1993 primary GHI encompassed typical Laron syndrome due to GHRD, post-receptor signal transduction abnormalities, and primary defects of synthesis of IGF-I, which had not yet been described. Considering all such defects to be ''primary GHI'' is not consistent with the meanings of primary and secondary (74). Secondary GHI included GH inhibiting antibodies, diabetes, malnutrition, liver disease, and renal disease, disorders that are not consistently associated with elevated serum GH concentrations or low levels of IGF-I, which should be requirements for classification as GHI. This imprecise terminology can lead to subjective interpretation and application. In 1999, we proposed a classification based on the fundamental role of IGF-I, in which primary IGF-I deficiency applied only to defects in IGF-I synthesis, secondary IGF-I deficiency was that due to GHRD or GH–GHR signal transduction defects, or acquired as a result of catabolic conditions such as chronic disease or undernutrition, and tertiary IGF-I deficiency included congenital and acquired GHD. However rational this classification may have been, the terms primary, secondary, and tertiary remained sufficiently removed from the precise abnormalities as to obscure rather than enhance understanding and communication (76). Some ambiguity may also arise from the perception of IGF-I as the principal, i.e., primary, growth effector. Thus, a clinical pathologic state, which is *primarily* the result of IGF-I deficiency devolves into *primary* IGF-I deficiency. Contemporary knowledge and the need for clarity in scientific communication must dismiss terminology that is subject to individual perspective and bias, i.e., subjective.

The classification used in this chapter begins with a global separation of pre-GHR and receptor/post-receptor loci (Table 3) consistent with knowledge of existing defects at each level of the GH–IGF-I cascade and adaptable to new discoveries. The general term GHI would apply to all receptor or post-receptor abnormalities, i.e., those that result in unresponsiveness to GH, while GHRD refers specifically to abnormalities of the GHR. Both GHRD and signal transduction defects result in the clinical and biochemical condition often referred to as Laron syndrome.

IGF-I DEFICIENCY DUE TO CONGENITAL GHD

Hypothalamic–Pituitary Malformations

Anterior and posterior pituitary deficiencies associated with various congenital syndromes affecting the hypothalamus, the pituitary, or both may be discovered through magnetic resonance imaging (MRI) of the hypothalamus and pituitary gland. These abnormalities may result in apparent deficiencies in infancy or not until later in childhood.

Anencephaly has long been recognized as a cause of an ectopic, hypoplastic, or malformed pituitary gland (77,78). Slightly less severe in the clinical spectrum of major cranial malformations, holoprosencephaly is commonly associated with hypothalamic defects resulting in pituitary hormone deficiency. Associated defects can range from cyclopia to hypertelorism, with varying coexistent defects, including palatal or lip clefts, nasal septal aplasia, or, in surviving older children, a single central incisor (79,80). Schizencephaly, sometimes identified by MRI during evaluation of gait disturbance, can also be associated with hypothalamic–pituitary malformation. Lastly, even isolated cleft lip or palate may be associated with hypothalamic or pituitary defects (81,82).

SOD is a malformation syndrome in which at least 50% of affected children have hypopitutarism (77,82–87). Children may have diminished visual acuity, nystagmus, color blindness, or bilateral blindness associated with varying degrees of bilateral or unilateral optic nerve hypoplasia. Other associated malformations can be absence or hypoplasia of

Table 3 Classification of IGF-I Deficiency and Resistance

	GH	GHBP	IGF-I	IGFBP-3	Fetal growth	Mental deficiency	Response to rhGH
Pre-GH receptor							
GHRH receptor deficiency	Low	Normal	Low	Low	Normal	No	Yes
Hypopituitarism (isolated GH deficiency, MPHD)	Low	Normal	Low	Low	Normal	No	Yes
Acquired GH inhibiting antibodies	Low	Normal	Low	Low	Normal	No	No
GH receptor/post receptor							
GHR deficiency	High	Low/normal/ high	Low	Low	Normal	No	No
GH–GHR signal transduction defect	High	Normal	Low	Low	Normal	No	No
IGF-I synthetic defect	High	Normal	Low/high	Normal	Reduced	Severe	No
IGF receptor deficiency	Normal/high	Normal	Normal/high	High	Reduced	Mild	Some
IGF-I-binding protein 3/ acid-labile subunit mutations	Elevated	Unknown	Low	Low	Unknown	No	Unknown
Catabolic states/chronic illness	Normal/high	Low/normal	Low	Normal/low	Normal	No	Variable

Abbreviations: GH, growth hormone; GHBP, growth hormone-binding protein; GHR, growth hormone receptor; IGF-I, Insulin-like growth factor-I; IGFBP-3, insulin-like growth factor-binding protein 3; GHRH, growth hormone-releasing hormone; MPHD, multiple pituitary hormone deficiency; rhGH, recombinant human growth hormone.

the septum pellucidum or corpus callosum, schizencephaly, autism, mental retardation, and holoprosencephaly. The varying presentations may represent a heterogeneous group of genetic disorders, including some HESX1 gene defects, as well as some fetal vascular accidents, perhaps involving the proximal trunk of the anterior cerebral artery, or maternal valproic acid exposure (88). A 2002 report from the National Cooperative Growth Study identified 65 children in its substudy with SOD. GHD was a frequent finding among patients, and 24% of those treated for GHD were ACTH deficient as well, while 27% of the treated group had TSH deficiency (89). A child identified as having SOD should therefore be referred to a pediatric endocrinologist for monitoring or testing. In its most severe form, SOD is associated with hypoplasia or absence of the optic nerves or chiasm, agenesis or hypoplasia of the septum pellucidum, and hypothalamic defects (80,83–85). Infants are generally born full-term with normal birth weights. The mothers were significantly younger than average in a study of Scottish women, in which the average maternal age for babies with SOD was 21 years, whereas the average maternal age in Scotland was 27 years (90). Typically, growth failure due to GHD becomes apparent between 6 and 18 months of age. Optic disc hypoplasia, even without malformations of the corpus callosum or septum pellucidum, has a 35% association with hypopituitarism in children with MRI-documented hypothalamic–pituitary defects. Therefore, even children with what appears to be isolated optic disc hypoplasia should be carefully scrutinized for GHD in isolation or with other pituitary hormone deficiencies (87,91). Neurological follow up should also be arranged. In one descriptive study of 25 patients, 14 of 20 patients with SOD had mild to moderate neurological disorders, and 7 of 19 had electroencephalography abnormalities. MRI analysis in these 25 patients revealed a number of abnormalities in addition to the classically reported anomalies and included abnormalities of the cortex and malrotated hippocampal structures (92).

Other specific, rare genetic syndromes are of uncertain or variable association with hypopituitarism. Pallister Hall syndrome is associated with hypothalamic hamartomablastoma, micropenis, cryptorchidism, and post-axial polydactyly (93). Rieger syndrome is an autosomal dominant disorder characterized by GHD, coloboma, an increased risk for glaucoma, dental hypoplasia, and a prominent umbilicus. The phenotype is produced by haploinsufficiancy for the PITX2 (RIEG) gene; this gene is expressed in Rathke's pouch, periocular mesenchyme, umbilicus, and maxillary and mandibular epithelia in a murine model (94). As MRI is used increasingly in the evaluation of children with unexplained neurological findings, more genetic syndromes affecting the structural integrity of the hypothalamic–pituitary axis are likely to be recognized. The use of MRI has led to the recognition that many children thought to have idiopathic GHD actually have abnormal posterior pituitary or stalk regions (95).

Some children found to have hypopituitarism have MRI findings of an interrupted pituitary stalk. The lesion may be congenital and accompanied by varying degrees of diabetes insipidus (DI) or anterior pituitary deficiencies. In children with Diamond-Blackfan anemia, GHD has been documented in association with pituitary stalk interruption and short stature (96). Interrupted pituitary stalk has also been reported in five patients with Fanconi anemia and GHD (97). Fanconi anemia is a congenital disorder

characterized by bone marrow failure, a 50% rate of malignancy by age 40 years, and thumb, kidney, and cardiac anomalies. Some of these patients also had TSH deficiency. Other instances are probably acquired during trauma, including at birth, and may show some recovery of function later.

Hypothalamic dysfunction may explain the not infrequent finding of the short child with abnormal growth velocity and delayed bone age who, to the surprise of the endocrinologist, has a normal response to provocative GH testing. Some investigators have surmised that these children, while able to produce GH in response to pharmocologic stimuli, fail to have adequate daily secretion of GHRH and thus, GH (98). This may be particularly true for a subset of children, those who have had prophylactic intracranial irradiation for leukemia (99). Children with GHD following cranial radiation were found to respond to GHRH administration with augmented levels of GH (100) (Vol. 2; Chap. 30).

Spontaneous GH secretion testing for this neurosecretory disorder can be cumbersome, requiring an indwelling catheter and the obtaining of frequent spontaneous GH samples over a 24-hour period or a nocturnal 12-hour period. For this reason and because GHRH is not a commercially available therapeutic option, common practice is to pursue a trial of GH therapy, usually for a six-month period, in the short child with poor growth velocity and normal provocative GH testing results.

Another example in which the phenotype of GHD may be hypothalamic in origin is the child with Prader–Labhart–Willi syndrome (PLWS), resulting from partial deletion of chromosome 15 or maternal uniparental disomy (i.e., the loss of paternally derived genetic material on chromosome 15). PLWS results in a phenotype of variable short stature, hypothalamic obesity, hypotonia, and hypogonadism (101). GH therapy results in an increase in lean body mass and growth velocity sustained over two years of therapy (102) (Vol. 2; Chap. 5). Interestingly, patients with PLWS have been demonstrated to have elevated ghrelin levels (103). These levels are higher than the levels found in similarly obese patients without PLWS. Apparently, ghrelin is suppressed by BMI in PLWS patients, but in response to an abnormal threshold (104). A recent study in a small cohort has demonstrated that SS infusion successfully suppresses ghrelin release in PLWS but does not alter appetite, failing to fully explain the relationship between ghrelin and obesity in PLWS (105).

The empty sella syndrome is frequently found by MRI during the evaluation of children with IGHD or panhypopituitarism, but is only a finding in 2% of healthy children (106). Mild elevation of serum PRL may be observed. The empty sella is the enlarged pituitary fossa, whose size has been altered by herniated arachnoid contents. An empty sella may be a primary or secondary finding (107). Secondary causes include surgery, irradiation, and tumor. The actual pituitary tissue is flattened against the fossa wall, leading to varying degrees of hypopituitarism.

Prenatal and postnatal injuries are known to result in the birth of some infants with hypopituitarism (108). Breech delivery is also associated with hypopituitarism, but may be the result rather than the cause of hypopituitarism (109). Support for this hypothesis includes the finding of microphallus in some newborn boys with perinatal asphyxia (110,111), suggesting inadequate prenatal gonadotropin, GH, or both. In some instances, however, perinatal asphyxia may compromise pituitary blood flow with subsequent ischemic pituitary damage.

Hereditary Forms of GHD

Although studies of GH response with GHRH and GHRPs indicate a hypothalamic basis for many instances of GHD, no defects have been described which affect GHRH synthesis (112). Numerous mutations have been described, however, affecting homeodomain transcription factors for pituitary development, the GHRH receptor, and GH genes.

Pituitary Differentiation Factors

As noted in the section on pituitary differentiation, *HESX*, *LHX3/LHX4, SOX3, PITX2,* and *GLI2* are factors involved in differentiation of the Rathke pouch into distinctive pituitary cell types. The gene for HESX1 is found on chromosome 3p21.2 and codes for a 185 amino acid protein. A missense mutation of *HESX1* has been described in a single family with SOD, agenesis of the corpus callosum, and anterior hypopituitarism. Heterozygous members of the family were unaffected. Three siblings having deficiency of all pituitary hormones except ACTH were found to be homozygous for a missense mutation in the *LHX3* gene, and a single patient with the same hormonal status in another family had an intragenic deletion, which predicted a severely truncated protein lacking the entire homeodomain (14). One of these patients had an enlarged anterior pituitary, whereas the others had pituitary hypoplasia. All four had rigidity of the cervical spine resulting in limited rotational ability of the head, but other families have been described without this limitation.

Some instances of MPHD have been attributed to dominant and recessive mutations of the gene for pituitary transcription factor, POU1F1 is critical for the differentiation of somatotrophs, thyrotrophs, and lactotrophs. The POU1F1 gene is on chromosome 3p11. The product of the POU1F1 gene binds to the GH gene promoter. POU1F1 defects are associated with deficiency of prolactin, TSH, and GH secretion and are identified more frequently in familial, rather than sporadic, combined hypopituitarism (113). Recessive mutations result in varying degrees of loss of DNA binding or transcriptional activation functions (9). Mechanisms for the effects of the four

dominant mutations are less readily explained, one involving enhanced binding of the mutant to GH and PRL promoter sites, another impairing distal enhancer activation; mechanisms for the other two mutations remain unexplained. Affected patients have intact ACTH, LH, and FSH function (114). Such occurrences have also been characterized by a small or normal sella turcica (24,115). Mutations include R271W and E230K in a Maltese population, ins778A, and R172Q (24,115).

The PROP1 gene encodes a paired-like homeodomain protein expressed briefly in embryonic pituitary and necessary for POU1F1 expression (116). It was demonstrated in several families, as well as in sporadic cases, that mutation of the PROP1 gene can cause gonadotropin deficiency in addition to GH, TSH, and prolactin deficiency (116–119). In the families described by Nader et al. (120), in 1975, and by Parks et al. (121) in 1978, now recognized to be families with PROP1 deficiency, MPHD was associated with sellar enlargement. In a few instances where this mass has led to surgical intervention, histological examination indicates proliferation of undifferentiated connective tissue (122). This phenomenon does not appear to be genotype specific and varies within the families of the same genotype (Fig. 3); the clinical importance is the need to differentiate this pituitary enlargement from adenoma, craniopharyngioma, or other tumor. ACTH deficiency may result from progressive deterioration of the pituitary or be specific to certain mutations, suggesting a role for PROP1 in the differentiation or maintenance of corticotroph cells (123).

Homozygosity for a GA or an AG deletion in the sequence 296GAGAGAG in exon 2 of PROP1, originally noted in one of the first four families with PROP1 deficiency reported by Wu et al. (117), is the most common of the eight recessive mutations that have been described, reported in patients from Russia (118,125), Turkey (125), Jamaica (122), the United States (125), Brazil (122,125), and Dominican Republic (124). The series of three dinucleotide repeats appears to be a mutational hot spot, with susceptibility to misalignment of DNA, generating slippage and deletion independently in different populations. Another mutational hot spot also results in a 2-bp deletion in exon 2 (149delGA) that leads to the same serine to stop codon change at codon 109 and is present as a compound heterozygote with the 296delGA mutation in children with MPHD from four Russian families (9). The 296GAdel mutation represents a severe loss of function mutation. The altered sequence predicts a protein of 108 amino acids with a frameshift and truncation in the second a-helix of the DNA-binding domain. When expressed in a mouse PROP1 context,

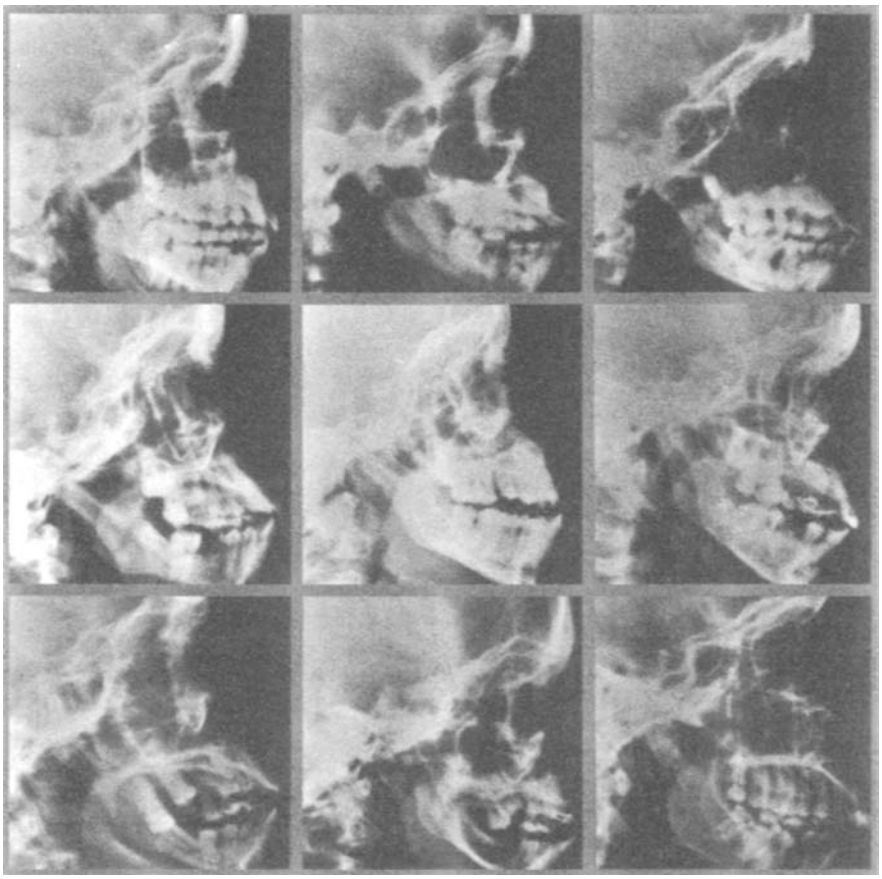

Figure 3 Lateral skull films for sellar area in patients with familial anterior hypopituitarism due to PROP1 deficiency, from left to right, ages 17, 21, 23, 27, 29, 33, 38, and 40 years and normal sister age 22 years (third row, right). The first, fourth, and fifth subjects have sellar enlargement for height and bone age. *Abbreviation*: PROP1, PROphet of Pit1. *Source*: From Ref. 124.

the recombinant protein lacks detectable DNA-binding and transcriptional activation activities (117). The severity of the hormone deficiency phenotype is compatible with the complete loss of PROP1 activity.

PROP1 mutations are an important cause of MPHD (126). Of 52 Polish patients, 33 had mutations in PROP1 and none in POU1F1; all 11 multiplex families had mutations, while 17 of 27 isolated cases did (9). Similarly, 35 of 73 subjects with MPHD in Switzerland had PROP1 mutations, including all who were affected in 17 multiplex families (127).

GHRH Receptor Defects

Reports of mutations of the GHRH receptor gene come from inbred populations in India, Pakistan, and Brazil (25–28,128–131). The first two families reported consisted of two children each from Indian Muslim families not known to be related, one of which was consanguineous (128,129). These patients were homozygous for a G to T transversion at position 265 of the GHRH-R gene which introduces a premature stop codon at residue 72, a mutation that was also found in 18 Pakistani patients who were part of a multiply consanguineous pedigree (25). The largest group of patients described thus far, 105 Caucasian individuals of Portuguese descent from an inbred population in northeastern Brazil, exhibit homozygosity for a different splice site mutation (130,131).

As expected, individuals with GHRH receptor mutations have severe short stature and high pitched voice. There is mild delay in sexual maturation but fertility has been demonstrated for both males and females (who require delivery by c-section). One affected couple had an obligatorily affected but otherwise normal youngster. There are a number of clinical features that distinguish those with GHRH receptor mutation from individuals with related conditions of severe IGHD or GHI. GHRH receptor deficient patients have a normal penis size before puberty, whereas microphallus is typical of GHD and GHRD. Unlike congenital GHD and GHRD, hypoglycemia has not been noted in any of the populations and there is minimal or no facial hypoplasia or prominence of the forehead. The Pakistani patients had asymptomatic low blood pressure and relatively small head sizes, but this has not been noted in the other reports. In further contrast to GHD and GHRD, central adiposity is uncommon and body proportions are normal in those with GHRH receptor mutation. A further interesting difference from GHRD is that there is no difference in the markedly low levels of IGF-I, IGF-II, and IGFBP-3, or the elevated levels of IGFBP-2 between prepubertal and adult individuals (25,132,133).

Isolated Growth Hormone Deficiency

Four autosomal recessive disorders, an autosomal dominant mutation, and an X-linked form of IGHD have been identified (134). The GH1 gene for GH is located at 17q22–24.

IGHD IA

IGHD IA is a recessive condition caused by heterogeneous defects of the gene for GH (GH1), and is the most severe form of GHD. Defects of the GH1 have included gene deletions, and frameshift and nonsense mutations (1,135–139). Because these individuals have complete GHD with no exposure to endogenous GH, the administration of rhGH results in formation of anti-GH antibodies and cessation of growth response after a few months in most, but not all patients (1,140–142). In an Italian family with three affected children, two developed high titre antibodies and ceased responding to exogenous GH, while the third sibling continued to respond and had low titre antibodies (143).

IGHD IB

IGHD IB is also an autosomal recessive condition differing from IGHD IA by the presence of detectable levels of endogenous GH and, consequently, continued response to rhGH administration without the development of anti-GH antibodies. This form of IGHD results from mutations affecting splicing of the GH1 gene (144) or mutations within the gene encoding the GHRH receptor (136). These rare mutations have been described in Middle Eastern consanguineous families. The result of some of these mutations can be complete absence of measurable circulating GH, thought to be due to loss of anti-GH antibody-binding sites necessary for the radioimmunoassay reaction. The presence of some GH related protein has been supported by the absence of development of anti-GH antibodies when these patients are treated with rhGH (129). In an Arab-Bedouin family from Israel with IGHD IB, carriers of the mutant GH-1 allele were significantly shorter than those who were homozygous normal; one-third of 33 heterozygotes but only one of the 17 normal homozygotes, had stature >2 SD below the mean (145).

IGHD II

IGHD II is distinguished from IGHD I because it is inherited in an autosomal dominant mode, the result of dominant negative mutations of the GH1 gene. Affected individuals typically have an affected parent and respond well to rhGH administration. Most of the mutations that have been described associated with this form of IGHD are in intron 3 of the GH gene and alter splicing of GH mRNA with skipping or deletion of exon 3. It is not clear why these mutations prevent expression of normal GH from the unaffected allele (the dominant negative phenomenon) (146–148). With one of the splice mutations, resulting in del32–71-GH, transfection studies in neuroendocrine cell lines demonstrated suppression of wild-type

GH by the mutant as a post-translational effect caused by decreased stability rather than decreased synthesis of the wild-type GH (149). It has recently been noted that 8 of 12 subjects from four families with the P89L mutated GH form developed other endocrine deficits as the pituitary gland became smaller with time, findings not detected in 17 subjects from five families with the R183H GH missense mutation (150). Thus, it is necessary to recognize that some forms of genetically determined IGHD require monitoring for the development of other deficiencies.

IGHD III

IGHD III is inherited in an X-linked manner. It is associated with agammaglobulinemia in some but not all families, suggesting contiguous gene defects on the long arm of the X-chromosome as a cause of some instances of IGHD III (151). No abnormalities of the GH-1 gene have been detected and the mechanism remains unknown (152).

Bioinactive Growth Hormone

The presence of immunologically detectable but biologically ineffective GH has been proposed for a number of reported patients with the appearance of GHD, normal levels of radioimmunoassayable GH in the circulation, and low concentrations of IGF-I (153). Radioreceptor assay for GH in these cases has indicated lower concentrations than in the radioimmunoassay, and therapeutic response to rhGH is comparable to that in IGHD (153,154). A mutation resulting in a heterozygous single amino acid substitution in the GH1 gene has been described in a patient thought to have bioinactive GH; while there was an abnormal GH peak on isoelectric focusing, there was also a normal one and the patient shared this mutation with his normal father, suggesting that it was not pathologic (155). In quest of a molecular defect in such patients, 200 children with short stature found to have GH sufficiency were reviewed; three were identified with short stature and growth velocities consistent with GHD, elevated basal and stimulated levels of immunoassayable GH, and low concentrations of IGF-I and IGFBP-3 in the serum. A rat lymphoma cell proliferation assay and an immunofunctional assay for GH gave abnormally low responses compared to the RIA. High-resolution sequencing of both strands of the coating region and display sites of genomic DNA and sequencing of the cDNA did not reveal GH1 gene mutation in any of the patients. It was postulated that the reduced biological activity of abnormal translation product could be due to post-translational processing in these patients (156).

A biologically inactive mutant GH with one of its two disulfide bridges disrupted by a homozygous missense mutation in the GH1 gene was the cause of short stature in the Serbian patient, confirming predictions from transgenic mice with absence of the first disulfide bridge (157).

ACQUIRED GHD

Tumors

By far the most common cause of acquired GHD in children is tumor. Craniopharyngiomas are most common in these children, but several other benign or malignant tumors can also be responsible for the deficient state (158–160). Benign lesions, which damage the hypothalamic–pituitary axis include pituitary adenoma, Rathke's cleft cyst, and arachnoid cyst (161). Primary malignant tumors, which can affect this region include dysgerminomas, germinomas, meningiomas, and gliomas (162). Although metastatic tumor is a more common cause of GHD in adults than in children, childhood Hodgkin disease or nasopharyngeal carcinoma can metastasize to the pituitary or hypothalamus (162). Signs and symptoms of primary or metastatic malignancies, as well as benign intracranial lesions, can be visual loss (particularly bitemporal hemianopsia, for optic chiasm lesions), papilledema, headache with sleep or on awakening, emesis, and behavioral changes (162). Lesions in the hypothalamus may additionally cause hypersomnolence, appetite changes, or DI (163). Weight loss may result from appetite loss or excess fluid losses from DI. Symptoms of PRL excess, including amenorrhea or galactorrhea, may occur, because of the loss of dopamine inhibition from the hypothalamus.

Craniopharyngioma

Two-thirds of craniopharyngiomas are found in a suprasellar location. The tumor most likely arises from remnants of the invagination of the embryonic stomodeum, which gives rises to the adenohypophysis in the fetus. However, some neuropathologists believe the tumor could instead arise from metaplastic squamous epithelial cells in the adenohypophysis (161). The tumor accounts for 6% to 10% of all pediatric brain tumors and is most common from ages 5 to 15 years, but a second peak in incidence is seen in adults age 50 to 70 years (158). MRI imaging often reveals a calcified lesion, and histolopathological examination reveals an encapsulated, oil-containing cystic tumor of squamous epithelial cells which show keratinization. The high cholesterol content of the tumor aids in identification on MRI. In one series, 95% of patients with craniopharyngioma had at least one anterior pituitary hormone deficiency at diagnosis and 38% had hyperprolactinemia (158). DI does not usually occur preoperatively (164). In the Jenkins series, nearly two-thirds of patients had tumor recurrence, and this recurrence correlated with cyst size. The best long-term prognosis is seen in patients with purely

cystic lesions, and patients with tumor symptoms before the age of five years may have a worse prognosis than older patients (160). After initial surgical resection, virtually all patients with craniopharyngioma develop hypopituitarism. Sleep and appetite centers in the hypothalamus may be damaged by the tumor or by the attempted resection. Hyperphagia and resultant obesity tend to correlate with damage to the ventromedial or paraventricular nuclei (161), and this excess nutrition may result in normal or supranormal growth velocity despite profound GHD.

Among 20 patients with craniopharyngioma reported by Jenkins, 19 had GH and gonadotropin deficiencies at diagnosis (158). Thirteen patients had secondary or tertiary hypothyroidism, and 10 had ACTH deficiency. None of the patients had DI before surgical resection.

With surgical resection, nearly all patients with craniopharyngioma will have at least temporary vasopressin deficiency. The development of further pituitary deficiency depends upon degree of tumor resection (165), which will also influence long-term survival. With attempted complete removal of the tumor (without subsequent radiation), 42% of patients have tumor recurrence (160). Gross total resection of the tumor had a 90% cure rate in another series, but about one-third of patients experienced behavioral problems, appetite or sleep dysfunction, and memory deficits post-operatively (162). Near total excision followed by fractionated radiotherapy may provide the best prognosis (166,167).

Rathke Cleft Cyst

A Rathke cleft cyst (RCC) is a benign lesion believed to arise from Rathke's pouch remnants, neuroepithelium, or metaplastic anterior pituitary cells (168). The lesion is generally intrasellar or suprasellar and may present with hypopituitarism, neurological findings, or visual deficits (161). The lesion is often mucoid and may contain cuboidal, columnar, or ciliated epithelium. It may be difficult to distinguish from a craniopharyngioma or arachnoid cyst. In one series, 81% of patients with RCC had preoperative hypopituitarism, 60% had neurological dysfunction, and 38% had visual losses (168). As in craniopharyngioma, hyperprolactinemia may result from loss of dopamine inhibition of PRL secretion. On MRI, most RCCs have a hyperintense signal on T1 and T2 (169).

Arachnoid Cleft Cyst

The arachnoid cleft cyst (ACC) usually occurs in an intrasellar location and may be difficult to distinguish preoperatively. ACC occurs typically in older patients than does craniopharyngioma or RCC. The cyst is lined by arachnoid cells and contains cerebrospinal fluid. It is either a congenital lesion or an acquired lesion that develops when the diaphragma sella permits herniation of the arachnoid membrane. The endocrine prognosis is worse for ACC than RCC or craniopharyngioma, most likely due to more chronic, high pressure damage to the adenohypophysis by the cyst (168).

Pituitary Adenoma

Pituitary adenomas are more common in adults than in children, and may cause hypopituitarism by pressure-induced damage to surrounding normal pituitary tissue. Five to six percent of childhood pituitary tumors are adenomas and may occur in isolation or as part of multiple endocrine neooplasia type I (170). More than half of these adenomas are prolactinomas; the remainder may secrete ACTH or GH, or may be nonfunctioning (163). Histopathological evaluation often reveals areas of hemorrhage within the adenoma; hemorrhage may cause pituitary apoplexy, and, if longstanding, the hemorrhagic areas may become cystic (171).

Other Causes of Acquired GHD

A variety of other causes of acquired GHD have been identified. Among the mechanical causes of GHD are trauma with or without skull fracture, irradiation for therapy of malignancy, and surgery. Hemorrhage into the pituitary or hypothalamus may cause hypopituitarism and has been reported in patients on chronic anticoagulant therapy, as well as in those with pituitary apoplexy. Inflammatory processes resulting in hypopituitarism include autoimmune hypophysitis, infectious diseases resulting in meningitis or encephalitis, and infiltrative processes such as sarcoidosis, histiocytosis X, and hemochromatosis. Cerebral edema occurring with diabetic ketoacidosis has also been reported to result in hypopituitarism (32). Interferon alpha therapy has recently been reported to result in hypopituitarism in an adult manifested by deficiency of LH, FSH, and GH (172).

Head Trauma

Following significant head trauma, 40% of patients may have transient or permanent vasopressin deficiency (163). On occasion, apparently permanent anterior hypopituitarism has spontaneously resolved months after the head injury (173). Varying degrees of other pituitary hormonal deficiencies may be seen. Hypopituitarism may result from direct structural damage to the hypothalamus or pituitary or because of hypovolemia with severe blood losses or increased intracranial pressure. Hypopituitarism may occur after seemingly minor head trauma (174) and should always be a diagnostic consideration in the evaluation of the child abuse victim with head trauma.

Radiation Therapy

Current CNS oncological radiation includes conventional radiation, as well as proton-beam, gamma knife, and yttrium-90 or gold-198 radiotherapy. All of these therapies may cause pituitary insufficiency (163,175). With conventional radiation of 50 Gy for

four weeks for pituitary adenoma, 11 of 22 patients developed hypopituitarism within 4.2 years (176). Head and neck irradiation, or ''mantle'' radiation for lymphoma, may lead to pituitary deficiencies as well (177). It is prudent to evaluate growth velocity, weight gain, and in pubertal age patients, pubertal progression, at frequent intervals in the first five years following cranial radiation.

Pituitary Apoplexy

Pituitary apoplexy has been best described in patients with pituitary adenomas and in women in the immediate post-partal period (178). It may also occur in severely dehydrated patients with diabetes mellitus, trauma, or intracranial pressure changes, or in patients undergoing chronic anticoagulation or radiation therapy (163,179). Post-partum apoplexy is known as Sheehan syndrome. Apoplexy is due to hemorrhage or infarct into an adenoma or into normal pituitary tissue, with subsequent hypopituitarism. Failure to recognize the signs of ACTH deficiency resulting from apoplexy can have fatal consequences. Patients may have severe headache (''the worst of my life''), diplopia or visual loss, deficits of cranial nerves III, IV, or VI, sensorium changes, or hypotension. Diagnosis is confirmed by CT scan or MRI.

Inflammatory Disease

Autoimmune or lymphocytic hypophysitis has been described in patients with autoimmune polyglandular syndrome, in post-partum women, and as an isolated finding (180,181) (Vol. 2; Chap. 26). Diagnosis can be difficult to confirm but may be based upon the finding of pituitary antibodies (not commercially available) or of lymphocytic infiltration on pituitary biopsy (182). Patients with meningitis or encephalitis may also develop hypopituitarism with bacterial, viral, or fungal infections (183). Infiltrative diseases uncommonly cause hypopituitarism in children, but may result from hemochromatosis due to chronic blood transfusions for thalassemia (184), histiocytosis X, or sarcoidosis. A thickened pituitary stalk may suggest the diagnosis on MRI. Children with histiocytosis X often initially have DI without other hormonal deficiencies and with a thickened pituitary stalk (185). The child with histiocytosis and DI often has unusual, desperate, water-seeking behaviors, such as drinking from flower vases or pets' water dishes. Subsequently, GHD, alone or with other pituitary deficiencies, may develop.

Deprivation Syndrome

Failure to thrive thought to result from lack of nurturing in infants raised in institutions was described as anaclitic depression (Fig. 4) (186). Talbot et al. (187) initially suggested that growth failure can occur beyond infancy associated with emotional deprivation and characterized by subtle nutritional inadequacy. The spectrum of pathology associated with growth failure in dysfunctional settings has been referred to as deprivation, maternal deprivation,

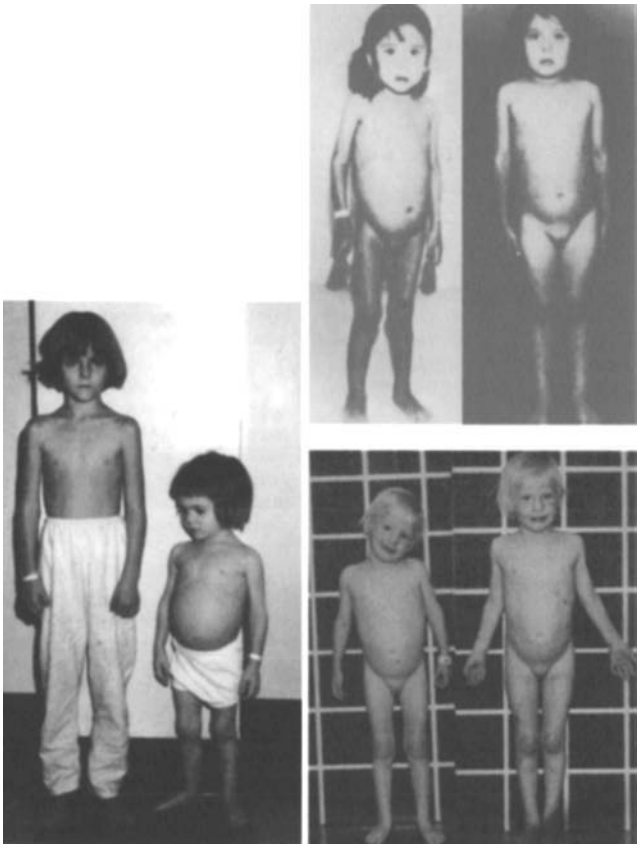

Figure 4 Three children with deprivation syndrome. (*Left*): Seven-year old daughter of psychotic parents with hepatomegaly and lack of normal social behavior, standing with a normal sized age mate. (*Right upper*): Daughter of alcoholic single mother. This five-year-old was thought to have gluten-induced enteropathy to explain her short stature (height SD −4.1, height age two years two months). In the care of her grandmother, she gained 20 lb in 10 months, nearly doubling her weight, and grew 7.25 in. (height SD −1.2, height age four years eight months). (*Right lower*): Five-year-old thought to have growth hormone deficiency to explain severe short stature (height SD −5); when she returned six months later to start pituitary GH injections, she had grown 4 in. and it was learned that her social situation had changed dramatically after the initial hospitalization. *Abbreviations*: SD, standard deviation; GH, growth hormone.

emotional deprivation, or environmental deprivation dwarfism, psychosocial dwarfism, and hyperphagic short stature. Fifteen years following the report of Talbot et al. (187), Patton and Gardner (188,189) stressed the importance of historical information in defining some instances of growth failure as a truly psychosomatic disorder. They speculated that the etiology could be attributed to hypothalamic influences on the pituitary secretion of GH, undernutrition as a result of neglect, diminished appetite resulting from depression and lethargy, or decreased intestinal motility and absorption due to nutritional and emotional factors.

Deprivation dwarfism was later described as a triad of extreme short stature, voracious appetite, and markedly delayed sexual maturation in nine patients who had had feeding difficulties in infancy,

persistent sleep problems, and whose parents had serious psychological disturbances; growth improved markedly with foster placement (190). The clinical syndrome was further described in 13 children initially thought to have idiopathic hypopituitarism to include, in addition to high degrees of social disruption and pathology in the family and severe growth failure, bizarre eating and drinking behavior by history, sleep disturbances, malabsorption with foul smelling stools, delayed speech, and abdominal protuberance. The children ate out of garbage cans, ''stole'' food, gorged, and ate from the pets' dishes. They drank from toilet bowls, glasses filled with dishwater, rain pools, and old beer cans holding stagnant water. Removal from the home was associated with growth acceleration and behavioral improvement. Emotional factors, malabsorption, inadequate nutrition, and hypopituitarism were all considered as possible contributors (191). Endocrinologic evaluation indicated frequent deficiency of ACTH and GH with return of GH responses to normal; occasional patients had normal GH values, but it was not recognized at that time that recovery of GH secretion might occur very early after removal from the home (192). Others confirmed deficient GH and adrenocorticotropic responses to hypoglycemia in children, but not infants, with the deprivation syndrome and noted that some children had normal or elevated serum GH concentrations at the time of admission, reminiscent of the range of possibilities with malnutrition (193).

Two important investigations published in 1969 addressed the question of whether environmental or maternal deprivation, in the presence of adequate nutrition, could result in growth failure. Rhesus monkeys, shown to be extremely dependent on maternal attention and physical play for normal behavioral development, were provided an ad libitum diet, but deprived of all social contact and environmental stimulation from birth, including direct human contact except that required for maintenance and experimental measures. Despite developing persistent behavioral abnormalities including autistic posturing, fear, inability to engage in social play and after maturity to engage in social and sexual activity, their growth was completely normal (194).

A remarkable prospective study was carried out by Whitten et al. of 16 human infants aged 3 to 24 months referred for growth failure, who were of normal weight at birth (195). During a two-week period of initial hospitalization with minimal mothering and plentiful calories, and a period of high intensity mothering, comparable weight recovery occurred. In the home setting, provision of adequate calories supervised by a home visitor, described to the family as a study protocol rather than supervision, also resulted in adequate gain. Some of the mothers admitted that the amount of food consumed was considerably greater than they had been providing. Similarly, other mothers have admitted intentional starvation as a form of abuse, or to avoid having the child get sick, bloated, throw up, or have loose stools (196). Extensive studies of mothers of affected infants and children have demonstrated a range of pathology affecting mothering skills and character (197,198).

In a review of 185 patients hospitalized for evaluation of failure to thrive, 50% were attributed to environmental deprivation, corresponding precisely to the percentage noted 20 years earlier by Talbot et al. (187). Organic etiologies were suggested by history and physical examination; only 1.4% of laboratory studies performed were of diagnostic value and only if there was a specific indication for the test from the clinical evaluation (199).

The frequent observation of disturbed sleep patterns in these children suggests that their growth failure might in part be related to a failure of nocturnal GH secretion, dependent on the attainment of deep sleep (200). Polygraphic sleep recordings soon after hospital admission in children with psychosocial dwarfism demonstrated a gross deficit of stage IV sleep and a decrease of the overall slow wave sleep episodes, both stage III and IV. After 3 to 15 weeks in the new environment and with growth recovery, stage IV sleep returned (201). More recent studies have demonstrated progressive improvement in GH pulse amplitude, with maintenance of the individual's characteristic pulsatility (202,203).

The deprivation syndrome can now be recognized as a social pathology resulting in chronic undernutrition and secondary endocrine deficiencies, primarily GH and ACTH, which are readily reversible with provision of adequate food intake. Removal from the home temporarily or permanently is usually necessary, particularly for older children. Infants with failure to thrive often have mothers who mean well, but are immature, dependent, and insensitive to their infants' needs; counseling and support may make it possible for the child to thrive in the home (198). In the few recognized instances where exogenous GH has been administered to children with deprivation syndrome, responses were nil to much less than typical of GHD, as one might expect in the context of noncompliance and undernutrition (204,205).

IGF-I DEFICIENCY DUE TO GH RECEPTOR/ POST-RECEPTOR ABNORMALITY

Congenital Growth Hormone Insensitivity

Growth Hormone Receptor Deficiency (Laron Syndrome)

Following the initial report (206) of three Yemenite Jewish siblings ''with hypoglycemia and other clinical and laboratory signs of GHD, but with abnormally high concentrations of immunoreactive serum GH,'' 22 patients were reported from Israel, all Oriental Jews, with an apparent autosomal recessive mode of transmission in consanguineous families (207). These reports preceded the recognition of the critical role of cell surface receptors in hormone action and

it was postulated that the defect was in the GH molecule that these patients were producing. This impression was substantiated by the observation of free-fatty acid mobilization, nitrogen retention, and growth in patients being administered exogenous GH. These effects may have been due to other pituitary hormones in the crude extracts administered or to nutritional changes in the investigative setting. In the first patient reported outside of Israel, in 1968, there was no response to exogenous GH, leading to the hypothesis that the defect was in the GHR (208). This hypothesis was substantiated by the failure to demonstrate sulfation factor activation with exogenous GH administration, reported in 1969 (209) and reports in 1973 and 1976 that the patients' GH was normal on fractionation, in its binding to antibodies, and in its binding to hepatic cell membranes from normal individuals (210–213). In vitro demonstration of cellular unresponsiveness to GH was demonstrated by the failure of erythroid progenitor cells from the peripheral blood of two patients to respond to exogenous GH (214). The failure of radioiodine labeled GH to bind to liver cell microsomes obtained from biopsy of two patients with Laron syndrome confirmed that the defect was in the GHR (215). The two reports of absent GHBP in sera of patients with Laron syndrome (44,45) appeared just before the publication of the finding that GHBP was the extracellular domain of the cell surface GHR (46).

Mutations of the GHR have provided insight into its physiology; over 40 distinct mutations in the extracellular and transmembrane domains produce a clinical picture of severe GH/IGF-I deficiency in the homozygous state or as compound heterozygotes, whereas two dominant negative mutations of the intracellular domain result in a milder clinical syndrome. Some individuals described with features of GH resistance but apparently intact GH binding have defective signal transduction by the GH–GHR complex. Knowledge of the biology and signaling of the GHR has attracted extensive research interest because GH is a powerful regulator of somatic growth, mitogenesis, and metabolism, with a wide variety of effects on target cells, all mediated by the GHR, which is expressed in 40 different tissues.

The structure of the human GHR gene is depicted in Figure 5. There are eight variants (V1–V8) contributing to the 5′-untranslated region (UTR), followed by nine coding exons (exons 2–10). Exon 2 encodes the last 11 base pairs of the 5′-UTR sequence, an 18 amino acid signal sequence, and the initial five amino acids of the extracellular hormone-binding domain. Exons 3–7 encode the extracellular hormone-binding domain, except for the terminal three amino acids of this domain, which are encoded by exon 8. Exon 8 further encodes the 24 amino acid hydrophobic transmembrane domain and the initial four amino acids of the intracellular domain. Exons 9 and 10 encode the large intracellular domain. Exon 10 also encodes the 2 kb 3′-UTR. Four of the alternative 5′-UTRs of the human gene have been cloned from genomic DNA indicating that each was encoded by a separate exon. It has been suggested that this variability serves to regulate translational efficiency of the mRNAs (216). The human GHR is unique in existing in an alternative splice isoform that excludes exon 3; this deletion has been thought to have no demonstrable effect on GHR function (217).

The report of the characterization of the GHR gene included the first description of a genetic defect of the GHR, a deletion of exons 3, 5, and 6 (47); recognition that the exon 3 deletion represented an alternatively spliced variant without functional significance resolved the dilemma of explaining deletion of nonconsecutive exons. In contrast to the alternatively spliced variant lacking exon 3, the first mutation of this exon has been described in a typical GHR deficient patient with heterozygosity for a nonsense mutation in exon 4, and family studies indicate that heterozygosity for the exon 3 mutant has no effect. This study also raises questions of the origin and function of the exon 3 deleted variant (218). More recently this isoform, present in either the homozygous or heterozygous state, was found to be associated with 1.7 to 2 times more growth acceleration from GH administration during two years of

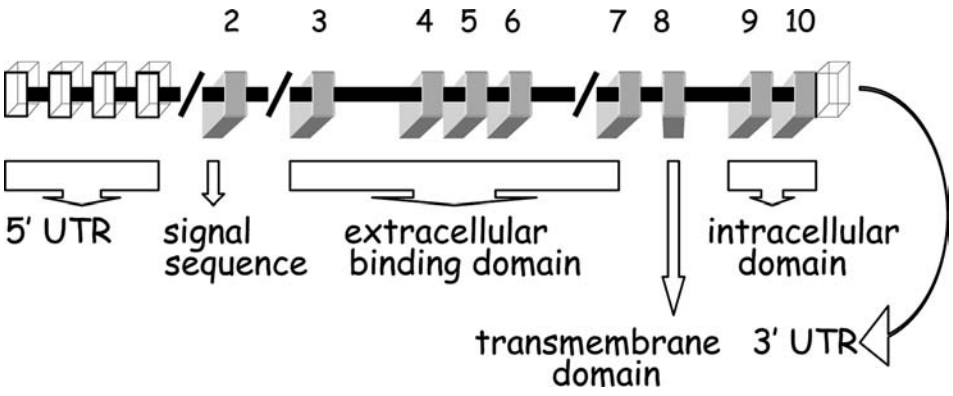

Figure 5 Representation of the GHR gene. The black horizontal line represents intron sequence; breaks in the lines indicate uncloned portions of the intron; and the boxes represent exons, which are enlarged for clarity. Exons represented by clear boxes designate untranslated regions (UTR) of the transcripts. The translated regions, exons 2–10, are represented by solid boxes.

treatment of children with short stature who had been small for gestational age or had idiopathic short stature (ISS) (219). In addition to the original exon 5, 6 deletion, another deletion of exon 5 has been described (220), along with numerous nonsense mutations, missense mutations, frame shift mutations, splice mutations, and a unique intronic mutation resulting in insertion of a pseudo-exon (76,221). A number of other mutations have been described which are either polymorphisms or have not occurred in the homozygous or compound heterozygous state.

The point mutations, which result in severe GHRD when present in the homozygous state or as a compound heterozygote, are all associated with the typical phenotype of severe GHD. All but a few of the defects result in absent or extremely low levels of GHBP. Noteworthy is the D152H missense mutation, which affects the dimerization site, thus permitting the production of the extracellular domain in normal quantities but failure of dimerization at the cell surface, which is necessary for signal transduction and IGF-I production (222). Two defects that are close to [G223G] or within [R274T] the transmembrane domain result in extremely high levels of GHBP (223–225). These defects interfere with the normal splicing of exon 8, which encodes the transmembrane domain, with the mature GHR transcript being translated into a truncated protein that retains GH-binding activity but cannot be anchored to the cell surface.

As noted, all these homozygous defects and the compound hetorozygotes, whether involving the extracellular domain or the transmembrane domain and whether associated with very low or unmeasurable GHBP, result in a typical phenotype of severe GHD. In contrast, the intronic mutation present in the heterozygous state in a mother and daughter with relatively mild growth failure (both SD for height -3.6), and resulting in a dominant negative effect on GHR formation, is not associated with other phenotypic features of GHD. This splice mutation preceding exon 9 results in an extensively attenuated, virtually absent intracellular domain (223). Japanese siblings and their mother have a similar heterozygous point mutation of the donor splice site in intron 9, also resulting in mild growth failure compared to GHRD, but with definite, although mild, phenotypic features of GHD (217). GHBP levels in the Caucasian patients were at the upper limit of normal with a radiolabeled GH-binding assay (224) and in the Japanese patients twice the upper limit of normal, using a ligand immunofunction assay (225).

These heterozygous GHR mutants transfected into permanent cell lines have demonstrated increased affinity for GH compared to the wild type full-length GHR, with markedly increased production of GHBP. When co-transfected with full-length GHR, a dominant negative effect results from overexpression of the mutant GHR and inhibition of GH-induced tyrosine phosphorylation and transcription activation (224,226). Naturally occurring truncated isoforms have also shown this dominant negative effect in vitro (51,227,228).

A novel intronic point mutation was discovered in a highly consanguineous family with two pairs of affected cousins with GHBP-positive GHI, severe short stature, but without the facial features of severe GHD or GHRD. This mutation resulted in a 108 bp insertion of a pseudo-exon between exons 6 and 7, predicting an in-frame, 36 residue amino acid sequence. This is a region critically involved in receptor dimerization (229).

Of approximately 300 reported cases of typical GHRD, ethnic origin is predominantly Middle Eastern, Mediterranean, and South Asian (221). Nearly 50% are Oriental Jews as described in the original report, or known descendants of Iberian Jews who converted to Catholicism during the Spanish Inquisition. The latter comprise the largest cohort ($n > 70$) and the only genetically homogenous group, all but one subject having the E180 splice site mutation which is shared with at least one Israeli patient of Moroccan heritage. Most of the other defects appear to be highly family specific, with the R43X mutation that is seen in a single Ecuadorian patient, two other nonsense mutations (C38X and R217X), and the intron 4 splice mutation being the only ones thus far described which appear in disparate populations, on different genetic backgrounds, indicating mutational hotspots (76). Because the molecular defect in the GHR has been identified in about one-third of the patients with GHRD outside of Ecuador, it is likely that the list of mutations will continue to grow and provide further insight into the function of the GHR.

Partial GHI

GH resistance might be expected to occur in an incomplete form, analogous to insulin resistance, androgen insensitivity, or thyroid hormone resistance. Affected children might have growth failure with normal or slightly increased GH secretion, variable but usually decreased GHBP levels, decreased IGF-I concentrations, but not as severely reduced as in GHD or GHRD, and might respond to supraphysiologic doses of GH. It might also be expected, given the need for dimerization of the GHR for signal transduction, that certain mutations could have a dominant negative effect in the heterozygous state.

Credibility for a heterozygous defect as a cause of short stature requires the demonstration of functional significance, not only by transfection of the mutant allele, but also by co-transfection with wild-type GHR gene, to approximate the circumstance in vivo. Goddard et al. (230) identified six mutations in eight children with short stature (SD for height -5.1 to -2.0) and normal or increased stimulated GH levels. One patient had compound heterozygosity involving a novel mutation in exon 4 (E44K) and a mutation in exon 6 previously associated with GHRD

in the homozygous state (R161C). Two other patients were heterozygous for this mutation. The other five patients included two who were heterozygous for the same novel mutation in exon 7 (R211H), and one each with novel mutations of exon 5 (C122X), exons 7 (E224D), and exon 10 (A478T). Expression in vitro of these four novel mutations involving the extracellular domain has shown functional effects, although co-transfection studies have not been reported. The defect involving exon 10 has not been expressed in vitro. Other defects without demonstrable significance have been described involving exon 10 (231–233). None of these putative partial GHI patients had the clinical phenotype of GHD. Five of the eight patients were treated with GH with variable improvement in growth velocity, from slight to dramatic, in the first year (230). This variable response could be due to GH resistance or to the fact that the patients were not GH/IGF-I deficient.

The subjects studied by Goddard et al. (230) were selected from the large Genentech National Cooperative Growth Study database in pursuit of the question raised by the observation that GHBP concentrations are low in children with ISS (i.e., short children without a recognizable syndrome or GHD). Using a ligand-mediated immunofunction assay Carlsson et al. (234) studied a large number of short children with known causes of growth failure such as GHD and Turner syndrome, or ISS, and compared their GHBP concentrations in serum to those of normal controls. Ninety percent of the children with ISS had GHBP concentrations below the control mean and nearly 20% had concentrations that were 2 SD or more below the normal mean for age and sex. In a further analysis of the ISS group in this database, Attie et al. (235) identified over 500 patients who had been treated with rhGH and had normal GH stimulation tests, of whom, as noted above, 20% had low GHBP concentrations. While those with the low GHBP levels had significantly lower IGF-I concentrations and higher mean 12 hour serum GH levels, the GH differences were numerically unimpressive ($2.8 \pm 1.1\,\mu g/L$ vs. $2.3 \pm 1.1\,\mu g/L$). Particularly relevant to the supposed GH resistance, there was no correlation of GHBP levels with the growth response to exogenous GH in these patients. The search for defects in the GHR to explain ISS in the 100 subjects with low GHBP yielded seven heterozygous mutations, but in studies of the families of these children short stature did not segregate with the heterozygous state (236).

More recently, the Genentech database has been analyzed for evidence of GHI among approximately 5000 patients entered between 1993 and 1996, with short stature (height SD score < -2) being treated with rhGH. Over 40% were deemed IGF-I deficient, and half of these to have 'primary IGF-I deficiency, i.e., normal GH responses with low IGF-I (237). The ISS group as a whole had a similar pattern of response to rhGH as did GHD patients during the first year of treatment, with growth response correlating inversely with IGF-I baseline levels, exactly the opposite of the correlation that would be expected if they had GHI (238). What cannot be appreciated from such a cross-sectional analysis of data from hundreds of pediatric endocrinologists is the clinical context in which the biochemical measures were obtained. Decreased circulating IGF-I with normal or elevated GH levels occurs with chronic illness and undernutrition. Many of the children seen with what is termed ISS (Vol. 2; Chap. 2) are poor eaters with decreased body mass index and may be receiving treatment for hyperactivity, which can suppress appetite and growth (239–241). Even acutely, IGF-I levels decline substantially with fasting, which is considered a means of protecting against potential insulin-like effects on glycemia (242). Clinical investigations of children with ISS and varying responses to GH stimulation tests or IGF-I generation tests (in which GH is given for several days to stimulate IGF-I synthesis) have indicated that GHI is, at most, an uncommon finding (243,244).

The possibility of an effect of heterozygosity for a mutation which causes GHRD in the homozygous state was explored in the unique Ecuadorian cohort with GHRD, which comprises a large population with a single mutation, permitting genotyping of numerous first degree relatives. There were no significant differences in stature between carrier and homozygous normal relatives, indicating a lack of influence of heterozygosity for the E180 splice mutation of the GHR (245). A more general indication of the lack of influence of heterozygosity for GHR mutations involving the extracellular domain on growth comes from studies of the large multicenter European-based GHRD study. In both the European and Ecudorian populations the stature of parents and of unaffected siblings does not correlate with statural deviation of affected individuals (245,246), while expected high correlation exists between parents and unaffected offspring (245). If the mutations that cause growth failure in the homozygous state also affected growth in heterozygotes, heterozygous parents and predominantly heterozygous siblings would have height SD values which correlated with those of affected family members. In the Ecuadorian families, there was no difference in height correlations with parents between carriers and homozygous normal offspring (245).

GH–GH Receptor Signal Transduction Failure

Evidence of a post-GHR defect in three siblings of Palestinian Arab origin has been presented by Laron et al. (247). The children had typical features of severe GHD, but normal GHBP and IGFBP-3 levels with severe IGF-I deficiency. The molecular defect has yet to be identified. In contrast to these patients, four GHBP positive children from two unrelated Pakistani families, who had high GH, low IGF-I, and low

IGFBP-3 serum concentrations, had no phenotypic features of Laron syndrome except for short stature which was less severe than in typical GHRD (248). Fibroblasts from the children in one of the families demonstrated failure to activate part of the GH signaling pathway (249). Two mutations have been described in the signal transducer and activator of transcription 5B (STAT5B) a critical step in the pathway from GH binding to IGF-I production, resulting in a typical GHI clinical and biochemical picture (250,251). One of these is a missense mutation resulting in aberrant folding and rapid disappearance of the abnormal protein (250,252). The other mutation results in total absence of the STAT5B (251).

IGF-Binding Protein 3/Acid Labile Subunit Mutations

Absence of ALS in a single patient resulted in markedly reduced circulating levels of IGF-I and IGFBP-3 with normal baseline and stimulated GH concentrations, and delayed adolescence with near-normal mature height (253).

Acquired Growth Hormone Insensitivity

Acquired resistance to GH is an ubiquitous adaptation in protein catabolic states such as malnutrition, poorly controlled diabetes mellitus, and chronic renal disease. Growth failure is a variable result. GHBP is diminished for reasons that remain unexplained in these conditions, with elevated GH concentrations, decreased IGF-I and normal to decreased IGFBP-3 concentrations. IGFBP-1 concentrations are elevated and reflect the catabolic state, and in those with diabetes, relative insulin deficiency (254). Children with end stage renal disease, in addition to having decreased GHBP, normal or elevated GH and low normal IGF-I concentrations, have high concentrations of IGFBP-3, IGFBP-1, and IGFBP-2, thereby decreasing IGF-I bioavailability (255). These catabolic or chronic disease conditions may affect the GH–IGF-I axis in a manner comparable to the sick euthyroid syndrome. In the case of renal disease, the excess binding of IGF-I is not sufficient reason for invoking a GH resistant explanation.

IGF-I Gene Mutation

The initial case of defective IGF-I synthesis due to a gene deletion was described in a patient with a homozygous partial deletion of the IGF-I gene. His profound intrauterine growth failure (IUGR) persisted into adolescence and he had sensorineural deafness with mental retardation (62). The absence of the craniofacial phenotype of severe GHD and the presence of normal IGFBP-3 in this patient, despite unmeasurable levels of IGF-I, indicated that the craniofacial features and low IGFBP-3 of GHD and GHRD are related to an absence of the direct effects of GH that do not act through the medium of IGF-I synthesis. It is also noteworthy that profound IUGR and mental retardation are not characteristic of GHD or GHRD (256,257), but IGF-I knockout mice have defective neurological development as well as growth failure (258). Thus, IGF-I production in utero does not appear to be GH–GHR dependent. Elevated GH levels would be expected in this circumstance as a result of the absence of IGF-I suppression of SS; diurnal levels of GH were high, with an overnight GH peak of 171 ng/mL (62). More recently, a second patient has been described with the same clinical phenotype of IUGR, sensorineural deafness, micrognathia, severe mental retardation, and severe postnatal growth failure, but with a defect in IGF-I synthesis resulting in production of a nonfunctioning IGF-I molecule circulating in high concentration (63).

IGF-I Receptor Mutation

Defects in the IGF type I receptor or in the IGF receptor signal transduction pathway have been hypothesized in several patients. IGF-I resistance has been invoked as a cause of short stature in the Pygmy, demonstrated by failure of response of T cells from such patients to IGF-I and reduced numbers of IGF-I receptors in vitro; the absence of IGF-I elevation that should accompany such resistance, however, requires explanation (259,260). Loss of a single allele for the IGF receptor caused by distal long arm deletion of chromosome 15 fails to abolish in vitro effects of IGF-I and, therefore, would not appear to explain short stature in affected patients (261).

Three patients have been reported with normal or high GH levels and elevated IGF-I levels. In two of these patients, normal bioactivity was demonstrated for the circulating IGF-I and fibroblasts from skin biopsies of these patients responded normally to recombinant IGF-I (262–264). It was hypothesized that these patients might have a tissue specific (i.e., skeletal) IGF-I unresponsiveness. Such an explanation could also be invoked to explain the findings of normal IGF-I levels, lack of response to exogenous GH, and normal fibroblast response to IGF-I in those patients with heterozygous deletion of the IGF-I receptor gene (261).

A systematic examination for mutations in the IGF-I receptor was pursued in two groups of children with IUGR who remained less than two SD for length after 18 months of age. This population was selected for study because IGF-I receptor knockout mice have more severe IUGR than do IGF-I knockout mice. Among 42 US subjects who did not have low IGF-I and IGFBP-3 concentrations, a single patient was identified with compound heterozygosity for mutations of the IGF-I receptor resulting in amino acid substitutions. She had severe IUGR (birthweight 1420 g at 38 weeks), poor postnatal growth, and elevated concentrations of IGF-I and integrated GH concentration when prepubertal, consistent with IGF-I resistance. The location of the mutations was within a putative ligand-binding domain and the heterozygous parents were subnormal in stature and had also had

low birthweight. A European group of 50 IUGR subjects was selected who had elevated circulating IGF-I concentrations, and a second subject identified who had a heterozygous nonsense mutation reducing the number of IGF-I receptors on fibroblasts; two other affected first-degree relatives were identified (64).

Acquired IGF-I Synthetic Defect

Alagille syndrome (chronic intrahepatic cholestasis) is associated with moderate growth failure in 50% of instances along with elevated GH and GHBP levels, and normal IGFBP-3 levels. Thus, this does not appear to be a GH resistant state but one involving failure of IGF-I generation due to hepatic dysfunction (265).

EVALUATION

Clinical Assessment for IGF-I Deficiency

Children should be evaluated for growth problems based on the following criteria:

- height velocity $<$25th percentile for $>$6 months
- downward crossing of percentiles on growth chart after age 18 months
- height below -2 SD for age
- height below genetic potential (-2 SD below midparental height)
- bone age delay comparable to height age.

Evaluation should begin with a complete history, including consideration of the mother's pregnancy (illness, toxins, alcohol/drugs used, and gestation), perinatal events, birthweight and length, signs of chronic disease or abnormality in psychosocial status, and growth history (Vol. 2; Chap. 1). Family history of growth and pubertal timing are important, and parental heights should be measured, if possible (266). Calculation of midparental and target heights provides a context for determining the child's growth potential, based on current bone age and height, and whether, in fact, there is an abnormality.

Midparental height is calculated as:

$$\text{Girls}: \quad \frac{(\text{Father's height} - 13\,\text{cm}) + \text{Mother's height}}{2}$$

$$\text{Boys}: \quad \frac{(\text{Mother's height} + 13\,\text{cm}) + \text{Father's height}}{2}$$

$$\text{Target height} = \text{Midparental height} \pm 2\text{SD} \quad (1\text{SD} = 5\text{cm})$$

Height needs to be measured against the wall, using a fixed scale and a right angle device against the head, with the back of the head, the spine, and the heels against the wall or device without flexion of the legs. Head circumference should also be measured and compared to standard curves for age and size. Body proportions can be determined by measuring span from middle fingertip to middle fingertip of the outstretched arms, and upper to lower segment calculated by measuring the distance from the top of the symphysis pubis to the floor with the legs slightly apart and straight. This distance is subtracted from total height to obtain the upper segment. Major and minor anomalies should also be sought as clues to syndromes associated with small stature; the presence of a dysmorphic syndrome, however, does not obviate the possibility of concomitant GHD.

Accurate weight is important because the endocrine causes of growth failure result in height deviation that is greater than that of weight, so that patients usually have greater than normal weight for height. The physical examination should also assess pubertal status and look for any signs of chronic illness, typically associated with low weight for height.

The typical somatic features of severe IGF-I deficiency, listed in Table 4, are not cause specific (Fig. 6).

Table 4 Clinical features of severe IGF-I deficiency due to GHD or GH receptor deficiency

Growth
Birth weight—normal; birth length—usually normal
Growth failure, from birth, with velocity 1/2 normal
Height deviation correlates with (low) serum levels of IGF-I, -II and IGFBP-3
Delayed bone age, but advanced for height age
Small hands or feet
Craniofacial characteristics
Sparse hair before age 7; frontotemporal hairline recession all ages
Prominent forehead (bossing)
Head size more normal than stature with impression of large head
"Setting sun sign" (sclera visible above iris at rest) 25% $<$ 10 yr of age
Hypoplastic nasal bridge, shallow orbits
Decreased vertical dimension of face
Blue scleras
Prolonged retention of primary dentition with decay; normal permanent teeth, may be crowded; absent 3rd molars
Sculpted chin
Unilateral ptosis, facial asymmetry (15%); only reported in GHRD
Musculoskeletal/body composition
Hypomuscularity with delay in walking
Avascular necrosis of femoral head (25% of GHRD)
High pitched voices in all children, most adults
Thin, prematurely aged skin
Limited elbow extensibility after 5 yr of age
Children underweight to normal for height, most adults overweight for height; marked decrease of ratio of lean mass to fat mass, compared to normal, at all ages
Osteopenia indicated by DEXA
Metabolic
Hypoglycemia (fasting)
Increased cholesterol and LDL-C
Decreased sweating
Sexual development
Small penis in childhood; normal growth with adolescence
Delayed puberty
Normal reproduction

Abbreviations: IGF, insulin-like growth factor; IGFBP-3, IGF binding protein-3; GHRD, growth hormone receptor deficiency; DEXA; LDL-C, low density lipoprotein-C.

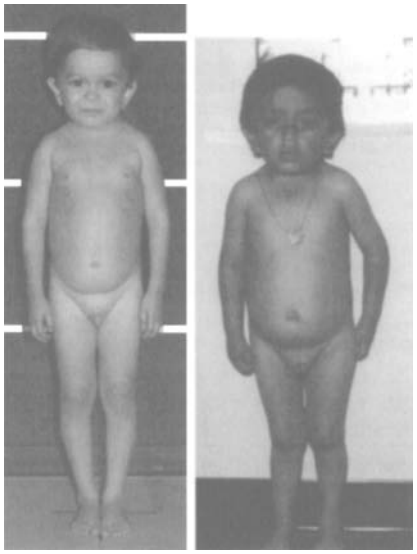

Figure 6 Similar phenotype for growth hormone (GH) receptor deficiency, (*left*), and idiopathic combined pituitary hormone deficiency (GH, thyroid), (right). The boy on the left, at 9.4 years of age, with a bone age of 3.5 years, has a height SD −9.1; despite the appearance of obesity, his weight for height is just under the 3rd percentile. The boy on the right, at 8.5 years, with a bone age of two years, has a height SD −8.5 and weight for height well below the 3rd percentile, approximately four SD below the mean. These patients are clinically indistinguishable despite their distinctive diagnoses; both show prominent foreheads, immature facies with foreshortening, the appearance of obesity, particularly truncal, despite reduced weight for height, and small phallus. *Abbreviations*: GHD: growth hormone deficiency; SD, standard deviation.

Many, if not most, such patients, including those with GHRH receptor deficiency, GH gene deletion, and GHRD, have normal intrauterine growth. SD for length declines rapidly after birth in severe GHD or GHRD (Fig. 7) indicating the GH dependency of extra-uterine growth. It was formerly thought that the first six months of infantile growth were not GH dependent, but this was based on inadequate data in severe GHD. Growth velocity with severe GHD or GHRD is approximately half normal (Fig. 8). Occasional periods of normal growth velocity, as noted in Fig. 8, may be related to improved nutrition (267).

Despite normal, if often delayed, sexual maturation in isolated IGF-I deficiency, the pubertal growth spurt is minimal or absent, as documented in some of the most extensive available data, in subjects with GHRD from Israel and Ecuador (35,269). The adolescent growth spurt is GH dependent, reflected in significantly elevated circulating levels of GH and IGF-I compared to preadolescence and adulthood (270). Among 24 Israeli patients with GHRD followed from infancy to adulthood, persistent growth beyond the normal time of adolescence was seen only in boys. In the Ecuadorian population girls also showed this phenomenon (Fig. 8). Adult stature in

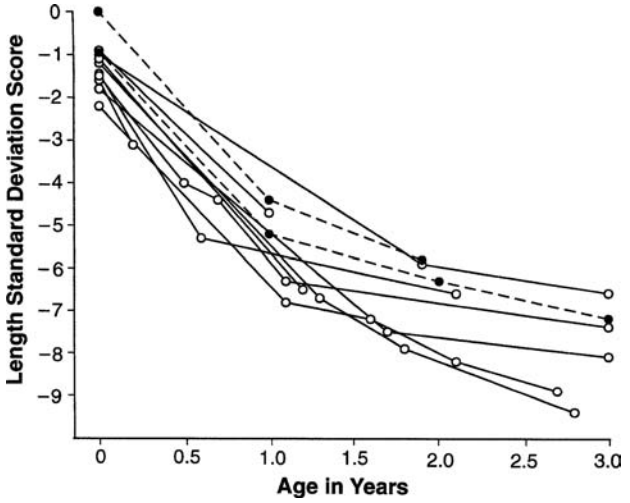

Figure 7 Length standard deviation scores of nine girls from Ecuador (*open circles and solid lines*) and two brothers from southern Russia (*solid circles and dashed lines*) with known birth lengths, followed over the first two to three year of life. *Source*: Adapted from Ref. 35.

GHRD varies from −12 to −5.3 SD in Ecuadorian patients and −9 to −3.8 SD in others in the literature, using the US standards (35). This is a height range of 95 to 124 cm for women and 106 to 141 cm for men in the Ecuadorian population. This wide variation in the effect of GHRD on stature was not only seen within the affected population but also between affected siblings, and this intrafamilial variability has also been described with severe GHD due to GH gene deletion (140) and between those having the same mutations in the GHRH receptor (25). With MPHD, resulting

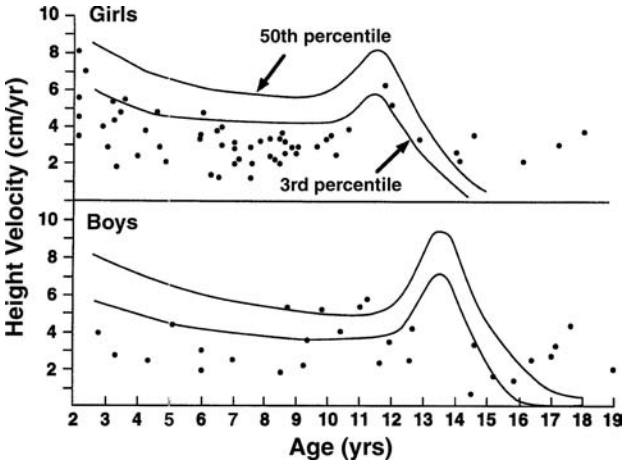

Figure 8 Growth velocities of 30 Ecuadorian patients (10 males) with growth hormone receptor deficiency; repeated measures were at least six months apart. *Source*: Adapted from Ref. 35, 268.

in failure of adolescence, growth may continue into the 30s and 40s (Fig. 9).

Children with familial causes of IGF-I deficiency may be recognized by knowledgeable family members at birth because of craniofacial characteristics of frontal prominence, depressed nasal bridge, and sparse hair, as well as small hands or feet, and hypoplastic fingernails. Decreased vertical dimension of the face is typical of severe GHD or GHRD and has been demonstrated in GHRD by computer analysis of the relationships between facial landmarks in all patients including those with normal appearing facies. This foreshortening is particularly apparent when patients are compared with their unaffected relatives (Fig. 4) (271). Blue scleras, the result of decreased thickness of the scleral connective tissue, permitting visualization of the underlying choroid, were originally described in Ecuadorian patients with GHRD, and subsequently recognized in other populations with GHRD, as well as in GHD (120,256,272,273).

Hypomuscularity is apparent in radiographs of infants with GHRD, and is thought to be responsible for delayed walking, despite normal intelligence and timing of speech onset (132). Although radiographs of the children suggest osteopenia and dual photon absorptiometry and dual energy x-ray absorptiometry in children and adults confirms this, dynamic bone histomorphometry in adults has been found to be substantially normal, suggesting that some of these findings are artifactual, based on small bone size (274). Limited elbow extensibility is an unexplained phenomenon seen in most patients over five years of age with GHRD and severe GHD. It is an acquired characteristic, absent in younger children and increasing in severity with age (124,132). Although children may appear overweight, those with GHRD, and some with GHD, are underweight to normal weight for height, while most adults, especially females, are overweight with markedly decreased lean to fat ratios (Figs. 4 and 8) (132).

Symptoms of hypoglycemia, including convulsions, particularly in infancy, are common with IGHD as well as with MPHD and GHRD, indicating the critical importance of direct metabolic effects of GH in the fasting state-increasing hepatic glucose output, decreasing glucose uptake, and increasing lipolysis (Table 2 and Fig. 3). Persistent physiologic jaundice of infancy has also been noted with GHD. Decreased sweating has been described in GHD and documented by pilocarpine electrophoresis in patients with GHRD. The subjects had significantly reduced sweat secretion rates and elevated sweat electrolyte concentrations, indicating that sweat gland function is under the influence of the GH–IGF-I axis (275).

Severe GHD is associated with small penis size with normal penile growth at adolescence or with testosterone treatment in childhood. This is also true of GHRD, but for as yet unexplained reasons, not in severe GH–IGF-I deficiency resulting from functional mutations of the GHRH receptor (25). Although puberty may be delayed three to seven years in some 50% of individuals with IGHD or GHRD, there is normal adult sexual function with documented reproduction by males and females (132). A couple with severe IGF-I deficiency due to GHRH receptor deficiency in Pakistan had an obligatorily affected, but otherwise normal child (25).

There is no evidence that GHD per se results in intellectual impairment. Studies that do not account for the ascertainment bias related to the cause of the GHD, or the effects of other deficiencies in MPHD, can be misleading. Intellectual impairment was

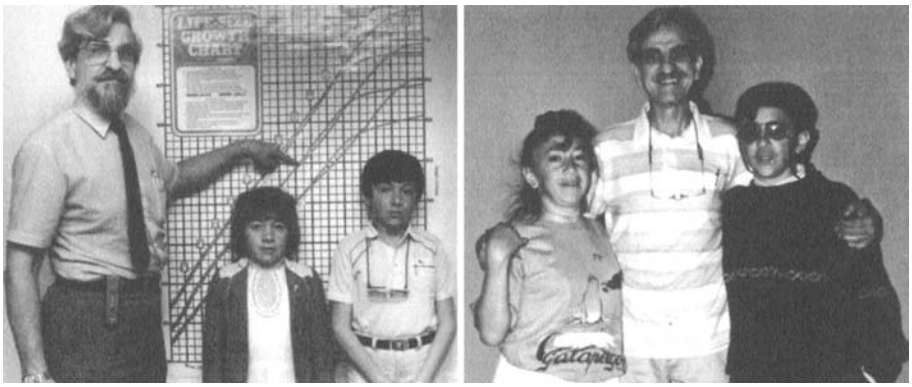

Figure 9 Adult Ecuadorian siblings with multiple pituitary hormone deficiency demonstrating persistence of responsiveness to growth promotion effects of exogenous growth hormone. (*Left*): 33.4-year-old woman with SD for height −5.9 and bone age 11-year and 31.7-year-old man with height SD −5.6 and bone age 13.5 years, with investigator at time of diagnosis. (*Right*): The woman is now 37.1 years of age with a bone age of 13.5 years and has received thyroid and cortisol replacement for 3.7 years, rhGH for 2.6 years (until 14 months previously) and low doses of estradiol for the past 2.6 years; her height SD is now −2.5, an increase of 3.4 SD. The man is now age 35.4 years with SD for height SD −3.0, an increase of 2.6 SD, and bone age 15.8 years; he has also received thyroid and cortisol replacement since diagnosis, rhGH until six months previously and incremental testosterone since one year after diagnosis. *Abbreviations*: rhGH, recombinant human growth hormone; SD, standard deviation.

originally considered a feature of the Laron syndrome (276). Among 18 affected children and adolescents with Laron syndrome administered the Wechsler Intelligence scale for Children, only three had IQs within the average range (90–110); of the remaining 15 subjects, three were in the low average range (80–89), three in the borderline range (70–79), and nine in the intellectually disabled range (< 70). These studies were done without family controls, so that the possibility of other factors related to consanguinity and social circumstances that might affect intellectual development could not be addressed. In a follow up study 25 years later, the investigators re-examined eight of the original 18 patients and four new patients with GHRD, excluding five patients with mental disabilities who were in the original study (277). This group had mean verbal and performance IQs of 86 and 92 on the Wechsler scale without evidence of visual motor integration difficulties that had been noted in the earlier group, but there was a suggestion of deficient short-term memory and attention. The investigators hypothesized that early and prolonged IGF deficiency might impair normal development of the CNS, or that hypoglycemia common in younger patients may have had a deleterious effect.

The recent description of intellectual impairment with severe IGF-I deficiency due to mutation of the IGF-I gene has added concern about potential effects of severe IGF-I deficiency in utero (62). Nonetheless, patients with severe IGF-I deficiency due to GH gene deletion or GHRH receptor deficiency have not been intellectually impaired (25,139). Sporadic anecdotal reports of patients with GHRD suggested a normal range of intelligence. The collective data from the European IGF-I treatment study group, which includes a wider range of clinical abnormality than either the Ecuadorian or Israeli population, notes a mental retardation rate of 13.5% among 82 patients, but formal testing was not carried out (246). Here again, the high rate of consanguinity was proposed as a possible explanation; hypoglycemia frequency or severity could not be correlated with these findings.

In the Ecuadorian GHRD cohort, exceptional school performance was reported among 51 affected individuals of school age or older who had attended school, with 44 typically in the top three places in their classes and most thought to be as bright or brighter than the smartest of their unaffected siblings (269). The first controlled documentation of intellectual function in a population with GHRD was in the Ecuadorian patients, a study of school age individuals compared to their close relatives and to community controls. No significant differences in intellectual ability could be detected among these groups, using non-verbal tests with minimal cultural limitations. It was hypothesized that the exceptional school performance in this population might have been related to the lack of social opportunities due to extreme short stature, permitting greater devotion to studies and superior achievement in school for IQ level (249).

The clinical findings of intellectual impairment with IGF-I gene deletion (62) and intellectual normality with GHRD is consistent with gene disruption studies in mice. The IGF-I deleted mouse is neurologically impaired, while the GHRD mouse is behaviorally normal (278). Thus, while GH-independent IGF-I synthesis appears necessary for normal brain development in utero, GH-dependent IGF-I production is not necessary for normal brain development and function.

Laboratory Evaluation

The methods for GH testing include provocative stimulation with arginine, insulin, clonidine, l-DOPA, and glucagon, frequently with propranolol priming, and physiologic testing with exercise or serial sampling. Priming with sex steroids is often done, especially in late prepubertal children. GH secretion is pulsatile throughout the day, with the usual concentration being low, typically below the limits of sensitivity of most assays. Thus, random GH samples are not helpful unless they are elevated, which might occur as a result of the stress of venipuncture. These various stimuli provoke GHRH release, suppress SS, or work in combination. Serial sampling studies have not been helpful in diagnosis (279). Exercise must be standardized in terms of VO_2max, which is difficult to accomplish.

The problems with GH testing include:

1. Response only correlates with successful GH treatment when there is unequivocal deficiency (280).
2. Deficient GH response to stimulation is seen without endocrine disease, for example in malnutrition, and during the slow growth phase of preadolescence.
3. There is great variability in the assay from laboratory to laboratory. The monoclonal immunoradiometric assay results in values that are approximately two-thirds those of the polyclonal assay (281).
4. There is great intraindividual variability in response from day-to-day, including nocturnal profiles (282).
5. Responses vary with age and body mass (283).
6. The definition of a normal response is arbitrary.

In view of these difficulties with GH testing, the measurement of GH dependent IGF-I and IGFBP-3 for the diagnosis of GHD has been proposed as a functional bioassay. These substances have low or no circadian variation, although prolonged fasting can significantly decrease IGF-I levels as the result of increase in IGFBP-1 (242). Although IGF-I and IGFBP-3 are present in sufficient concentration to minimize difficulties with assay sensitivity, there can be as much as a twofold variation in IGF-I results between laboratories (284) and IGFBP-3 has been noted to be highly specific (100%), but inadequately sensitive (27%) for diagnostic use (285). These measurements also vary with nutritional status and in disease states, and there is wide age variation. They may also be normal in children with GHD resulting from brain tumors.

Several studies have looked at the diagnostic usefulness of IGF-I and IGFBP-3 measurements.

Based on results of peak stimulated GH and mean nocturnal GH concentrations, Nunez et al. (286) separated 104 short statured patients into three groups, GH deficient (<7 mcg/L), borderline (7–10 mcg/L, or nocturnal GH below -2 SD of mean for age and pubertal stage), and ISS (>10 mcg/L). Confirmed was the earlier finding that nocturnal GH monitoring had little diagnostic value (279). IGF-I and IGFBP-3 concentrations, expressed as SD scores, correlated with peak stimulated GH but with wide scatter and primarily as a result of the strong correlations in the GH deficient group. Although means differed, there was not a significant difference between IGFBP-3 concentrations among patients in the three diagnostic groups, and only the GHD group differed in IGF-I mean concentration from the other two. The practical application of these findings was to use IGF-I for initial testing, with a criterion of -1.0 SD to identify the group that would include 88% of those with GHD, 71% of borderline GHD, and 46% of ISS. With IGF-I and IGFBP-3 above -1.0 SD, 68% of ISS would be identified as not requiring GH testing. Evaluation of growth velocity over the next three to six months will identify those children requiring further testing.

In a study that separated 203 children into two groups, GH deficient and normal on the basis of response to provocative testing, IGF-I and IGFBP-3 concentrations were found to correlate with peak GH response. In children <10 years of age with GHD, however, IGF-I concentrations were below the cutoff of -2 SD in only half, for a sensitivity of 53.3%. IGFBP-3 gave a comparable sensitivity of 60% and the combination was even less sensitive (46.6%). Specificity, however, was nearly 100% for both IGF-I and IGFBP-3. In 10–20-year-olds specificity and predictive value of these measures was generally lower (287).

Considering that the specificity of each of the measurements (IGF-I and IGFBP-3) is $>90\%$, subnormal concentrations undoubtedly support the diagnosis of GHD and combining two measurements of IGF-I with evaluation of growth velocity in another study gave a sensitivity and specificity $\geq 95\%$ for the diagnosis of GHD (288).

Growth Hormone Deficiency

GHD may be considered with the following findings or circumstances:

- other systemic causes of growth failure ruled out
- subnormal growth rate
- progressive decline in height percentile
- delayed bone age
- low IGF-I, IGFBP-3 [>1 SD below laboratory mean for developmental (bone) age]
- poor GH response to stimulation
- predisposing condition (e.g., brain irradiation, optic hypoplasia)
- other evidence of pituitary dysfunction (e.g., neonatal hypoglycemia, microphallus, mid-facial hypoplasia, central adiposity, single central incisor).

Consensus guidelines for the diagnosis and treatment of GHD in childhood and adolescence were developed by the GH Research Society in October 1999 (289). The process of evaluation of the GH–IGF axis was summarized as follows:

> In a child with slow growth, whose history and auxology suggest GHD, testing for GH/IGF-I deficiency requires IGF-I/IGFBP-3 levels and GH provocation tests after hypothyroidism has been excluded. In suspected IGHD, two GH provocation tests (sequential or on separate days) are required. In those with defined CNS pathology, history of irradiation, MPHD or a genetic defect, one GH test will suffice. In addition, an evaluation of other pituitary function is required. In patients who have had cranial irradiation or malformations of the hypothalamic–pituitary unit, GHD may evolve over years and its diagnosis may require repeat testing of the GH–IGF axis.
>
> It is recognized, however, that some patients with auxology suggestive of GHD may have IGF-I and/or IGFBP-3 levels below the normal range on repeated tests, but GH responses in provocation tests above the cut-off level. These children are not classically GH deficient but may have an abnormality of the GH–IGF axis and, after the exclusion of systemic disorders affecting the synthesis or action of IGF-I, could be considered for GH treatment.
>
> A magnetic resonance imaging (MRI) [or computed tomography (CT) scan] of the brain with particular attention to the hypothalamic–pituitary region should be carried out in any child diagnosed as having GHD.
>
> *Conclusion*: The diagnosis of severe GHD is usually straightforward, as there are well-defined clinical, auxological, biochemical, and radiological abnormalities. However, the diagnosis of moderate GHD can be associated with normal values within the IGF axis and a normal MRI. It is very important that the response to GH treatment be carefully reviewed, particularly in those patients with moderate GHD.

Growth Hormone Insensitivity–Growth Hormone Receptor Deficiency

GHRD is readily diagnosed in its typical and complete form because of: severe growth failure; the somatic phenotype of severe GHD; elevated serum GH levels; and marked reduction in IGF-I, IGF-II, and IGFBP-3 concentrations, with increased concentrations of IGFBP-1 and IGFBP-2. Most such individuals will also have absent to very low concentrations of GHBP, although the less common GHBP positive forms make absence of GHBP an important but not essential criterion. As noted in Table 3, some of the biochemical features of GHRD may be shared by conditions associated with acquired GHI, such as malnutrition and liver disease. In a large

multinational study designed to identify patients for replacement therapy with rhIGF-I, a scoring system was developed which assigned one point for each of the following:

- height >3 SD below mean height for age
- basal GH >2.5 mcg/L
- basal IGF-I $<$ 50 mcg/L
- basal IGFBP-3 <-2 SD
- IGF-I rise with GH(0.05 mg/kg/day $\times$ 4 days) $<$ 15 mcg/L
- IGFBP-3 rise with GH stimulation <0.4 mg/L
- GH binding $<10\%$ (based on binding of ^{125}I-hGH)

A score of 5 out of the possible 7 was considered diagnostic for GHR deficiency. This standard resulted in identification of 82 patients from 22 countries who reflect a wide variability for each criterion. Particularly noteworthy was that height SD range was up to -2.2 (290). These criteria recognize the age and sex (after seven years) variation of IGFBP-3 by using a standard of <-2 SD but, oddly, designate a fixed standard for IGF-I which falls within the range of normal for children under age 7.

As noted above, the presence of a homozygous mutation or a compound heterozygous mutation affecting the GHR usually provides definitive diagnosis. Thirty-one of the 82 patients reported by Woods et al. (246) had a genetic study of the GHR, of whom 27 had abnormalities affecting both alleles of the GHR gene, in association with clinically and biochemically unequivocal GHRD. Identification of heterozygous mutations, however, is not necessarily helpful because, as noted earlier, polymorphisms have been described which appear to have no phenotypic consequences.

TREATMENT

GH Replacement

The history of and current therapeutic recommendations for recombinant GH therapy in children and adults are discussed in detail in the chapter by Nathan and Allen (Vol. 2; Chap. 5).

IGF-I Therapy

Soon after the cloning of the human IGF-I cDNA, human IGF-I was synthesized by recombinant DNA techniques (rhIGF-I) (291,292). Subcutaneous preparations of rhIGF-I became available in 1990 and have been used since then for the treatment of severe IGF-I deficiency resulting from GHRD/Laron syndrome and for GH gene deletion in which inactivating antibodies to exogenous rhGH have developed. In total, approximately 150 patients have been treated (70,71,293–300). Data for 58 of these patients were used for US Food and Drug Administration approval of rhIGF-I (Increlex^{TM} (mecasermin), Tercica Inc., Brisbane, California, U.S.A.) and for another 25 patients, approval of the binary protein complex of rhIGF-I and rhIGFBP-3 (iPlex^{TM} (mecasermin rinfabate), Insmed Inc., Glen Allen Virginia, U.S.A.) as orphan drugs (72,73,301).

Dose Response

In the European multicenter study (296), twice daily dosages varied according to response, but average dosage was similar to that used in the North Carolina (71,297,298,300) and Ecuadorian (70,293,294) study populations. As predicted from short-term studies, IGFBP-3 levels did not increase during long-term treatment with rhIGF-I. In the only direct comparison of dosages, there was no difference in growth response between 80 mcg/kg body weight and 120 mcg/kg twice daily; apparently defining a plateau effect (70). Improvement in mean height SD over two years was 1.2 in the European study, 1.5 for the higher dose and 1.3 for the lower dose in Ecuador, and 1.3 in the North Carolina study. The European multicenter study and Ecuadorian study patients achieved two-thirds of their improvement in the initial year. In the Israeli patients treated with single daily injections of rhIGF-I (120 mcg/kg), there was an improvement of only 0.4 SD during the first year of treatment with no further improvement for the six patients completing two years of therapy (295). This supports the rationale for twice daily administration, which was based on kinetic studies in normal controls.

Comparison of growth response of 22 rhIGF-I-treated GHRD patients and 11 GH-treated GHD patients in the same setting demonstrated growth velocity increments in those with GHRD to be 63% of that achieved with GH treatment of GHD in the first year and less than 50% in the second and third years (70). The inadequate growth response compared to GH treatment of GHD persisted over this longer term treatment period, with a mean improvement in height SD of only 1.4, from -5.6 to -4.2, thus only sustaining the improvement of the first two years of treatment. The report from the European study described 17 patients treated for 48 months or longer. Overall increase in height SD was 1.67 ± 1.16, suggesting modest continued improvement over time in this group, but still markedly less than expected with GH replacement therapy (296). Studies in Ecuador noted correlation of growth acceleration with trough levels of serum IGF-I, obtained before the 12 hourly injection (70). Also noted was an increase in body weight for height; in the European study, the gain in BMI correlated with improvement in SD for height. This increasing BMI with treatment likely reflects high-dose IGF-I insulin-like effects during peak times following IGF-I injection.

The lesser response to IGF-I in GHRD than to GH in GHD could be attributable to failure to increase IGFBP-3 and ALS levels, a direct GH effect, but three children who had defective IGF-I synthesis attributed

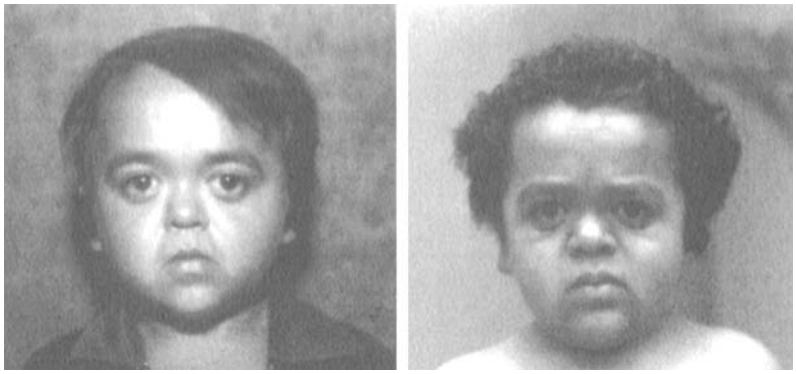

Figure 10 Face and hair changes in 17.7-year-old patient (bone age 13 years) with GHRD during six months treatment with IGF-I, 120 mcg/kg bid and depot GnRH agonist begun at age 16.5 years. *Abbreviations*: GHRD, growth hormone receptor deficiency; IGF-I, insulin-like growth factor-I; GnRH, gonadotropin releasing hormone. *Source*: Adapted from Ref. 159.

to a post-receptor defect did not grow better while receiving IGF-I than did subjects with GHRD, despite their normal IGFBP-3 levels (295). Furthermore, acromegaloid facial changes in some patients (Fig. 10) indicate that the amount of IGF-I reaching tissues is supraphysiologic (61,293,299). Inadequate binding of exogenous rhIGF-I should increase the risk for hypoglycemia in IGFBP-3 deficient patients, as free IGF-I would bind to the insulin receptor; however, placebo-controlled study of IGF-I treatment of children with GHRD showed no increase in hypoglycemia over a 6-month period (294). More recent study of children with low IGF-I and IGFBP-3 levels monitored over a 24-hour period at the end of two weeks of rhIGF-I treatment failed to demonstrate IGF-I induced hypoglycemia (302). The more likely explanation for the relatively modest growth response is the absence of the direct GH effects at the growth plate. These effects include epiphyseal prechondrocyte differentiation, increased responsiveness to IGF-I, and enhancement of local production of IGF-I that stimulates clonal expansion of the differentiating chondrocytes (68,69). Of great interest would be studies of the administration of IGFBP-3 with IGF-I, and as noted above, an equimolar preparation of rhIGF-I–rhIGFBP-3 was approved in late 2005 for use in severe IGF-I deficiency due to GHRD or GH inactivating antibodies. The advantages of the combination were considered to be the possibility of single daily injection, lesser risk of hypoglycemia, and possible improved growth response resulting from sustained circulating levels of IGF-I. In subjects with molecularly proven GHRD, twice daily rhIGF-I and once daily rhIGF-I–rhIGFBP-3 in comparable dosage produced similar maximal concentrations of IGF-I and areas under the curve, but the T_{max} for the combination was double that of the IGF-I alone (303). These data and the observations in rhIGF-I-treated GHRD suggest alternative binding in the absence of IGFBP-3, perhaps from the increased IGFBP-2 seen in GHRD patients given rhIGF-I (58). Considering that the IGF-I–IGFBP-3 combination lacks the stabilizing ALS component of the physiologic circulating ternary complex, the advantage of the preparation remains unclear.

Adverse Events

Hypoglycemia is frequent in children with GHRD, and was a concern with rhIGF-I treatment because of the very low IGFBP-3 levels resulting in greater amounts of free IGF-I. Severe hypoglycemic episodes have been reported in the European treatment study (296). During a six-month placebo-controlled trial of rhIGF-I treatment in children with GHRD, however, there was no difference in the frequency of hypoglycemia between placebo and treatment groups (294). Headache is a frequent complaint among treated patients, but also did not vary in frequency between placebo- and rhIGF-I-treated patients (296). Pain at the injection site is common and injection into lumps may result in cessation of response. Tachycardia, reflecting the inotropic effect of IGF-I (304), is uniformly present early in treatment, but clears after several months (305). Less frequent side effects include parotid swelling, facial nerve palsy, lymphoid hyperplasia, which may require tonsillectomy or adenoidectomy, papilledema, and pseudotumor cerebri. Coarsening of the facial features with mandibular hyperplasia and excessive weight gain are also seen in some patients (Fig. 10) (61,299,306). Hyperandrogenism with oligomenorrhea or amenorrhea, acne, and elevated serum androgens has been described in prepubertal and young adult patients given single daily injections of rhIGF-I (307).

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4

Growth Hormone–Deficiency in the Adult: Transition from Adolescence to Adulthood

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FINAL ADULT HEIGHT REACHED BY CHILDREN WITH GROWTH HORMONE DEFICIENCY

With the introduction of recombinant human growth hormone (GH), GH-deficient (GHD) children have been treated with larger doses and in a continuous fashion and have been followed to final height. Near-adult height data were reported by Blethen et al. (1) in 121 GHD children who were prepubertal at the beginning of treatment; GH was administered at a dose of 0.3 mg/Kg/wk, initially three times weekly and then daily. The adult height standard deviation (SD) score was found to be -0.7 ± 1.2, which was significantly greater than the pretreatment height SD score, the predicted adult height SD score, and the height SD score at the start of puberty. The etiology of GH-deficiency and the presence or absence of spontaneous puberty did not affect the outcome. Adult height in this study was positively dependent on height and negatively dependent on age at the start of the study; statistically significant variables included duration of treatment with GH, age and height at the start of GH and the growth rate during the first year of therapy.

In an effort to assess the efficacy of GH therapy in GHD children treated before the age of three, Rappaport et al. (2) treated a cohort of 49 children with isolated GH-deficiency or multiple pituitary hormone deficiency with a GH dose of 0.6 IU/Kg/wk (0.2 mg/Kg/wk). The mean height SD score of treated patients increased from -3.6 ± 1.0 to -0.9 ± 1.2 after four years of treatment, reaching a plateau after five years of therapy with a height SD score -0.8 ± 1.2 SD.

Combined GH and gonadotropin-releasing hormone analog (GnRHa) treatment, in an attempt of preserving the height potential of patients with GH deficiency and early puberty, was evaluated by Adan et al. (3) and subsequently by Mericq et al. (4). The latter, in a prospective, randomized study compared the effects of GH treatment combined with a GnRHa with that of GH alone on the near final height of 17 pubertal GHD patients and concluded that the addition of a GnRHa to GH in pubertal GHD adolescents increases their final height (-1.3 ± 0.5 SDS vs. -2.7 ± 0.3 SDS in children treated with GH alone). These findings will have to be confirmed in a larger population of GHD children, because the combined use of GnRHa and GH has proven to be less effective and remains controversial in short non-GHD patients entering into puberty at an early age (5).

TRANSITION FROM CHILDHOOD TO ADULTHOOD GROWTH HORMONE DEFICIENCY

Rationale for Continued Growth Hormone Treatment

Until recently, GH treatment was discontinued in GHD adolescents once they reached their final adult height. It is becoming clear, however, as demonstrated by studies by Baroncelli et al. (6) and Attanasio et al. (7), that muscle and bone mass continue accumulating during the phase of development that occurs after the end of linear growth, reaching adult values during this period. Attanasio et al. (7) showed that patients with childhood-onset GHD who were treated only to final height suffered a significant maturational deficit. The body mass, skeleton, muscle, and probably fat were suboptimally acquired and there was a relative failure of anabolism in terms of achieving the genetically determined cell mass, tissue, and organ growth. Although continued gains in lean body mass are a feature of healthy physical development after linear growth ceases, recent studies by Carroll et al. (8) showed that lean body mass remains unchanged over 12 months after GH cessation in GHD patients treated to final height, reducing potential exercise capacity and muscular strength in adult life. On the other hand, the accrual of lean body mass continued in GHD patients who remained on GH replacement after completion of linear growth. As to bone metabolism, Baroncelli et al. (6) reported that GHD adolescents have delayed timing and reduced mean values of both lumbar peak BMD (pBMD) area and pBMD volume,

as well as a rapid decline of lumbar BMD volume in comparison with controls. They concluded that the discontinuation of GH treatment before the attainment of lumbar pBMD could be the main cause of the reduced BMD in patients with GHD during the transition from late adolescence to early adulthood. Recent studies have also demonstrated a decrease of both bone formation and bone resorption markers, including osteocalcin, carboxy-terminal propeptide of type 1 procollagen (PICP), bone-specific alkaline phosphatase, and the carboxy-terminal cross-linked telopeptide of type 1 collagen (ICTP) two years after discontinuation of GH treatment in GHD adolescents who had reached final height (9).

In addition to an abnormal body composition, untreated GHD adolescents into adulthood have elevated fasting total and low-density lipoprotein (LDL) cholesterol and triglycerides levels (10,11) and increased postprandial triglyceride concentrations (12). Additionally, the concentrations of the independent cardiovascular risk factor, homocysteine, have also been reported to be elevated (12,13). Recent studies by Lanes et al. (14) also demonstrated an increase in peripheral inflammatory and fibrinolytic markers in GHD subjects, beginning in adolescence. These markers seem to be related to the elevated levels of fasting and postprandial triglyceride-rich lipoproteins (TRPs), suggesting that lipoprotein remnants may induce an inflammatory response in endothelial cells and macrophages. GHD adolescents have a reduced left ventricular mass (15,16) and may present vascular abnormalities manifested by lower flow-mediated endothelium-dependent vasodilation (17). These findings together with an increase in the epicardial adipose tissue of GHD adolescents, a good indicator of abdominal/visceral fat, demonstrate the presence of a number of potentially important differences between GHD patients and controls, which may contribute to an increased long-term cardiovascular risk.

One could therefore argue based on these studies that continued GH treatment from adolescence into early adulthood may contribute to the attainment of a normal bone and muscle mass and contribute to a decrease of the cardiovascular risk of GHD adults. However, very recent data by Mauras et al. (18) question the need of GH treatment in GHD adolescents properly treated in childhood who are at near completion of their linear growth. Continuation of GH therapy for two years in a group of these patients did not change their BMD, body composition, cardiac function, muscle strength, carbohydrate and lipid metabolism, and quality of life (QoL) measures, as compared to placebo-treated or control subjects. Therefore, controversy still exists on whether GH should be discontinued in adolescents who have reached their final height until the deleterious effects of GH-deficiency in adults become apparent or whether it should be continued without interruption into adulthood. However, the continuation of GH treatment after final height is reached should be based on a careful assessment of the status of the patient, including a determination of the persistence of GHD after adult height is achieved and/or the development of alterations of the adult GH-deficiency syndrome.

Reevaluation of the Growth Hormone Status of Growth Hormone–Deficient Adolescents

Adolescents or young adults with a diagnosis of childhood-onset GHD must have this diagnosis confirmed when the final adult height is reached, because persistence of severe GHD is only present in less than 50% of retested patients (19,20). The gold standard GH stimulation test is the insulin tolerance test (ITT), because it provokes a pronounced GH response in normal individuals and it allows for simultaneous pituitary–adrenal axis to be tested. The diagnostic cut-off value of either 3 μg/L or 5 μg/L GH level attained after this provocative stimuli has been recommended. The cut-off value of 5 μg/L yields a specificity of 97%, a sensitivity of 100%, a positive predictive value of 99%, and a negative predictive value of 100% (21). This test, however, may be associated with undesirable side effects and can be dangerous in patients with a history of seizures or cardiovascular disease. Thus it should only be performed in carefully supervised setups.

Other stimulation tests can be used provided that test-specific cut-off points are employed. Biller et al. (21) compared six different tests for the diagnosis of GHD deficiency in adults with hypothalamic–pituitary disease and demonstrated that the greatest diagnostic accuracy was obtained with the ITT and the Arginine-growth hormone–releasing hormone test (ARG-GHRH), while there was more overlap with other tests including arginine, larodopa, and arginine-larodopa. The GHRH-pyridostigmine (GHRH-PD) test can be used to assess the GH status in patients with pituitary rather than hypothalamic alterations. No side effects were noted with the ARG-GHRH test, while the GHRH-PD test showed unpleasant side effects as a consequence of an increased cholinergic tone. While Aimaretti et al. (19) have suggested a cut-off point of 9 μg/L in GH levels for these tests, a more recent study by Biller et al. (21) suggested using a cut-off point of 4.1 μg/L. These levels provided 95% sensitivity and 91% specificity. One can conclude after reviewing the literature that the ARG-GHRH test should be used for the reevaluation of patients with childhood-onset GHD of assumed pituitary origin, while the ITT remains the most valuable alternative for the reevaluation of patients with childhood-onset GHD of hypothalamic origin. The ARG-GHRH test, however, seems not to be a reliable test for GHD due to craniospinal radiation therapy (22).

In patients with organic hypothalamic–pituitary disease, the likelihood of persistent GHD increases when there is an increasing number of pituitary hormone deficiencies. The probability of GH-deficiency in patients with three to four pituitary hormone deficiencies and low IGF-1 levels ranges from 91% to 100% (21).

IGF-1 SDS estimation by itself could be considered adequate in patients with childhood-onset GHD and multiple hormone deficiencies (23). IGF-I levels should serve as one of two tests of GH status in individuals with isolated GHD. While IGF-1 levels are diagnostic in acromegaly, they are less accurate for diagnosis in GHD, particularly in adults, as serum IGF levels decline with age (24–26); the typical IGF-I considered to indicate GHD being 70 to 80 mcg/L.

Recent studies have demonstrated that provocative testing for the diagnosis of GHD in patients with any degree of obesity will not accurately distinguish normal from deficient responses (27). Therefore large population studies are required to establish appropriate GH cut-off points for individuals with elevated body mass index values.

It has been suggested that patients with childhood-onset GHD and ectopic posterior pituitary hyperintense signal (EPPHS) in a magnetic resonance imaging (MRI) should not be retested when adult height is achieved. In a recent study, Leger et al. (28) showed that it is not necessary to reconfirm GHD by GH provocative testing once final height is reached in patients who have no visible pituitary stalk on MRI, a location of EPPHS at the median eminence, and multiple anterior pituitary hormone deficiencies. However, all other patients, including those with isolated GHD and those who present another anterior pituitary hormone deficiency, regardless of the location of the EPPHS, should be retested. It is important to keep in mind that patients with isolated GHD could present with multiple pituitary hormone deficiencies at any age (29).

ADULT GROWTH HORMONE DEFICIENCY

Causes

Adult GHD is encountered mainly in patients with pituitary or hypothalamic disease. It may be seen in patients who have genetic, congenital, or acquired alterations beginning in infancy and childhood, which persist into adulthood with various degrees of GHD and/or hypopituitarism (Table 1). These conditions are reviewed in detail in other chapters of this book (Vol. 2; Chaps. 2, 3 and 5). In children, GHD is usually an isolated alteration though it may be associated with one or more pituitary hormone deficiencies. GHD can be secondary to neoplasms, which can be developmental in origin, among which a craniopharyngioma is the most common. There may be pituitary or extrasellar tumors (pituitary macroadenomas, germinoma/teratoma, meningioma, astrocytoma, ependymoma, dermoid cyst, or metastatic neoplasias). The likely mechanism of the hormonal deficits in patients with tumors is compression of the portal vessels in the pituitary stalk, either due to the expanding tumor mass or due to raised intrasellar pressure. It could also result from infiltrative/granulomatous disease of the hypothalamus and stalk, which can cause hypopituitarism and diabetes insipidus (sarcoidosis, tuberculosis, eosinophilic granuloma, Wegener's granulomatosis, lymphoma, or Langerhans cell histiocytosis). Hypopituitarism may also be due to infarction, autoimmune disease (lymphocytic hypophysitis, which may involve the pituitary and the stalk), cranial radiation, which evolves with time and is dose dependent, surgery, or trauma. GHD can also be associated with brain structural defects (such as agenesis of the corpus callosum, septo-optic dysplasia, empty sella syndrome, and hydrocephalus) and with midline facial defects. However it is often idiopathic in nature and this type accounts for most individuals with childhood-onset GHD, many of which when reassessed in adulthood are found to have normal GH responses (30).

Table 1 Major Causes of Growth Hormone Deficiency

Genetic
(a) Leading to multiple hormone deficiency
Transcription factor defects (PIT-1, PROP-1, LHX3/4, HESX-1, PITX-)
(b) Leading to isolated growth hormone deficiency
Growth hormone–releasing hormone receptor gene defects.
Growth hormone (GH) secretagogue receptor gene defects
GH gene defects
GH receptor/postreceptor defects
Neoplasm
Craniopharyngioma, Rathke's pouch and arachnoid cyst
Glioma/astrocytoma
Germinoma
Ependymoma
Pituitary adenoma (functioning and nonfunctioning)
Dermoid/epidermoid cyst
Metastatic
Others
Infiltrative
Granulomatous disease
Sarcoidosis
Tuberculosis
Syphilis
Wegener's granulomatosis
Langerhans cell hystiocytosis
Autoimmune
Lymphocytic hypophysitis
Isolated adrenocorticotropic hormone deficiency
Cranial Radiation
Surgery
Acquired
Perinatal trauma
Postnatal trauma
Central nervous system infection
Associated with midline defects
Cleft-lip/palate
Single central incisor
Associated with brain structural defects
Septo-optic dysplasia
Agenesis of corpus callosum
Empty sella syndrome
Hydrocephalus
Encephalocele
Holoprosencephaly
Prader-Willi syndrome
Infarction
Idiopathic

Clinical Manifestations of Adult Growth Hormone–Deficient Syndrome

Changes in Body Composition

Individuals with adult GH-deficiency secondary to pituitary or hypothalamic disease exhibit nonspecific symptoms such as lack of energy and decreased vitality and develop abnormalities in body composition characterized by a significant increase in fat mass, particularly visceral fat, and a decrease in lean body mass, which may account for diminished strength and exercise capacity (31,32). Decreased GH secretion has been implicated as a risk factor for abdominal and visceral adiposity and visceral adiposity has been associated with increased inflammatory cardiovascular risk factors, dyslipidemia and insulin resistance (32).

Insulin sensitivity is reduced in adults with GHD and this is probably explained by increased central adiposity, reduced muscle mass, and low levels of serum IGF-1. Short-term GH replacement leads to a further decrease in insulin sensitivity, but most available data suggest a return to baseline values with prolonged treatment. A generally accepted hypothesis for the return of insulin sensitivity toward baseline levels after 3 to 12 months of GH replacement is the beneficial effect of GH on body composition with a sustained increase in lean mass and a sustained reduction in body fat. The short-term effect of GH therapy with increased lipolysis, lipid oxidation, and circulating free fatty acid concentrations deteriorates insulin sensitivity; the long-term effect with a reduction in body fat is beneficial for insulin sensitivity (33).

Muscle mass increases in GHD patients on GH treatment, and an increase in muscle strength and an improved exercise performance have been noted in these patients. Svensson et al. (34) showed that GH replacement therapy in adults with adult-onset GHD normalized isometric and isokinetic knee flexor and extensor strength, while handgrip strength increased. Ter Maaten et al. (35) demonstrated an increase in maximal workload and oxygen consumption in GHD adults after long-term GH therapy.

Cardiovascular Risk Factors

A number of cardiovascular risk factors have been found to be increased in GHD adults, placing them at a higher risk for cardiovascular events. Untreated GHD adults have been found to have elevated fasting cholesterol and triglyceride levels (32,36). Abnormalities in serum lipids of GHD patients may be due to an increase in the secretion rate and a reduction in the clearance rate of very low–density lipoproteins (VLDL). The increased VLDL-apo B secretion is probably related to the abdominal obesity of GHD patients, because central adiposity when combined with insulin resistance increases VLDL-apo B secretion from the liver (37).

In recent years, considerable evidence suggesting a positive correlation between the postprandial triglyceride response to an oral lipid load and atherosclerosis of the carotid arteries and coronary arteries was found in adults (38). Al Shoumer et al. (39) and Twickler et al. (40) reported increased fasting and postprandial levels of triglycerides and TRP in adult-onset GHD patients, suggesting that these changes may contribute to an observed increased vascular morbidity and mortality. The accumulation of postprandial TRP in adult-onset GHD may be explained by a decrease in their removal from the circulation via hepatic lipoprotein receptors, because the expression of several hepatic surface receptors such as LDL and LDL-receptor–related protein receptors is lower in GHD states than in healthy subjects.

Abnormalities of coagulation factors, suggestive of a defective fibrinolytic system, such as elevated tissue plasminogen activator inhibitor-1 (PAI-1), fibrinogen, and factor VII concentrations have been reported in GHD adults (41). Obesity and in particular abdominal fat is associated with increased concentrations of fibrinogen and PAI-1 activity; in subjects with GHD, this high activity may be linked to their markedly higher waist–hip ratio and high triglycerides might contribute to the elevated PAI-1 activity in GHD men (42,43).

The plasma levels of proinflammatory cytokines such as interleukin (IL) -6 and tumor necrosis factor-α are increased during the postprandial period in GHD adults and are related to the presence of elevated levels of lipoprotein remnants (44). Lipoprotein remnants may induce an inflammatory response in endothelial cells and macrophages. In addition, untreated GHD patients have increased levels of C-reactive protein (CRP) and IL-6 (45). The IL-6 concentrations are independently associated with the degree of common carotid artery intima-media thickness, so that the increased inflammatory activity of the vessel may be the cause of the high IL-6 levels.

Independent cardiovascular risk factors such as lipoprotein(a) and homocysteine have also been found to be elevated in GHD adults (46,47). Lipoprotein(a) is an atherogenic lipoprotein that can be thrombogenic and may be used as a plasmatic marker for individuals at risk for cardiovascular events. Homocysteine is prothrombotic and the circulating concentrations correlate with vascular endothelial injury and dysfunction. The median level of homocysteine at baseline of GHD adults was found to be at the 90th percentile of a comparable subset from a large cross-sectional U.S. study in non-GHD adults (47).

Adults with GHD have been shown to have an impairment of cardiac performance as manifested by a reduction in left ventricular mass, an inadequate ejection fraction, and abnormalities of left ventricular diastolic filling (48–50). Cardiac performance was correlated with the severity of the GHD, because a more significant impairment was found in patients with severe GHD as compared with partial GHD, and was not present in non-GHD hypopituitary patients (48). Other studies reported increased

intima-media thickness, with more atheromatous plaques in the carotid and the femoral arteries of GHD adults as compared with controls matched for age, sex, and body weight (51,52). This increased intima-media thickness, which represents the earliest morphological change in the arterial wall in the process of atherogenesis, has been detected even in the absence of clear-cut alterations of classic vascular risk factors. Adult GHD has also been found to be associated with endothelial dysfunction and with an increase in large-artery stiffness and this involves a reduced availability of endothelial nitric oxide (NO), a vasodilatory compound. IGF-1 has a direct NO-releasing effect in cultured human endothelial cells and low basal IGF-1 levels in serum are associated with low basal urinary nitrate and cyclic adenosine monophosphate excretion (52).

Life expectancy is reduced in hypopituitary patients with GHD (53–55) largely as a consequence of cardiovascular disease (53). GH treatment normalized cardiac mass and function and resulted in a reduction in arterial stiffness (56–58). Thereby it may also result in enhanced life expectancy and decreased morbidity of cardiovascular disease (59).

Bone Metabolism

GHD adults have a low bone mass, despite prior GH substitution (60,61). Osteopenia is present in both patients with isolated GH deficiency and multiple pituitary deficiencies, supporting the view that GH deficiency per se is responsible for the observed deficit. The pathogenesis of the reduced bone mineral content (BMC) and density in patients with childhood-onset GHD is more likely due to deficient build up of the bone mass, rather than from premature bone loss, whereas in subjects with adult-onset GHD, the pathogenesis is less clear. GHD adults have been found to have low levels of bone formation markers (osteocalcin, procollagen-III, and PICP) and of bone resorption markers (ICTP), suggesting decreased bone turnover in this group of patients (60).

The degree of GHD seems to be correlated with the severity of bone mass and turnover impairment (61). A significant reduction of BMD associated with abnormalities of bone turnover parameters was found only in patients with very severe or severe GHD. The effect of severe GHD on BMD seems to be dependent on age, so that low bone mass was observed in young patients, while adults over the age of 60 demonstrated a mean BMD Z score above that of the reference population and significantly greater than that of young GHD adults (62). A recent study suggests that it is mainly the BMC that is affected in GHD adults, so that the BMC of the lumbar spine, femoral neck, and total body of patients with either isolated GHD or multiple GHD where 52% to 71% of expected values of that of age- and sex-matched controls, while the calculated true bone density was normal (63). The prevalence of lifetime low-energy fractures was not increased in patients with isolated GHD, but it substantially exceeded the expected prevalence in patients with multiple pituitary hormone deficiencies.

Quality of Life Issues in Growth Hormone Deficiency

The definition of QoL is complex and has been the subject of several recent reviews (64). Older studies have relied on well-validated, generic tests of overall health and psychiatric well-being such as the Nottingham health profile or the general health questionnaire applied to hormone-deficient patients. Another approach to the measurement of QoL in adult GHD patients is the development of disease-specific questionnaires based on in-depth relatively unstructured interviews, such as the QoL-assessment of GH-deficiency in adults. This addresses seven areas of concern to GHD patients, such as body image and fat distribution, energy level, concentration and memory, irritability and temper, strength and stamina, coping with stress and physical and mental drive.

Poor body image and socialization, low energy, irritability, poor concentration and diminished memory, mental fatigue, social isolation, decreased self-esteem, and decreased life satisfaction appear to be more prevalent in the GHD population (64,65). In untreated GHD patients, decreased physical mobility and general health, as well as a trend toward a reduction in well-being were described. Although most studies of QoL have been performed in adult-onset GHD, analysis of social indices and psychological parameters suggests that QoL may also be impaired in childhood-onset GHD. However, when patients with childhood-onset GHD were compared to their same sex sibling, virtually all of the differences noted when compared to norms tended to disappear; the most marked deficits in the adaptive functioning where those of patients with multiple anterior pituitary hormone deficiencies as compared to subjects with isolated GHD. When examining an unselected cohort of elderly GHD adults, impaired QoL is not universally present; while some QoL domains deteriorated in the patients with GHD studied by Gilchrist et al. (66), the control subjects also had worsening QoL scores after two years, which were similar to the GHD patients.

Morbidity and Mortality

Several retrospective studies have revealed an increase in mortality in hypopituitary adults (67,68); three of these studies found that the premature mortality was due to an increase in cardiovascular mortality, while one study found a small nonsignificant increase in cardiovascular deaths. Another reported a small increase in overall mortality, but cardiovascular mortality was decreased. Whether the increased mortality in hypopituitary adults is due to untreated GHD, inappropriate or insufficient replacement of other pituitary hormones or is rather related to the surgery or radiation often utilized in the treatment of the underlying disease is unknown.

In a review of the long-term mortality of pituitary-derived GH recipients, Mills (69) reported data from the National hormone and pituitary program of over 6000 patients. There was an alarming rate of mortality, 433 deaths compared to an expected number of 114. Only patients with isolated GHD had a death rate similar to that expected for the general population. The highest risk categories included patients with tumors, adrenal insufficiency, and hypoglycemia. There were 26 deaths from Creutzfeldt-Jacob disease. In 25% of the fatalities, death was sudden and unexpected. Thus it appears that adrenal insufficiency played an important role leading to death even in the adult. Inappropriate steroid replacement even during trivial infections may have played a role, so that these episodes must be aggressively treated. There were 20 sudden deaths in patients without adrenal insufficiency, thus suggesting that other factors like uncontrolled diabetes insipidus may also play a role.

In a report by Svensson et al. (59) in a large population of hypopituitary adults without GH replacement, overall mortality and morbidity and the rates of myocardial infarctions, cerebrovascular events, and malignancies were increased when compared with the normal population. While the data on both fatal and nonfatal morbidity confirm the previous observations of an increase in the relative risk and vascular mortality in hypopituitary patients, the 3.8 times increase in the relative risk for total mortality in this population of hypopituitary adults without GH replacement is larger than previously observed. The rate of cerebrovascular events was increased in both men and women without GH replacement therapy, but this could be secondary to radiation angiopathy.

Conflicting results regarding the incidence of malignancies in patients with pituitary disease have been reported. While some studies have found a decreased rate of malignancies in hypopituitary men without GH replacement, an increased rate was observed in hypopituitary women who did not receive GH treatment. In retrospective studies by both Popovic et al. (67) and Nilsson et al. (68), the rate and the mortality from neoplasia were increased in patients with pituitary tumors. In the study by Svensson et al. (59) in hypopituitary adults without GH replacement, the incidence and the overall mortality from malignancies were increased; it is, however, possible that this increased incidence could be partly explained by factors other than hypopituitarism and its treatment, because these patients were older and may have presented with brain tumors secondary to surgery and radiotherapy.

THERAPY

General Considerations

In the adolescent patient who has reached final adult height, the timing and the dose of GH therapy remain to be established. The GH doses used to treat adolescents toward the end of linear growth are three to six times higher than the average dose used in adult GH replacement. The timing of resumption of GH plays an important role in determining the dose to be used. If GH therapy had been stopped for a long time after the attainment of final height, restarting GH at a low dose and titrating up according to the IGF-1 response would seem logical. However, if GH is to be continued without interruption after reaching final height, it is likely that the dose necessary to normalize IGF-1 levels be much closer to the pediatric dose being used. Thus one could continue treatment at the pediatric dose and gradually titrate down according to serum IGF-1 levels (70–72). This may allow for a maximal accrual of bone and muscle mass before transitioning to adult replacement doses (6–8).

Initial studies of GH replacement in adults were carried out using a dose calculated based on body weight or body surface area. This lead to a high incidence of side effects mainly related to fluid retention given the potent antinatriuretic effect of GH. The dosing in adults has to be adjusted taking into consideration the spontaneous GH secretion rates of normal subjects. This declines with increasing age and is inversely proportional to body fat mass. Side effects following a high dose of GH occurred more frequently in subjects who were older and more obese and in those with adult-onset, rather than those with childhood-onset GHD. In addition, hypopituitary female patients require higher maintenance GH doses than do males, to achieve a similar clinical and biochemical response (73).

It should be kept in mind that many symptoms and alterations in patients with GHD and other pituitary deficiencies may improve by replacement of the deficient hormones, other than GH. For example sex hormone administration may lead to clinical well-being and improvement in body composition.

Dose

The GH dose initially used to treat adult GHD had been adopted from pediatric practice and was found be supraphysiologic causing high rates of side effects. The most common complaints were edema, arthralgia, and myalgias. Even taking into consideration the gender, body composition, and age of onset of GHD, the individual response to GH replacement remains variable, prompting the use of individual dose titration. Several studies have demonstrated that adverse effects were less than half as frequent in the dose titration group as in the weight-based dosing group (74,75) and that the final maintenance doses were somewhat lower in the dose titration group. In this context, Drake et al. (74) titrated the GH replacement dose aiming for IGF-1 levels to remain in the upper half of the age-related reference range and compared the results with data from subjects treated with a weight-based regimen. A median maintenance dose of 0.27 mg/day in male subjects and 0.4 mg/day in

female patients was determined. It was significantly lower than the maintenance dose of 0.5 mg/day calculated with the weight-based regimen. However Underwood et al. (75) concluded that it is not appropriate to shift immediately from the higher dose of GH commonly used for children and adolescents to those recommended for adults. A significant dose response was detected for the percent increase in spine BMD and for the body composition end points at 24 months, with a significant decrease in low density–lipoprotein cholesterol noted only in the higher-dose group. Young adult GHD patients may benefit from high initial doses (400–500 μg/day or even higher in subjects in the transition period from pediatric treatment), while a starting dose of 300 μg/day in subjects 30 to 60 years and of 100 to 200 μg/day in older patients (>60 years) may be appropriate. Doses should be increased by 100 to 200 μg/day every one to two months until obtaining a good clinical response and attaining an IGF-1 level within the age-adjusted reference range, with no side effects (70–72).

Dose requirements may have to be adjusted by gender (6), as in males a usual adult dose is adequate, while in females a dose that is like the one used in the pediatric range is necessary to achieve the same IGF-1 response, possibly due to the attenuation of GH action by estrogen (76). As women come off estrogen or are switched from oral to transdermal estrogen, the GH dose may have to be lowered. Older patients may require lower GH doses as they have an increased susceptibility to GH-related side effects. Monitoring of patients should be performed at one to two month intervals while on dose titration and every six months thereafter and should include a physical examination, assessment of side effects, and measurement of IGF-1 levels. Serum lipids, fasting glucose, and insulin levels need to be checked yearly, possibly accompanied by the measurement of CRP and homocysteine concentrations. Individualized GH replacement therapy, beginning with a low dose that is gradually increased based on clinical response, will result in a slow gradual reduction in body fat and lower lipid oxidation and circulating free fatty acids concentrations, therefore minimizing the transient decrease in insulin sensitivity noted during the first months of GH therapy. A DXA scan may be performed initially and then followed if abnormal. QoL assessment may also be useful in monitoring response to therapy.

Table 2 Clinical and Laboratory Features that May Be Associated with Growth Hormone Deficiency

Body composition
Increased body fat
Increased truncal fat
Decreased lean body mass
Decreased extracellular water
Decreased muscle strength and exercise capacity
Decreased psychological well-being
Bone mineral
Decreased bone mineral content
Decreased bone mineral density
Decreased bone turnover
Lipid profile
Increased total cholesterol
Increased low-density lipoprotein cholesterol
Decreased high-density lipoprotein cholesterol
Increased fasting and postprandial triglycerides
Coagulation factors
Increased fibrinogen
Increased plasminogen activator inhibitor 1
Proinflammatory markers
Increased C-reactive protein
Increased interleukin-6 and tumor necrosis factor-α
Cardiac
Decreased left ventricular mass
Decreased left ventricular function
Vascular
Increased carotid artery intima-media thickness
Increased large artery stiffness

Side Effects and Risks Associated with Growth Hormone Therapy

Older and heavier subjects and female patients have a greater chance of developing complications and these are dose related. The most common side effects are related to fluid retention, occur in 5% to 18% of patients, and include peripheral edema, arthralgia, joint stiffness, myalgia, and paresthesias (77). Carpal tunnel syndrome occurs in 2% of treated GHD adults (78) and increased blood pressure may also be seen; most adverse side effects improve with dose reduction.

Benign intracranial hypertension has been linked to GH treatment in children, but is very rare in adults (79). Insulin resistance and diabetes have been found in a small number of patients, but one must bear in mind that variability in insulin sensitivity exists due to differences in age and body composition (33). With the current low-dose GH regimens, there appears to be no excess risk of diabetes mellitus (80). Gynecomastia has been reported in some elderly subjects receiving GH (78).

There has been a concern regarding tumor recurrence or development of malignancies following GH therapy and the subsequent increase in IGF-1 concentrations. An increase in the recurrence of intracranial or extracranial tumors has not been reported in adult GHD (81). Mortality from colorectal cancer and Hodgkin's disease was found to be increased in a cohort of 1848 GHD subjects who had received GH during childhood; however, only two cases of each were reported (82). Although an increased incidence of leukemia in children treated with GH was reported, this excess risk could be due to the presence of other tumors and/or radiotherapy, as there was no increase in leukemia in children with idiopathic GHD who were treated with GH (83).

The hypothalamic–pituitary–adrenal axis should be reassessed in GHD patients during GH treatment as a recent study has demonstrated a lowering of serum cortisol during therapy, that had been masked while untreated probably due to the enhanced conversion

of cortisone to cortisol during the GHD state (84); this can be done using the low dose adrenocorticotropic hormone test or the ITT. It is important to reassess patients periodically as they may develop other pituitary deficits over time and need to be treated with steroid during stress (85).

CONCLUSIONS

The continuation of GH treatment after final height is reached should be based on a careful assessment of the status of the patient, including a determination of the persistence of GHD after adult height is achieved. If one decides to discontinue GH therapy at final height, careful follow-up should determine whether and when the adult GH deficiency syndrome develops and whether GH treatment is required.

It is clear that adult GHD can contribute to an increased cardiovascular risk, and an increased incidence of mortality due to cardiovascular disease has recently been reported in this population. GHD is associated with central adiposity, dyslipidemia, an increased thrombotic tendency, peripheral insulin resistance, and abnormal cardiac structure and performance. In addition, GHD adults have been shown to have vascular disease manifested by increased intima-media thickness and stiffness of the carotid arteries and impaired flow-mediated endothelium-dependent dilation of the brachial arteries. GH replacement has been shown to induce beneficial effects on body composition, the lipid profile, cardiac performance, and atherosclerosis and may contribute to the protection from serious myocardial infarctions. In addition, GH seems to play an important role in the achievement of peak bone mass after the completion of linear growth and in the maintenance of bone mass throughout adult life.

Even taking into consideration the gender, body composition, and age of onset of GHD, the individual response to GH replacement remains variable, prompting the use of individual dose titration. While individualized GH replacement therapy, beginning with a low dose that is gradually increased based on clinical response and IGF-1 concentrations, will minimize side effects, careful follow-up of patients is required during GH-treatment.

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Growth Hormone Treatment

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INTRODUCTION

Human growth hormone (GH) was first used more than 30 years ago to stimulate growth in a child with hypopituitarism (1). Subsequently, a limited supply of pituitary glands from which GH could be extracted and purified required that GH therapy be restricted to children with the most severe and unequivocal GH deficiency (GHD). Strict, arbitrary laboratory criteria were established to identify patients likely to derive the greatest benefit from scarce GH. Delays in diagnosis and treatment, interruptions in therapy, and dosage restrictions were common during this time. Consequently, while GH accelerated growth of these individuals, adult statures were usually less than average (2–4).

In 1985, the first case of Creutzfeld–Jakob disease (CJD) in patients who had received GH was recognized; investigation disclosed that pituitary glands from which the GH was derived were contaminated with subviral particles. Distribution of pituitary-derived GH was stopped. Subsequently, in the United States, CJD was diagnosed in seven recipients of GH distributed by the National Hormone and Pituitary Program (NHPP) (5,6). Fortunately, 192 amino-acid biosynthetic GH, first tested in the United States in 1981, was approved by the Food and Drug Administration (FDA) in 1985 (7) and a second 191 amino-acid biosynthetic GH was approved in 1987. The production of GH by biological systems [*Escherichia coli* and, more recently, mammalian cells (8)] transplanted with the GH gene yields a virtually unlimited supply of GH.

Biosynthetic GH therapy eliminated the risk of CJD and offered children with severe GHD an opportunity for optimal treatment. Children with milder forms of inadequate GH secretion, previously excluded from GH, could be treated. Increased availability of recombinant DNA-derived GH has also allowed investigation of its growth-promoting effects in poorly growing children who do not fit traditional definitions of GHD, many of whom were previously believed to be unresponsive to GH treatment. In addition, metabolic effects of GH apart from linear growth promotion are now being studied extensively, leading to new indications for GH therapy.

Abundance of GH has added complexity to decisions about treatment of stature disorders. Human GH augmentation therapy has now been added to GH replacement therapy, thus expanding the traditional boundaries of endocrinology endeavors, where missing hormones are replaced and excessive hormones production suppressed. Advantages conferred by increased height in social, economic, professional, and political realms of Western society are well documented. Concern about social and psychological harm of short stature, and hope for effective therapy, has resulted in increased referrals for growth-promoting therapy. However, evidence for an association between short stature and psychosocial maladjustment remain predominantly unfounded (9,10) and concerns about the potentially deleterious effects of short stature on psychological health and behavior may be fueled more by parents than children themselves (11). Still, others maintain a higher frequency of underachievement, behavior problems, and reduced social competency in short-statured children (12). Central to the core of this debate are methodological limitations inherent to most of these analyses including definition of short stature; presence of gender specific differences; appropriateness of self versus parental versus peer perceived study instruments; selection biases for study population; and the appropriate age at which to evaluate subjects. Neuroendocrine dysfunction (e.g., classic GHD), rather than stature itself, may correlate most closely with psychological and scholastic impairment (13). While the physiologic benefits of GH supplementation to children with severe GHD appear obvious, data confirming the efficacy of GH therapy in

improving the quality of life of non–GH-deficient recipients are scarce (14,15) and deserve additional study.

For many children, GH treatment will be appropriate therapy after the cause, the concerns of patients and parents, and likelihood of success have been assessed. For most short children, however, efforts to build self-esteem through parental support, judicious selection of activities, and counseling will be more effective than injected GH therapy. The decision to institute long-term GH therapy should include both careful physical and psychological evaluation, to determine whether the degree of disability and likelihood of therapeutic benefit justify investment of the required emotional and monetary resources. Although experience has shown that side effects appear minimal, other possible effects remain unknown. Unexpected benefits may also be found.

The spectrum of disorders for which GH has been prescribed and the number of children receiving treatment (Table 1) continue to increase. In this chapter, we review aspects of GH treatment for severe, ''classic'' GHD, including recent information on nongrowth metabolic effects of GH for adult GH-deficient individuals. Treatment of various short-stature conditions and catabolic disorders with GH is also considered, reflecting the recent proliferation of investigations of the metabolic and growth-promoting effects of GH in non–GH-deficient individuals. Possible risks and ethical issues related to GH therapy are also addressed.

Table 1 Stature Disorders and Metabolic Conditions for Which GH Treatment Has Been Investigated

Idiopathic short stature[a]	Regenerative or reparative states
Constitutional delay of growth	Fractures
Intrauterine growth retardation/SGA[a]	Peripheral nerve damage
Russell–Silver syndrome	Neural tube defects
TS[a]	Spina bifida
Skeletal dysplasias	Myelomeningocele
Hypochondroplasia	Chronic Illness
Achondroplasia	Glucocorticoid-dependent disorders
Spondyloepihyseal dysplasia	Renal transplantation
Multiple epiphyseal dysplaisa	Juvenille rheumatoid arthritis
XHR	Asthma
Miscellaneous syndromes	CRF[a]
NS	Cystinosis
PWS[a]	Cystic fibrosis
Down syndrome	AIDS wasting[a]
Catabolic states	Inflammatory bowel disease
Postoperative wound healing	Congenital adrenal hyperplasia
Burns	Adult GHD[a]
Critical illness	Aging

[a]FDA-approved indication for GH therapy.

Abbreviations: SGA, small for gestational age; TS, Turner syndrome; NS, Noonan syndrome; CRF, chronic renal failure; GHD, growth hormone deficiency; PWS, Prader–Willi syndrome; XHR, X-linked hypophosphatemic rickets; FDA, Food and Drug Administration; GH, growth hormone.

GROWTH HORMONE PHYSIOLOGY

Human GH is secreted by the anterior pituitary gland throughout life under the primary influence of stimulatory GH-releasing hormone (GHRH) and inhibitory somatostatin (SRIH). Both of these regulatory peptides are synthesized in and released from the hypothalamus. The cyclical pulsatile secretion of GH is a response to a coincident decline in SRIH release and abrupt increase in GHRH secretion, modified by the input of neurotransmitters, and the metabolic status upon the hypothalamus and somatotroph (16). Both GH and free insulin-like growth factor (IGF)-1 (17,18) exert positive feedback on SRIH secretion, and GH exerts negative feedback on GHRH secretion. GH is released in response to sleep, exercise, and relative hypoglycemia. Sleep-associated pulses of GH release usually occur in the first 30 to 60 minutes of sleep, and can occur at any time of day when individuals reach stages three and four of slow-wave sleep. Vigorous exercise for 15 to 20 minutes provokes a significant GH surge in 90% of normal children. Because surges in GH are more prolonged in physically unfit than in fit individuals performing comparable work, exercise-induced GH release appears more related to physical stress than to exercise per se. Psychological stresses, such as venipuncture or general alarm, also produce GH elevations. Rises in GH occur with the postprandial decline of blood glucose concentration, and GH secretory surges occur more often in the hours preceding meals than those following meals (19). Ghrelin, a recently identified 28 amino acid peptide secreted primarily by cells of the gastric mucosa, has emerged as an important and potent GH secretagogue (20,21). Ghrelin appears to act synergerstically with GHRH through the GH Secretagogue Receptor at the level of the hypothalamus to stimulate pituitary GH secretion (22,23).

Human GH is synthesized and stored in acidophils of the pituitary gland and accounts for as much as 8% of pituitary weight. About 80% of secreted GH has a 191-amino acid sequence and molecular weight of 22 kDa; the other 20% is approximately 20 kDa and is produced by alternate gene splicing, which deletes amino acids 32 to 46 from the RNA. Human GH is found in pituitary gland and plasma as monomers, dimers, and oligomers. Many other variants of GH, including proteolytically cleaved, *N*-acetylated, and deamidated forms, may also be found either as physiological variants or products of the extraction process. Because GH is species specific, animal GH other than that from primates is ineffective in humans. Circulating high affinity GH-binding protein (GHBP) complexes about 50% of basal GH and in humans, is generated through proteolysis of the extracellular domain of the GH receptor (GHR) likely via TNF-α converting enzyme activity (24). GHBP modulates GH activity by both enhancing and inhibiting release and distribution of GH to tissues through complex interactions at the level of the GHR that are

still not completely understood (25,26). Concentrations of GHBP are low in patients with GHR deficiency (27), and often low in other GH resistant states such as chronic renal failure (CRF), malnutrition, and hypothyroidism (28–30). The regulation of GHBP remains uncertain; pharmacologic doses of androgens appear to lower GHBP levels (31,32) as opposed to estrogens which increase GHBP activity (16); GHBP levels are increased in overweight children and adults (33,34) further highlighting the relationship between GHBP and nutritional status; GH secretory status appears to play only a minor role (35). The clinical utility of GHBP levels in the evaluation of short stature are currently limited to identifying severely affected children with abnormalities of the extracellular or GHBP domains of the GHR (Laron dwarfism), and are not predictive of individual responsiveness to GH therapy in idiopathic short stature (ISS) (36).

Serum GH concentrations are high in full-term and premature infants during the first 24 hours of life, averaging 50 to 60 ng/ml and resulting from both enhanced frequency and amplitude of pulses (37). In full-term, but not in premature infants, GH levels fall after 48 hours. Thereafter, GH concentrations reflect pulsatile secretion, which occurs more often and with higher peaks during infancy, diminishes during childhood, and is lowest in late prepubertal childhood and in adults. Spontaneous puberty in boys or (aromatizable) androgen treatment of prepubertal boys results in significant increases in GH release (38). This sex-hormone induced augmentation of GH secretion is primarily an amplitude-modulated phenomena, though more frequent GH peaks do occur (39). Interestingly, however, testosterone's effect on pubertal growth may be largely independent of changes in circulating GH (40). Stimulation of the somatotropic axis by testosterone is partly dependent upon its aromatization to estradiol; GH levels in adult women are higher than in men and rise in men when they are given estrogens (41). This gender specific difference in GH levels is related to an estrogen induced GH resistant state, and has been shown to occur with either oral or transdermal preparations of estradiol in postmenopausal women (42).

The very short half-life (>20 minutes) of circulating GH requires that blood sampling be carried out at frequent intervals to identify peaks. Studies of both normal and short statured children are associated with a broad range of GH secretion patterns (43). Whereas it was previously thought that a bimodal distribution of GH secretion discretely separated normal from abnormal, it is now clear that (with the rare exception of complete GHD secondary to GH gene deletion or abnormalities of expression) a continuum of ''inadequate'' GH secretion likely spans classic and partially GH-deficient children, children with delayed growth and puberty, and other poorly growing children who pass provocative tests but still secrete less GH than their peers. The spectrum of GH insensitive states now includes several novel mutations involving recently identified factors that regulate the GH intracellular signaling cascade rather than the extracellular or transmembrane portion of the GHR such as signal transducers and activators of transcription (STAT) -5 and STAT-5b (44,45). An asymptotic relationship between growth velocity and spontaneous GH secretion has been postulated for short children (46). This spectrum of GH insufficiency and responsiveness in which there are varying degrees of abnormal GH secretion and action, creates enormous difficulty in interpreting tests of GH secretion. As our understanding of the complex mechanisms underlying some patients with ISS continues to evolve, diagnostic modalities such as the IGF-1 generation test, may serve as a valuable tool for identifying those with even mild forms of GH insensitivity and individualizing GH therapy options (47).

GROWTH HORMONE EFFECTS

The objective of GH therapy traditionally has been to increase the growth rate and adult height of short-statured GH-deficient children. The effectiveness of this therapy has been assessed through achievement of normal growth velocity and height. While linear growth promotion remains the focus of GH therapy, additional metabolic effects of GH are being actively investigated for their potential clinical application. The major indirect actions of GH are anabolic and growth-promoting, mediated by IGFs (principally IGF-1), and include cell proliferation and protein synthesis in both skeletal and nonskeletal tissues. The IGFs are a family of peptides with molecular weight similar to insulin, which have insulin-like activity. Circulating IGFs are produced primarily by the liver in response to GH stimulation, and circulate bound to a family of six larger carrier proteins [IGF binding proteins (IGFBPs)], with molecular weights of 28 to 150 kDa. The majority of IGFs (~70–80%) circulate in a ternary complex comprised of IGFBP3, IGF, and the acid-labile subunit (ALS), while a small amount (<5%) of IGFs circulate in their free form. Most organs synthesize IGFs. Their action may occur within their cells of synthesis (autocrine), on cells in the immediate area (paracrine), and at distant sites (endocrine). A complex interplay of IGF production rates, clearance, and degree of binding to various IGFBPs modulate the levels and systemic activity of free IGFs (48).

The most apparent metabolic effect of GH is stimulation of linear growth in children prior to epiphyseal fusion. The relative roles of GH and IGF-1 in stimulating bone growth may be most accurately described by a ''dual effect'' model of GH action, in which GH stimulates cartilage precursor cells first to differentiate and subsequently to produce, and become responsive to, autocrine and paracrine mitogenic effects of IGF-1 (49). The wide distribution of receptors for IGF-1, and the fact that blood levels

of IGFs are higher than in any tissue suggest that the endocrine function may also be important. However, administration of IGF-1 to hypophysectomized rats does not promote growth equivalent to that of GH (50). IGF-1 also participates in negative feedback regulation of GH secretion by stimulating hypothalamic SRIH secretion (51) and by inhibiting the action of GHRH (52).

In a recent series of elaborate mouse experiments, the roles of hepatic and nonhepatic, localized IGF-1 production have been better elucidated. The importance of localized IGF-1 production on growth was demonstrated in liver IGF-1 knockout mice that grew normally despite having a 75% reduction in serum IGF-1 levels as compared to controls (53). Low IGF-1 levels and slightly lower body weights were observed in ALS knockout mice, demonstrating the importance of the ALS in stabilizing the IGF-1/IGFBP3 complex in serum (54). In a later experiment, double IGF-1 and ALS knockout mice had serum IGF-1 levels that were more severely reduced to 10% to 15% of normal and underwent significant postnatal linear growth retardation when compared to either of the single knockout mice or controls, suggesting that a threshold level of bioavailable IGF-1 is needed for normal growth in the mouse (55).

IGF-1 levels correlate with the clinical state of GHD, sufficiency, or excess. Serum IGF-1 levels are low in utero and in infancy, increase with age in both boys and girls, reach maximum values during puberty (earlier and higher in girls than boys), and decline to adult values as adolescence is completed. Although often used as part of the assessment of hypopituitarism, IGF-1 levels do not exclusively reflect GH production and are dependent on age, stage of pubertal maturation and nutritional status. Concentrations of IGF-1 correlate more closely with bone age and puberty (56) than with chronological age. Hypothyroidism, malnutrition, poorly controlled diabetes, and chronic disease diminish secretion of IGF-1. The normally low levels of IGF-1 in infants and young children preclude its diagnostic utility for classic GHD in this age group and an IGFBP3 level has been suggested as a supportive diagnostic test in infants (57). Thus, IGF-1 levels, while highly specific for GHD, have relatively low sensitivity. Given these limitations, the use of IGF-1 alone as a screening test for GH status is of little diagnostic value. However, an IGF-1 level in combination with calculation of growth velocity and an IGFBP-3 level may serve as a useful diagnostic panel to select which patients require additional stimulation testing (58). The Growth Hormone Research Society has recommended measurement of IGF-1 and IGFBP3 in addition to provocative GH testing as a means of identifying children with abnormalities in the GH/IGF axis not detected by standard tests (57).

Other metabolic effects of GH can be described as anabolic, lipolytic, and diabetogenic (Fig. 1). GH-induced growth acceleration is facilitated by concomitant enhancement of protein synthesis in bone, cartilage, skeletal muscles, the erythropoietic system, and other major organs. Administration of GH produces positive nitrogen balance, increased amino acid transport into cells, increased intracellular RNA, and decreased urea production and blood urea nitrogen levels. The metabolic efficacy of total parenteral nutrition is also enhanced by GH (59). A high-normal or mildly elevated blood urea nitrogen level and low serum phosphorus and alkaline phosphatase level

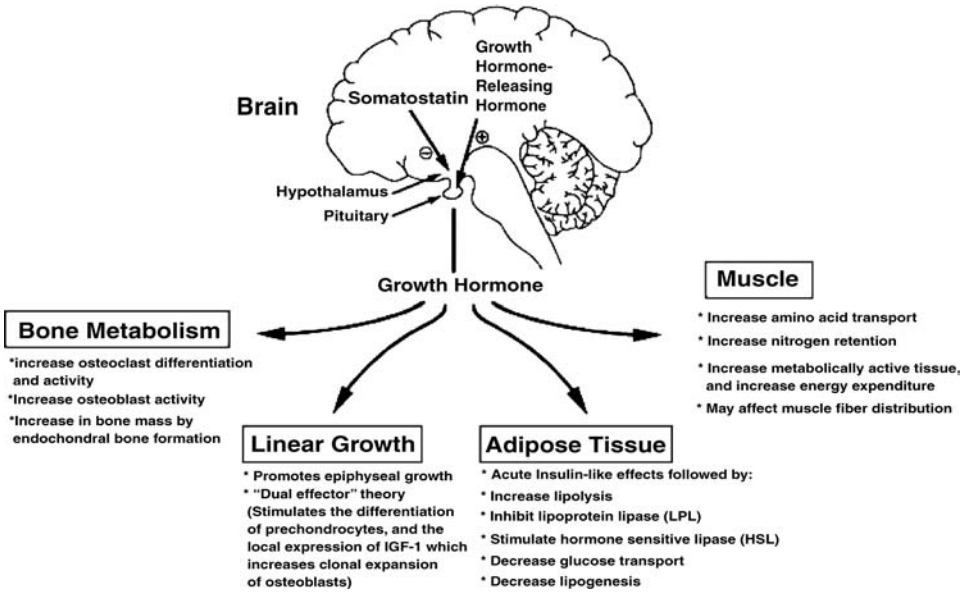

Figure 1 Multiple sites of growth hormone action.

are usually observed in GHD and reverse with GH treatment. Effects on mineral metabolism include increase intestinal calcium absorption and urinary calcium excretion and reduced urinary phosphate excretion (60). Bone density and levels of osteocalcin, procollagen type 1, and 1,25-dihydroxyvitamin D are reduced in GH-deficient children and increase with long-term GH therapy (61).

GHRs and expression of GHR mRNA have been demonstrated both in preadipocyte cultures and in mature adipocytes. Some actions of GH in adipose tissue are mediated directly through interaction with the GHR, while others are mediated indirectly through IGF1. These actions include: (i) inhibition of differentiation of immature adipocytes to mature adipocytes; (ii) enhancement of lipolysis and site-specific free fatty acid release from adipose tissue; and (iii) inhibition of lipoprotein lipase and stimulation of hormone sensitive lipase (62). Consequently, GH-deficient children tend to demonstrate increased, predominantly abdominal, subcutaneous fat, which lessens and becomes more peripheral during therapy with exogenous GH. Non–GH-deficient children also display reduction in overall body fat during GH therapy. The mechanism by which GH reduces adipocyte size in vivo remains unclear, but in vitro studies suggests that GH increases the basal rate of lipolysis and depresses reesterification of free fatty acids (63).

The effects of GH on carbohydrate metabolism are complex. Intravenous administration of GH causes an acute fall in blood glucose, most likely reflecting enhanced transport of glucose into adipose and skeletal muscle cells. GH is pivotal for maintenance of normal glucose homeostasis in infants, but this critical role is markedly diminished in older children and adults. In a child with GHD, insulin secretion is diminished, related in part to pancreatic islet cell hypoplasia. Glucose tolerance tests may reveal impaired ability to dispose of a carbohydrate load. However, fasting hypoglycemia can also occur in such patients because of heightened sensitivity to insulin. Administration of GH reduces sensitivity to insulin, thereby correcting hypoglycemia, and increases insulin secretion. Chronic administration of GH results in compensatory hyperinsulinemia and is associated with the development of insulin resistance. In non-GHD children, GH may also induce an insulin resistant state. In particular, children born small for gestational age (SGA) or with Turner syndrome (TS), who are at an increased risk for development of type 2 diabetes at baseline, are more likely to develop reversible, GH dependent hyperinsulinemia during therapy (64,65). Thus far, similar effects on carbohydrate metabolism have not been observed in GH-treated children with ISS (66). In summary, the combined effects of pharmacologic dosages of GH on both the release of and response to insulin can create laboratory evidence of altered carbohydrate intolerance, but the clinical relevance of this remains unresolved.

GROWTH HORMONE DEFICIENCY

The classic presentation of severe GHD is characterized by marked growth retardation, diminished growth velocity, delayed skeletal maturation, absence of other explanations for growth retardation, and subnormal secretion of GH, both physiologic and in response to provocative stimuli. Genetic (e.g., altered GH or GHRH gene), anatomic or congenital [e.g., midline cranial defects, septo-optic dysplasia (SOD), vascular malformations], and acquired (e.g., craniopharyngioma, glioma, histiocytosis) abnormalities of the hypothalamus and pituitary are identifiable in many affected children. Evaluation of pituitary gland anatomy using magnetic resonance imaging (MRI) has revealed a spectrum of more subtle morphologic abnormalities associated with the diagnosis of idiopathic GHD (67) or hypopituitarism (68). Irradiation or chemotherapy for malignancies and traumatic brain injury also cause organic hypopituitarism, and account for an increasing incidence of GHD as survival of these individuals improves. In spite of this growing list of etiologies, most children with GHD still are designated as idiopathic, often due to apparently defective hypothalamic regulation of GH release, rather than inability to synthesize GH. It is likely that further improvement in imaging techniques will reveal additional organic lesions contributing to this dysfunction.

Idiopathic GHD usually occurs sporadically but may be familial (69). Its frequency has been reported to be from 1:4000 children to 1:60,000 (70,71). Although the latter figure is similar to the treated population in the United States before 1985, the true incidence of classical GHD is probably in the range of 1:10,000. These estimates are complicated by the fact that, with the exceptions of GH gene deletion and severe pituitary or hypothalamic dysfunction, GHD is partial, rather than complete. The incidence of GHD is likely to continue to rise due to improved prolonged survival of children who have received radiation therapy for malignancies.

Currently, arbitrary laboratory criteria provide a technical distinction between GHD and GH-sufficiency, even though it is generally accepted that there is no meaningful physiologic distinction between children whose provoked GH levels fall slightly above or below laboratory threshold values. Evidence is accumulating to support the notion that GHD is not a distinct entity, but rather a spectrum of disorders of GH pulsatility. A continuum of GH secretion may span essential absence of GH, partial GHD, and a spectrum of ''normal'' GH secretion. Some investigators have found significant correlations between spontaneous GH secretion and statural growth rates (72) while others have not (73). The blurring of what was once thought to be a clear distinction between GHD and sufficiency has combined with the luxury of available, expensive GH to create new opportunities, uncertainties, and controversies in GH therapy.

Profoundly GH-deficient infants and young children may present initially with hypoglycemia. This is more commonly associated with adrenocorticotrophic hormone (ACTH) deficiency and hypocortisolism, but hypoglycemia may persist in spite of glucocorticoid (GC) replacement. Prompt administration of GH is necessary to prevent the neurological sequelae of persistent or recurrent hypoglycemia. Microphallus occurring in the newborn with GHD most often, but not invariably, is associated with gonadotropin deficiency. Because GH is largely responsible for phallic growth after the first few months of life, (untreated) isolated GH deficient males may display poor phallic growth during early childhood. This problem can be effectively treated with GH and very small doses of androgen. Growth velocity may be mildly or severely impaired, depending upon the degree of GHD and/or presence of accompanying hormonal deficiencies. Bone age is usually delayed in GHD, but is often less delayed than height age (age for which the child's height is average). Bone age is less delayed in isolated GHD than with multiple pituitary hormone deficiencies. ''Catch-up growth,'' a period of supra-normal growth velocity often observed particularly during early GH treatment, is accompanied by skeletal maturation proportionate to growth achieved, leading to the *appearance* of accelerated bone age advancement.

At least half the children with GHD were described in 1968 as having an isolated hormonal defect (74). This proportion has steadily risen as more children with partial or neurosecretory GHD have been recognized and diagnosed. A rise in GH secretion and increase in growth velocity observed following pulsatile GHRH administration in many of these patients indicates that deficient hypothalamic regulation of pituitary GH secretion is the etiology of GHD in many patients with isolated GHD (75). However, GHD is often accompanied by deficiencies of other anterior pituitary hormones—ACTH, thyroid stimulating hormone (TSH), luteinizing hormone (LH), follicle stimulating hormone (FSH) and posterior pituitary antidiuretic hormone (ADH), synthesized in hypothalamic nuclei. Hypopituitarism, the deficient production or release of one or more hormones from the pituitary, may be primary or secondary to hypothalamic dysfunction. When deficiencies other than GH occur, they are, in decreasing order of frequency: LH and FSH, TSH, and ACTH (76).

Advances in molecular genetics have led to the identification of mutations in several pituitary specific transcription factors important in pituitary gland organogenesis as well as other GH specific genes that result in what was once thought to be idiopathic cases of isolated GHD or hypopituitarism. Mutations in Lhx3, Lhx4, Prop-1, and POU1F1 (Pit-1) can result in combined pituitary hormone deficiencies whereas others may result in isolated deficiencies or specific syndromes [Hesx1 (SOD), Pitx2 (Rieger syndrome)] (77). Several different mutations have been identified in the gene encoding GH (GH-1) that have been associated with varying degrees of GHD and may be inherited in an autosomal recessive, Autosomal dominant, or X-linked patterns.

ADH deficiency, manifested as diabetes insipidus, usually occurs in acquired GHD (e.g., craniopharyngioma, surgical trauma) or congenitally as part of the SOD syndrome. In contrast to anterior pituitary hormones, ADH is synthesized in the hypothalamus and transported to and stored for release in the posterior pituitary, which is embryologically distinct from the anterior pituitary. Consequently, with the exception of SOD, deficiency of ADH is rarely seen with idiopathic hypopituitarism. On the other hand, extensive surgery for pituitary tumors will usually result in ADH deficiency (if it was not present before) and variable, but usually progressive loss of other pituitary hormonal secretion (78). A combination of less extensive surgery and irradiation, or irradiation alone, has been recommended as a more moderate approach to treatment of such patients. However, the gradual development of impaired GH secretion is also observed in many children who receive cranial irradiation for neoplasms of the central nervous system (79). With radiation of the hypothalamic-pituitary axis, GH is the first hormone to be affected and the degree of hormonal deficit is related to the radiation dose (80). In one study, five years following 3.75 to 42.5 Gy radiation therapy, all patients were GH deficient; gonadotropin, ACTH, and TSH were deficient in 91%, 77%, and 42% of patients, respectively (81). Adjuvant chemotherapy may also increase the risk for GHD as evidenced by a recent multicenter study of 1607 children with a history of a brain tumor (82). This analysis revealed that five years post-therapy, children receiving chemotherapy, radiation, and surgery (37.7%) were more likely to develop GHD than those who underwent surgery and radiation therapy (21.1%) or surgery alone (2.4%).

Timing of the onset of puberty normally is related most closely to a child's reaching a state of maturation (rather than chronological age) corresponding to a bone age of 10 to 11 for girls, and 11.5 to 12.5 for boys. Late recognition and treatment of GHD often results in delayed bone age and pubertal development. In addition, late diagnosis of GHD may not allow sufficient treatment time for height age to catch up to bone age prior to puberty; these children may experience rapid pubertal development without an adequate pubertal increment in height, resulting in reduced adult stature. LH-releasing hormone agonist therapy, which can slow or stop pubertal advancement, or aromatase inhibitor therapy, which may reduce estrogen-mediated epiphyseal plate closure, may offer effective means of ''reclaiming'' the time required for sufficient growth (83,84) but further controlled studies are needed to evaluate the efficacy of these treatments. A preferable option is prompt recognition of GHD and optimization of GH dosage and schedule, which facilitates both

the achievement of normal prepubertal height and entrance into puberty at a more appropriate chronological age.

TREATMENT OF GROWTH HORMONE DEFICIENCY

For over 30 years, the diagnosis of GHD was based on the analysis of serum GH levels following at least two provocative stimuli. The limited amount of available pituitary-extracted GH dictated that few short children could be treated. Criteria was established by national committees of the National Pituitary Agency (which later became the NHPP) to ensure that the most severely affected GH-deficient children would receive GH treatment. Thus, only very short, very slowly growing children who had very low GH levels on stimulation tests qualified for GH therapy, and these children were treated only until a height within −2 to −2.5 standard deviations (SD) of normal adult height was reached. This meticulous rationing of scarce GH to the most severely affected children maximized the overall benefit that could be derived from this therapy.

Improved pituitary collection and extraction strategies increased availability of GH during the 1970s and, as a result, criteria for treatment were relaxed. Whereas stimulated levels of GH less than 3 to 5 ng/dl were originally considered to be sufficiently subnormal to indicate GHD, the threshold GH level required for this diagnosis gradually rose to 7 ng/dl, then 10 ng/dl, and in some clinics, 12 ng/dl. Currently, children with levels in the higher subnormal range and children with normal stimulated GH levels but low spontaneous GH secretion are now designated as having ''partial'' GHD. Interpretation of tests for GH is also complicated by laboratory variation: whereas the NHPP originally provided uniform material, standards, and methodology for GH testing, later commercialization of GH assays methods created significant variation in laboratory values for GH in a single blood sample. Today, expanding definitions of ''partial'' GHD variations in GH assays, and unlimited GH availability have transformed the historically clearly defined and tightly regulated practice of diagnosing and treating GHD into a rapidly evolving and controversial endeavor.

Growth failure caused by severe GHD is a universally accepted therapeutic indication for GH treatment. Treatment of growth failure due to ''partial'' GHD, defined by subnormal stimulated GH levels, has also become accepted practice. However, it is now widely recognized that children with partial or subtle defects in the secretion of GH are difficult to identify, and no individual assessment of GH secretion or GH-associated biochemical finding unerringly detects such subjects (85). A single provocative test for GH (a necessary but not sufficient criterion for these diagnoses) appears to lack both specificity and sensitivity in identifying GH insufficiency. In children of normal stature, stimulated GH levels may be less than 7 ng/ml in as many as 20% (86). Specificity may be increased by performance of a second provocative test. On the other hand, a normal GH response to provocative stimuli does not guarantee sufficient spontaneous GH to maintain normal growth.

Attempts to define milder forms of GH through frequent blood sampling and analysis of spontaneous GH secretion pattern have led to the development of elaborate mathematical techniques for their interpretation. Children with subnormal GH secretion following cranial irradiation, who may pass provocative GH testing, may be identified solely by this frequent sampling method. [Over time, classic GHD develops in many of these patients (87)]. However, determination of spontaneous GH secretion requires considerable time, expense, and technical help, and is complicated by technical difficulties, disturbed daily and nighttime routines of the patients and lack of reproducibility (88). Current methods have been criticized for their inability to discriminate normal children from short, GH-responsive children, and even from classic GH-deficient children. In summary, measurement and analysis of spontaneous GH secretion is sometimes helpful in identifying GH-sufficient subjects, but adds little in most instances to provocative tests in the identification of the GH-deficient child. With either test, the notion of a discrete cutoff level of GH secretion that reliably distinguishes GHD from normal is historical, and has limited relevance to current practice of pediatric endocrinology. The diagnosis of mild forms of GH insufficiency depends primarily upon clinical perception; laboratory tests of GH secretion provide ancillary information which help to confirm or disprove that clinical diagnosis.

Whom to treat for isolated idiopathic GHD and for how long is further complicated by the fact that most children who are treated with GH do not have permanent or complete GHD, but rather have insufficient secretion of GH to support normal childhood growth. When children with isolated GHD are retested after GH replacement therapy has been interrupted, between 30% and 70% will have a normal GH response. Children with a previous diagnosis of partial GHD (i.e., peak stimulated GH levels of 5–10 μg/L or low 24-hour integrated GH secretion) are particularly likely to have normal results on posttreatment testing (89). While MRI findings of ectopic posterior pituitary (EPP) and hypoplastic infundibular stalk are classic signs of isolated GHD (or multiple pituitary deficiencies) in childhood, newer evidence suggests that a spectrum of subtle anatomic abnormalities and hormone deficient states likely exists. Indeed, patients with proximally located EPP and visible or thin infundibular stalks are more likely to have normal GH stimulation tests as adults and may not require long-term adult GH replacement therapy (90).

Various studies of time of initiation, dosage, and frequencies of GH administration have advanced progress toward optimal treatment of GHD during childhood. Subcutaneous daily GH administration is currently preferred, with the average GHD child in the United States receiving initial treatment with 0.3 mg/kg/wk divided into six to seven doses, although many children show early satisfactory growth on substantially smaller doses. GH preparations are essentially equivalent, but vary in preparation and delivery. They include lyophilized GH that is mixed with a sterile diluent, premixed GH solutions in a multiple dose vial, GH pen/needle delivery system, AND a nonneedle delivery system. A depot GH preparation administered every two to four weeks was approved for pediatric use in 1999, but was recently discontinued by its manufacturer. Intranasal preparations continue to be developed, but are not currently available (91). Because growth before puberty is a major determinant of final adult height, early initiation of GH treatment allows more complete normalization of height prior to puberty and an improved final height prognosis (92). While there can be a temptation to defer injection therapy in young children in order to minimize discomfort and inconvenience, the available evidence strongly supports early recognition, referral, diagnosis, and treatment of severely GH deficient patients as an important step toward optimizing their growth potential.

Increasing the dose of GH improves growth rate; a dose-response equation derived from treatment of children of all ages with various degrees of GHD reveals a logarithmic relationship between GH dose and growth rate for thrice weekly doses ranging from 0.015 to 0.1 mg/kg/tiw (93). The variation around the mean of each of these growth rate points is great, attesting to the poorly understood contribution of factors other than GH levels to the normal linear growth process. Other studies implementing daily GH injections confirmed the relationship between growth rate and ''conventional'' doses of GH (94). A recent study using 0.025, 0.05, and 0.1 mg/kg daily in prepubertal severely GHD children showed significantly greater growth velocities and gains in cumulative height SDS in the 0.05 and 0.1 mg/kg/day groups compared with the 0.025 mg/kg/day group after two years of treatment. There were no significant differences between the 0.05 and 0.1 mg/kg/day groups (95). The serum IGF1 response to GH is also dose-dependent, continuing to rise as GH is administered in doses above currently prescribed levels (96). Epidemiological studies that suggest an association between high serum IGF1 levels and the incidence of malignancy have prompted a recommendation that IGF1 and IGFBP3 levels be monitored on a regular basis. A younger age, greater delay in height age and bone age, and greater severity of GHD based upon provocative testing each correlate with improved initial response to GH therapy. Using multiple regression analysis, variables that have positive effects on adult height of GH-treated patients include taller parents, more frequent GH injections, longer duration of GH treatment, taller height at start of GH treatment, and greater severity of GHD.

Increased frequency of GH administration improves growth rate, suggesting the ''pulsing'' message of GH to its target cells, in addition to adequacy of GH levels, enhances linear growth. When the same weekly dose is given in daily injections, rather than three times per week, an improvement in first-year growth rate of about 1.5 cm/yr is observed (97). While not presently available for pediatric use, subcutaneous administration of depot preparations of GH at every-other-week intervals, has also been shown to effectively increase growth rates nearly to the same degree (98). Nocturnal administration, which more closely mimics physiological GH secretion, may also add to efficacy (99), although this is not consistently observed. Regardless of the regimen chosen, the effect of GH wanes with time, and the first year of treatment usually produces the greatest growth increment. Following this early phase of rapid growth, short-term increased replacement doses of GH renews catch-up growth without adverse metabolic effects (100). Seasonal variation in growth rate during GH therapy, with peaks in the summer and nadirs in the winter (North American population) has also been described (101).

Children who have had removal of craniopharyngioma frequently experience growth acceleration in the absence of measurable GH. This phenomenon may be attributable to postoperative hypothalamic dysfunction resulting in nutritional excess and hyperinsulinemia (102), although other mechanisms (e.g., GH variants, IGFs) are also postulated (103). Polyphagia and significant weight gain are usually also observed. While supplementation with GH is not required to sustain linear growth, body composition analysis reveals increased fat mass and decreased lean mass typical for the GHD state. Unfortunately, post surgical treatment with GH, while promoting linear growth, does not appear to slow the excessive weight gain commonly seen in this population (104). Avoidance of excessive cortisol replacement therapy is extremely important in these individuals. This growth pattern may persist, allowing attainment of normal adult stature without GH therapy, but GH may nevertheless be indicated to improve body composition and other metabolic consequences of GHD.

The distinct augmentation of GH pulsations and increased production rates which occur during normal puberty raise the question of whether GH replacement doses also should be increased during puberty. Insufficient dose and frequency of GH administration has been shown to permit epiphyseal closure in GH-deficient adolescents prior to adequate catch-up growth, thereby reducing expected adult height (105). In one study, doubling the dose of GH during puberty did not significantly change growth rate, but did tend to advance pubertal maturation (106). A proposed model of predicting

pubertal growth has demonstrated that the GH dose administered during puberty is only a minor factor in determining total pubertal growth as compared to gender, chronologic age at pubertal onset, and prepubertal height relative to mid-parental height (107) On the other hand, a larger randomized trial showed that an increase in dose to 0.7 mg/kg/wk (in contrast to conventional dosage recommendations of 0.18–0.35 mg/kg/wk) improved growth rates, near-adult height and height SDS in GHD adolescents without evident adverse effects (108). Because the cost of such treatment and the potential for inducing IGF-1 levels in the supra-physiologic range must be balanced with the possible added benefit achieved, the most effective frequency and dose of GH therapy during puberty is yet to be determined.

Children treated with GH may experience transient or persistent declines in serum thyroxine (T4) levels; in approximately 25%, T4 levels become abnormally low and may impair response to GH. Thyroid function tests should be monitored periodically (especially early) during GH therapy to ensure detection of secondary T4 deficiency and prevent this treatable cause of a poor response to GH. Cortisol supplementation may also impair the growth response to GH; as little as 7.5 to 10 mg/day of hydrocortisone may be growth-suppressive in a school-aged child. Thus, when ACTH deficiency has been documented, the dosage of daily cortisol replacement therapy should be reduced to a level sufficient to prevent symptoms of fatigue and lack of energy. In prepubertal children, these replacement levels are quite low, and some children with idiopathic hypopituitarism, even with evidence for ACTH deficiency, will not need cortisone replacement in the absence of illness or stress.

Testosterone or other anabolic agents will enhance the growth velocity of a prepubertal GH-deficient child taking GH, but (except for boys with microphallus) should not be given if the bone age is less than nine years, and then in very low doses initially and with preference to nonaromitizable forms (e.g., oxandrolone). Treatment for the purpose of virilization in a boy who is gonadotropin-deficient should be initiated after bone age approximates 11 to 12 years with low dosage (e.g., 50 mg testosterone enanthate i.m. q month) to prevent accelerated epiphyseal maturation. Doses can be gradually increased to adult replacement levels (e.g., 200–300 mg testosterone enanthate every two to four weeks) over the next several years in those boys with evidence for gonadotropin deficiency. It has recently become clear that estrogens have a potent effect on bone age acceleration in TS patients, and may have an accelerating effect on the growth resulting from GH therapy. The androgen oxandrolone, which cannot be aromatized to estrogen, in low dosages has also been useful in accelerating growth rate in both boys and girls. Future management of short stature during puberty may include other measures (e.g., aromatase inhibitors) designed to reduce epiphyseal-maturing estrogen effects while preserving growth-accelerating effects of GH and androgens (109).

With early diagnosis, careful attention to accompanying hormonal deficiencies, and progressive dose adjustments, children with GHD reach normal adult height (Fig. 2) (110). Bone age will advance with GH treatment, but usually not more than height age. Linear growth often accelerates faster than bone age following initiation of GH therapy, leading to increases in predicted final height. Even with successful long-term GH therapy, however, correction of disabling short stature does not consistently normalize the psychosocial outcome for adults with GHD (111). Psychosocial counseling, which increases both therapeutic compliance during childhood and social outcome during adulthood (112), should be a consistent adjunct to parenteral GH administration.

The consequences of severe GHD in adult life and the beneficial effect of replacement therapy are increasingly well established (Vol. 2; Chap. 4). Accurate selection of appropriate candidates for adult GH treatment, and the transition of their care from pediatrics to adult medicine require careful consideration of several issues. Because the majority of children who are diagnosed as GHD and treated with GH do not have permanent GHD, anticipatory

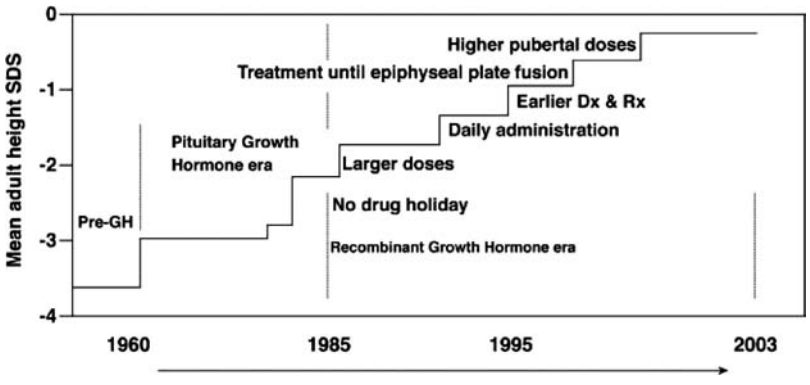

Figure 2 Progress in efficacy of childhood growth hormone treatment. *Source:* Adapted from Ref. 109.

counseling regarding possible lifelong treatment should be focussed on children with panhypopituitarism and those with severe isolated GHD associated with central nervous system abnormalities. Appropriate timing for termination of ''growth-promoting'' GH therapy should be guided by efforts to balance the high cost of late-adolescent treatment with the attainment of reasonable stature goals. Confirmation of GHD following cessation of GH therapy for at least one month and provocation with appropriate stimuli (i.e., insulin tolerance test, arginine or glucagon stimulation tests) and measurement of IGF-1 levels are appropriate for all candidates for adult GH therapy who do not have well documented multiple pituitary hormone deficiencies. Testing of the GH axis can be performed within weeks of GH cessation, but confirmation of an emerging adult GHD state with body composition, blood lipid panel, bone densitometry testing, and quality of life assessments may require one or more years of pretreatment observation (113). While it remains unclear whether such a period of observation is needed, advisable, or safe for late adolescents with unequivocal panhypopituitarism, a transition from growth-promoting to adult replacement doses of GH therapy without interruption has recently been recommended by a consensus panel (114). Those adolescents with a history of less severe GHD or those with ''partial GHD'' (i.e., stimulated GH values of 5–10 ug/L on retesting as an adult) pose a greater diagnostic and therapeutic challenge. If therapy is interrupted in this group, a minimum of close clinical, radiologic (if not previously performed), and laboratory monitoring for evidence of an evolving GHD state and/or other pituitary hormone deficiencies is required. Thus, selection of patients for lifelong adult GH replacement therapy will present diagnostic, therapeutic, and ethical dilemmas similar to those arising from treatment of childhood GHD. The experience and expertise of pediatric endocrinologists in diagnosing and treating GHD should be offered and utilized in the identification and transitioning of appropriate patients to adult GH therapy (115).

CAUSES OF SHORT STATURE OTHER THAN GROWTH HORMONE-DEFICIENCY THAT MAY RESPOND TO GROWTH HORMONE TREATMENT

For every short child who has impaired GH secretion, many more are short for other reasons. Parental concerns about the disadvantages of their child's short stature are legitimized by a ''heightist'' premise in modern America: to be tall is good and to be short is to be stigmatized (116). The preponderance of Caucasian males in the GH-treated population suggests that these social pressures give rise to ascertainment of short statured patients biased by sex, race, and socioeconomic status (117). Stigmatization based upon height begins in childhood. Feelings of incompetence and low self-esteem may arise from the short child's struggle with stature, although these problems may depend as much on the projection of parental perceptions of their child's vulnerability and immaturity as on short stature per se (118). On the other hand, recent studies of nonreferred short children have failed to reveal any predictable stature-associated morbidity (9). Future studies investigating improvement in quality of life, as well as improved stature growth, of non–GH-deficient children treated with GH will be of paramount importance.

Since the previous edition of this text, new indications for GH therapy have merited FDA approval, and the medical literature continues to be replete with reports of non–GH deficient children treated with GH, and numerous studies continue to investigate new indications for GH (Table 1). Many extraordinarily short children have overlapping diagnostic conditions [e.g., familial short stature and constitutional growth delay (CGD)], which complicates interpreting the response of a specific condition to GH therapy. Below, specific disorders that have undergone investigational trials to date, some of which are currently approved indications for GH therapy, are discussed.

Idiopathic Short Stature and Constitutional Growth Delay

The prospect that GH therapy may accelerate growth and increase adult height of markedly short, but otherwise healthy children has generated great interest and debate Vol. 2; Chap. 2. Serum GH concentrations are usually normal in these children; however, children with severe CGD may demonstrate temporary failure to secrete GH in response to stimuli, which normalizes with pubertal progression or induction (119). The finding that 30% to 70% of adults diagnosed as GH deficient during childhood have normal GH levels later suggests that this phenomenon is more common than generally recognized. Thus, repeat provocative testing for GH following short-term sex hormone treatment is advisable before considering a commitment to GH therapy for most near-pubertal patients who fit clinical criteria for CGD.

Administration of GH at dosages used to treat GHD increases growth velocity in the majority of these children throughout at least three years of treatment. A controlled study reported that mean growth velocity increased during the first year from 5.3 to 7.4 cm/yr and height SD score increased by 0.63 in GH-treated children versus no change in velocity or SD score for untreated children (120). Growth rates declined during each successive year of therapy, and in one study, approximated pretreatment growth velocity by the fourth year (121). In a more recent study, however, daily GH therapy during years two and three sustained growth rates of 7.6 and 7.2 cm/yr, respectively, compared with baseline growth rate of 4.6 cm/yr (122). Short, non–GH-deficient children who demonstrate sustained acceleration of growth rate on GH therapy tend to attain prepubertal

heights that are closer to, but do not exceed their genetic height potential (123).

Information regarding the efficacy of GH in improving the final height of children with ISS or CGD is now accumulating. Because height at onset of puberty is a more important determinant of adult height than pubertal growth (because prepubertal growth normally contributes 85% of final height), the most effective GH therapy will require substantial growth acceleration prior to puberty. Increases in standardized Bayley–Pinneau predictions of adult height have been consistently reported during prepubertal GH therapy, but these gains do not improve substantially during treatment of pubertal subjects. Puberty generally appears to occur at expected time in GH-treated children with ISS or CGD, but an accelerated tempo of puberty has been noted in boys (124) and an earlier age of onset in girls (125). This possible decrement in height acquired during puberty probably accounts for reports of actual final heights of GH-treated children that fall short of earlier more optimistic height predictions (126). Alternative strategies include administration of a higher dose of GH for two years prior to puberty to achieve normalization of adolescent height, precluding the need for GH therapy during puberty (127). Long-term GH therapy in non-GHD adolescents may increase insulin resistance, but not impaired glucose tolerance or hyperlipidemia (128).

How effective is GH therapy at increasing adult height of non-GHD individuals? Interpreting the results of studies aimed at answering this question have been difficult due to inter-study differences in GH dose and frequency of administration, age of initiation of therapy, inclusion criteria (i.e., heterogeneity of subject population) and the length of therapy. Of several studies published in the mid-1990s, only two reported final heights greater than pretreatment predicted heights and only one reported an improved proportion of subjects with final height greater than the midparental target height (129). On the other hand, a subsequent study of 80 non-GHD children treated with GH (highly variable duration of treatment) showed a mean increase in SD score for height from -2.7 to -1.4. The mean ($\pm$SD) difference between predicted adult height (using Bayley-Pinneau method) before treatment and achieved adult height among boys was 5.0 ± 5.1 cm, and 5.9 ± 5.2 cm for girls; still, only a few subjects achieved their midparental target height (130). An ensuing meta-analysis suggested an average gain in adult height of approximately 4 to 6 cm in ISS children treated with GH (131). More recently, a well-designed randomized, placebo control trial of short statured, peripubertal children without evidence of GHD demonstrated a mild, yet significant improvement in adult height outcomes by 0.51 SDS although the significance of this difference was lost when adjusted for midparental height SDS (132). The effect of GH dose was highlighted by a recent study comparing a dose of 0.24 mg/kg/wk versus 0.37 mg/kg/wk in prepubertal children, demonstrating a gain of 0.57 SDS in adult height for the high dose group (133).

On the basis of these data, the FDA concluded that long-term GH treatment of non-GHD short children at recommended doses could lead to statistically significant increases in final height in a sufficient number of children to justify approval for this indication. Interestingly, this particular ISS indication for GH therapy is the only one that includes a threshold height criterion; less than 2.25 SDS for height. Unfortunately, in this and other studies, no clinical (e.g., pretreatment growth rate) or biochemical determinants (e.g., overnight endogenous GH secretion) reliably predicted the individual response to GH therapy as well as degree of delay in skeletal maturation. Consequently, the effectiveness of GH in improving height in ''pure'' genetic short stature as opposed to delayed growth remains unresolved. Future research should focus on minimizing the bias from variables identified above as well as identifying ''responders'' versus ''nonresponders'' in order to maximize treatment efficacy. Further, whether improvements in final height due to GH therapy alone are sufficiently likely or clinically significant to justify cost and commitment to several years of therapy is still debatable.

Novel approaches to the treatment of ISS include concomitant suppression of pubertal hormones using gonadotropin releasing hormone (GnRH) agonist therapy and reduction of estrogen production using aromatase inhibitors. A recent analysis showed that therapy with a GnRH agonist in children with ISS provided a minimal increase in adult height (0.6 SD) but at the potential cost of lower bone mineral density (134). Another study reported that combined GnRH/GH therapy for three years resulted in gains in predicted height of 8 to 10 cm without demonstrable side effects (135). Final height data on these patients is not yet available, and current expense of such treatment is formidable. Anecdotal evidence regarding effectiveness and safety of aromatase inhibitor therapy exists (110,136), but long-term studies in pubertal boys are still needed.

Turner Syndrome and Noonan Syndrome

Between 95% and 100% of girls with TS experience growth failure, and the untreated mean final height of these patients is 143 cm (137), approximately 20 cm below the female average of the corresponding ethnic group. Growth curves for TS have been developed by European investigators, and North American patients closely match this data (138). Individual's height percentiles on this TS curve do not change from childhood to adulthood if they remain untreated. During childhood, average growth velocity is 4.44 cm for each year of bone age advancement. The lower final height results from the combined effects of mild intrauterine growth retardation (IUGR), a lack of a pubertal growth spurt (due to ovarian failure to produce

estrogens), and growth failure during childhood due to abnormalities in growth plate cartilage (139) and a possible resistance to the action of GH. Studies clearly show that TS patients do not have classic GHD, although endogenous GH levels (140,141) and urinary GH levels (142) are below normal after the age of eight years.

With the exception of GH deficient children, girls with TS have received the longest trials of GH therapy (up to 10 years). Numerous studies have demonstrated that GH, with or without anabolic steroids, can accelerated growth in girls with TS (143–145). During subsequent years of treatment, growth rates declined but remained higher than those of untreated girls. In one cohort, 14 of 17 girls receiving GH alone equaled or exceeded their original projected adult heights, while 41 of 45 girls receiving combined oxandrolone-GH therapy did so. The mean height of girls who completed a mean of 7.6 years of GH therapy ($n = 17$) was 150.4 cm, a gain of 8.4 cm over the expected average height, while those treated with GH plus oxandrolone ($n = 43$) achieved a mean final height of 152.1 cm, an average gain of 10.3 cm over predicted height without treatment (Fig. 3) (146). These results suggest that an adult height above the lower limit of normal for American women (150 cm) is now an attainable goal for many girls with this syndrome.

Somewhat more modest effects on total height gain (e.g., mean 0.7 SD increase) are also reported, particularly when estrogen treatment was initiated prior to 14 years of age (147). For instance, a recent report of the Canadian experience showed a mean height increase, estimated by analysis of covariance, of $+7.2$ cm. The treatment group achieved a height that was, on average, only 5.1 cm closer to their MPH than the control group. This more modest effect than was observed in prior studies can most likely be attributed to the relatively advanced age of the subjects (7 to 13 years at entry), the modest GH dosage (by current standards), and the use of estrogen at a set age of 13 (rather than individualization of estrogen replacement) (148).

These data have led to regulatory approval for the use of GH to treat the short stature of TS in many countries worldwide (including the United States). Critical factors for a successful outcome appear to be GH dosage and the number of years of GH treatment before estrogenization (149). As a result, initiation of GH therapy should be considered as soon as a patient with TS has dropped below the fifth percentile of the normal female growth curve. This may be as early as two years of age. For girls below 9 to 12 years of age, the recommended starting dose is 0.05 mg/kg/day, although individualization of dosing is appropriate based upon response. In older girls or in girls greater than eight years of age in whom therapy is started when short stature is extreme, concomitant administration of a steroid, which cannot be aromatized to estrogen, such as oxandrolone (0.05 mg/kd/day), should be considered. Girls given oxandrolone should be monitored by potential side effects including clitoral enlargement, and glucose intolerance, and liver toxicity. GH and oxandrolone therapy is continued until a satisfactory height is achieved or until bone age is greater than 14 and annual growth rate is less than 2 cm. Estrogen is not recommended as a growth-promoting agent, and the initiation of estrogen therapy should be timed to minimize negative effects on growth and adult height (150). Critical evaluation of each of these options is still warranted, however, because the long-term benefits of GH on

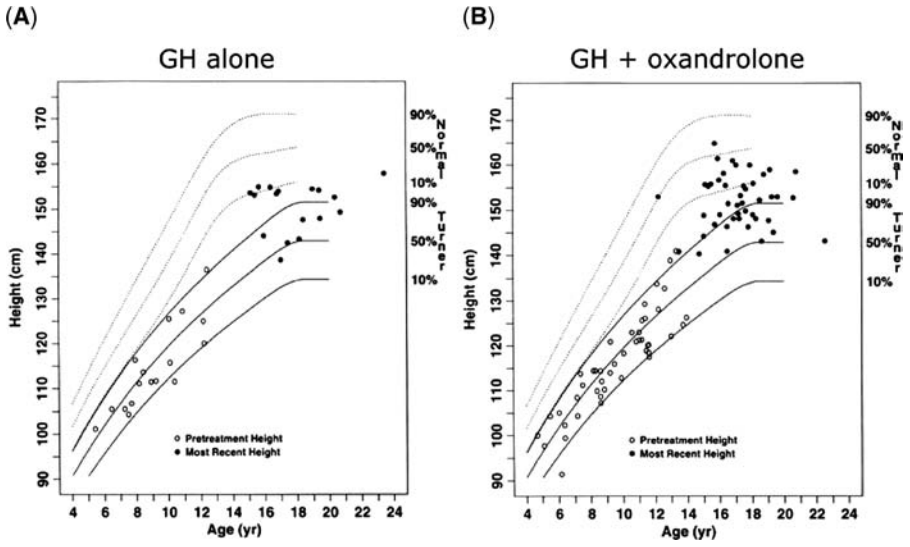

Figure 3 Effect of growth hormone therapy alone and with oxandrolone on last available height in girls with TS. *Abbreviation:* TS, Turner syndrome. *Source:* From Ref. 146.

improving adult quality of life (regardless of height and apparent bone mineral improvements) in TS has been difficult to show (151,152).

Adverse effects of GH therapy in TS patients have been minimal. While osteopenia is more prevalent in TS, bone mineral status is not impaired (and may be improved) in GH-treated adolescents with TS (153). Autoantibodies to endocrine organs also occur in TS with increased frequency; GH therapy does not alter immune function in TS (154). Glucose tolerance is of particular interest given the increased incidence of diabetes mellitus in adults with TS and the diabetogenic action of GH. Frequency of impaired glucose tolerance is increased in children with TS over normal. Investigations to date have generally revealed no significant change in glucose tolerance tests or levels of glycosylated hemoglobin during GH therapy (155), although one short-term study documented increases in fasting glucose, fasting insulin, and 24-hour insulin levels after only a two months period of GH (156). Obese patients or patients with high insulin concentrations before start of GH therapy may be at greater risk for deterioration (157). Elevations in plasma insulin concentrations are more frequent when GH therapy is combined with oxandrolone, which may also impair glucose tolerance by induction of insulin resistance.

Noonan syndrome (NS) is an autosomal dominant disorder that shares clinical features with TS, including growth retardation usually in the absence of GHD, resulting in adult heights that, on average, are 2 SD below the population mean (158). Gain of function mutations in PTPN11 (protein tyrosine phosphatase, nonreceptor type 11) have recently been identified as the genetic cause for approximately half of all NS cases (159,160). PTPN11 encodes for SHP2, a tyrosine phosphatase critical to postreceptor signaling of several developmental processes including the GHR signaling pathway.

Preliminary studies of GH in NS demonstrated a moderate effect of GH on growth velocity. A three-year controlled trial showed a mean increase in growth rate from a baseline of 4.4 cm/yr to 8.4, 6.2, and 5.8 cm/yr during years one, two, and three of GH therapy, respectively. Mean height SD score increased from −2.7 to −1.9 in GH-treated NS patients compared with a change from −2.7 to −2.4 in nontreated NS children. However, height acceleration was not significant during the second or third years when pubertal subjects were excluded (161). Others report that initial GH-induced growth acceleration is followed by a waning of effect with long-term therapy; in a small group of 10 patients, a mean increment in final height of only 3.1 cm was observed (162). In both studies, no deleterious effects on myocardial thickness were observed.

Thus far, there does not appear to be a correlation between severity of most clinical phenotypes or with growth disturbances and presence of a PTPN11 mutation (161,163,164). However, a recent three year prospective trial evaluating the effects of GH therapy on 7 PTPN11 mutation positive and seven mutation negative NS patients found a greater IGF-1 response and change in height SDS after three years in the patients without an identified mutation (161). A similar trend of greater IGF-I levels and a one year increased growth response to GH was found among mutation negative children compared to those with documented mutations (165). This data supports the hypothesis that PTPN11 mutations may interfere with normal growth and that mutation negative patients may have a more robust response to GH therapy.

Small for Gestational Age

For approximately 10% of children born SGA, the pattern of growth continues to be abnormal from intrauterine life to full maturity (165). Included in this group are children with dysmorphic features compatible with Russell-Silver syndrome. Even though bone age is often delayed early in childhood, the adolescent growth spurt usually occurs early and is reduced in magnitude (166). Although a minority of children with IUGR display subnormal spontaneous or provoked GH concentrations, results of these tests do not predict their responsiveness to GH.

In a study reported more than 30 years ago, approximately 50% of prepubertal children with SGA responded to twice- or thrice-weekly GH injections with significant growth acceleration (167). Studies of daily administration of GH reveal consistent increases in short-term growth velocity (e.g., from 6.7–10.4 cm/yr) (168); greater increments in growth velocity have been achieved with higher GH doses (169) though the dose response is likely limited to the prepubertal period (170). Prolonged (three years) GH treatment of children born SGA sustains an accelerated growth rate, which is proportionate and results in normalization of height and other anthropometric measurements, including HC, in contrast to untreated SGA control subjects (171). While one early study reported no increase height for bone age score, and thus an unaltered final height outcome (172), subsequent reports of five years of treatment (173) and a separate epianalysis of four randomized studies of six years duration (174) both indicate that administration of GH (0.033–0.067 mcg/kg/day with both continuous and discontinuous regimens employed) can normalize stature of short, non-GHD SGA children, at least during childhood and early puberty (Fig. 4). Recent studies have demonstrated an improvement in adult height outcomes in SGA treated GH children. Age at initiation and length of therapy remain important predictors of overall response. Initiation of high dose GH (0.47 mg/kg/wk) in the peripubertal period results in a significant though slight improvement (0.6 SDS) in adult height outcomes (175). Another recent dose-response study reported significant improvement in adult height SDS in treated children compared to a control group, but that the effect was not dose

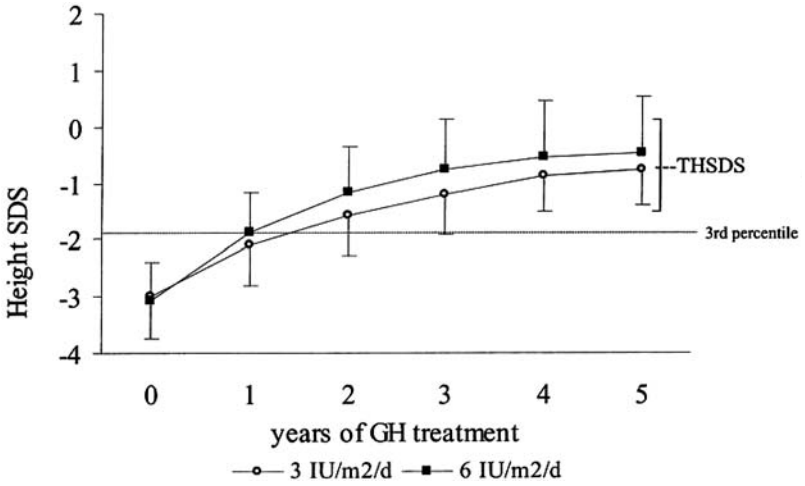

Figure 4 Effect of varying doses of long-term GH therapy on heights in children born SGA. *Abbreviations:* GH, growth hormone; SGA, small for gestational age. *Source:* From Ref. 173.

dependent (176). Furthermore, a recent study, utilizing either a control group of nontreated IUGR children or observational group study design, reported that nearly 90% of the GH-treated children achieved a FH within 1 SD of the target height, whereas only 52% of the untreated children achieved this target (171). The greatest gains in height were made by those children treated for longer than two years prior to the onset of puberty.

The overwhelming evidence points to a clear acceleration of growth rates in most SGA children treated with GH, with more dramatic improvements in adult height outcomes when started at an early age. However, similar to the response seen in the ISS group, GH effects are variable in this heterogenous group of patients. Thus, GH dosing recommendations should be used as an initial guide but regimens should ultimately be individualized.

Growth failure in SGA children has recently been approved as an indication for GH therapy by the FDA. Further, the successful and novel use of intermittent high-dose GH therapy in this group of patients is an important observation that could potentially be applied to other GH treatment indications. Whether children born SGA derive substantial psychological benefits from a more rapid tempo of growth during childhood (even without enhanced final stature) remains uncertain. On the other hand, one recent well-designed study used a randomized, double-blind design to (preliminarily) demonstrate improvements in intelligence and psychosocial functioning after long-term GH therapy in children born SGA (177). Whether these improvements in intelligence are indirectly related to the increase in growth velocity/stature or from a more direct GH effect on brain development remains to be determined.

Thus far, GH treatment in the SGA population has not been associated with any significant, long term detrimental side effects. Higher-dose GH therapy has also led to hyperinsulinemia in some of these children (178). However, this hyperinsulinemia appears to be predominantly transient and resolves three months after therapy (64). Given the baseline risk for development of diabetes in this population, close monitoring of glucose intolerance in those patients on GH therapy is warranted.

Long-term GH therapy in short SGA children does not appear to influence the age at onset and progression of puberty compared to AGA controls, regardless of treatment dose (179). However, given the inherent accelerated tempo of puberty that occurs in some of these patients, concomitant administration of GnRH analogue or aromatase inhibitors to permit a longer period of growth may be indicated for severely height-disabled individuals with IUGR.

Chronic Renal Failure and Hypophosphatemic Rickets

Poor nutrition, anemia, and chronic metabolic acidosis contribute to the growth failure characteristic of CRF. In addition, elevated fasting GH levels, exaggerated responses to provocative stimuli, depressed serum IGF-1 levels, and increased levels of IGF-1 binding protein (in particular IGFBP-1 and IGFBP-2) suggest resistance to the action of GH (180,181). Pharmacologic doses of GH are able to overcome these growth retarding influences in some patients with CRF through metabolic effects of GH [e.g., enhanced renal acid excretion (182)], increases in IGFs and ternary complexes which overcome inhibitory effects of excess IGFBPs (183), and direct growth-promoting effects (Table 2). A randomized, double-blind, placebo-controlled study of 125 prepubertal growth retarded children with CRF revealed first year (10.7 ± 3.1 cm/yr) and second year (7.8 ± 2.1 cm/yr) growth rates in the GH-treated group, significantly greater than those seen in the placebo group (184). The beneficial effect on final height potential predicted by this study was confirmed in one study of 38 GH-treated children with

Table 2 Final Height Outcomes in Growth Hormone Treated Children from North American Pediatric Renal Transplant Cooperative Study (Minimum Age at Last Visit was 18 Years)

CRI registry	Number	Height SDS at enrollment	Height SDS at last visit	Δ SDS
Prior rhGH	9	−3.00	−2.2	+0.70[a]
No Prior rhGH	335	−0.97	−0.99	−0.02[a]
Dialysis registry	Number	Height SDS at dialysis	Height SDS at last visit	Δ SDS
Prior rhGH	22	−3.59	−3.24	+0.35[b]
No Prior rhGH	377	−1.88	−1.82	+0.06[b]
Transplant Registry	Number	Height SDS at Transplant	Height SDS at last visit	Δ SDS
Prior rhGH	72	−2.95	−2.46	+0.49[c]
No Prior rhGH	1480	−1.74	−1.70	+0.04[c]

[a] $p = 0.036$.
[b] $p = 0.09$.
[c] $p = 0.001$.
Abbreviations: CRI, chronic renal insufficiency; rhGH, recombinant human GH.
Source: From Ref. 186.

CRF who reached a final height 1.4 SD above standardized height at baseline compared with mean final height of 50 nontreated matched control children with CRF that was 0.6 SD *below* standardized height at baseline (Fig. 5) (185). Recent follow-up data from the North American Pediatric Transplant Cooperative Study database showed a benefit in adult height outcomes among GH treated CRF patients, dialysis patients, and transplant patients (Table 2) (186). In general, responsiveness to GH appears to be inversely related to the degree of renal function impairment and metabolic compromise. Growth failure due to CRF is an FDA-approved indication for GH therapy.

GH excess produces hyperfiltration and increases glomerular sclerosis in the setting of uremia (187), raising a theoretical concern that GH-treatment of children with CRF could accelerate deterioration in renal function. However, current information suggests no adverse effect of GH therapy on glomerular filtration rate (GFR); loss of GFR is unchanged during the first year of GH treatment when compared to the year before treatment (188). Reports of accelerated rises in serum creatinine may reflect increased body size and creatinine production without a commensurate increase in GFR (189). Thus, growth itself, rather than GH, may place additional metabolic demands upon compromised, but stable renal function. Hyperinsulinemia, often present before GH therapy due to uremic insulin resistance, may remain stable or worsen during GH therapy. However, glucose homeostasis, assessed by oral glucose tolerance testing and glycosylated hemoglobin levels, has remained stable (190). Transient intracranial hypertension may occur during GH treatment of children with CRF, but is relatively rare and is reversible with temporary cessation of drug or reduction in dose.

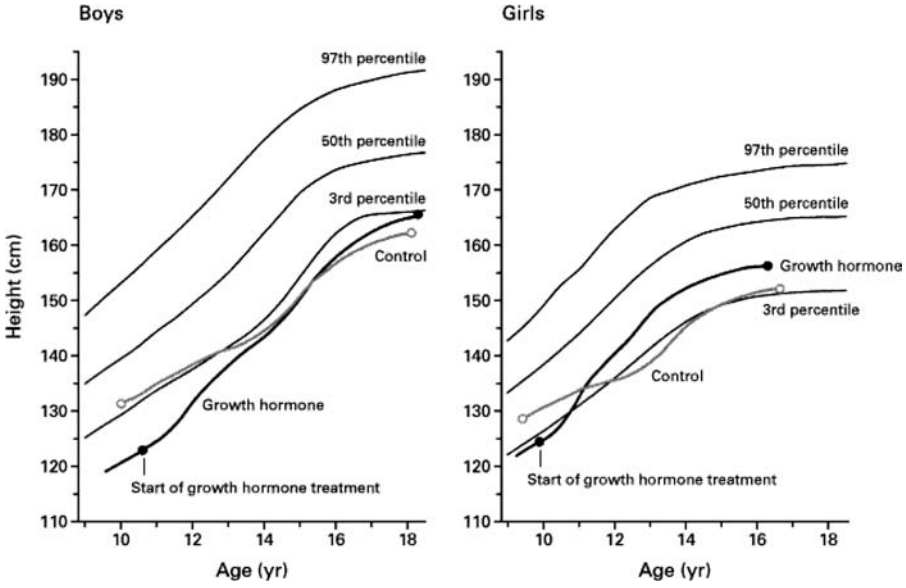

Figure 5 Beneficial effects of growth hormone therapy on long-term growth in children with chronic renal insufficiency. *Source:* From Ref. 185.

In normal kidneys, GH increases renal phosphate retention. Consequently, GH has been administered to poorly growing children with X-linked hypophosphatemic rickets (XHR). In a recent study reporting final height outcome, GH therapy combined with conventional treatment resulted in a change in height SD score in six children with XHR (mean baseline −3.4, mean posttreatment −2.4) whereas no change in height SD score was observed in six XHR patients not treated with GH. Phosphate retention, bone markers, and radial bone mineral density increased only in the GH-treated group (191). Additional studies using small patient numbers have observed a similar beneficial effect on phosphate and Vitamin D levels (192) as well as height outcomes (193). More powerful, long-term studies are needed to verify the value of long-term GH therapy for this disorder.

Skeletal Dysplasias

Numerous forms of skeletal dysplasia may cause significant growth retardation (Vol. 2; Chap. 6). Detailed descriptions of clinical and radiologic features and inheritance patterns belie a lack of understanding of the basic pathophysiology for many of these disorders, although associated genetic mutations (e.g., fibroblast growth factor receptor-3 in achondroplasia, and short stature homeobox mutations in Leri-Weill dyschondrosteosis and TS) are steadily being recognized (194,195).

A short-term study of GH therapy in children with hypochondroplasia showed an increase in first year growth rate that was proportionately distributed between limb and spine growth (196). Variation in response to GH with regard to rate and proportionality of growth have also been reported, and may be related to defects in the IGF-1 gene in some patients (197). Growth response to GH is generally less than that observed in children treated for classic GHD and declines after initial acceleration. Thus, modest improvement in adult height might be expected from currently used regimens of GH therapy. Higher GH dosage, concomitant GnRH analog treatment, and use of aromatase inhibitors could conceivably alter this prognosis. Patients with achondroplasia demonstrate normal secretion of GH, IGF-1 levels, and IGF-1 receptor activity (198). Reports of response to GH are variable. Some data indicate little change in growth rate, even when higher than conventional doses are used (199). More recent studies suggest that GH therapy can increase growth rate and height z score in a dose-dependent manner without significant side effects (200). However, effects on disproportionate growth remain a concern (201) and ultimate height outcomes remain unknown. Initial reports of GH therapy in limited numbers of patients with Leri–Weill dyschondrosteosis show a modest benefit in growth rate but no controlled studies have been performed to date (202,203). Preliminary trials of GH therapy for spondylo-epiphyseal dysplasia and multiple epiphyseal dysplasia are in progress.

Glucocorticoid-Treated Children

Treatment of many chronic disorders (e.g., juvenile rheumatoid arthritis, asthma, renal transplantation, inflammatory bowel disease), which themselves may lead to growth failure, includes GC therapy. GCs impede linear growth through several mechanisms, including promoting protein catabolism, inhibiting collagen synthesis, impairing the action of IGF-1, and suppressing endogenous GH secretion through augmentation of hypothalamic SRIH tone (204). Thus, these patients could be considered logical candidates to benefit from both the growth-promoting and anabolic effects of GH therapy.

Early studies, implementing relatively low dose and infrequent administration of GH, demonstrated marginal and inconsistent beneficial effects (205). Subsequent investigations of daily GH therapy for children with stable GC-treated illness (206) or postrenal transplantation (207) show more consistent resumption of normal growth velocity during one to three years of treatment. Biochemical markers of growth (e.g., type 1 procollagen levels) are also normalized by GH administration. Responsiveness to GH appears greatest in those on moderate-dose GC regimens with stable, nonarthritic underlying disease. Persistence of disease activity and higher GC dosage (e.g., prednisone dose $>0.35\,mg/kg/day$) (208) interfere with GH-responsiveness.

Recent analysis of larger numbers of GC-dependent children ($n = 83$) followed over a 12-month period reveal a mean response to GH therapy (mean dose $= 0.3\,mg/kg/wk$) of doubling of baseline growth rate (e.g., 3.0 ± 1.2 to $6.3 \pm 2.6\,cm/yr$)(Fig. 6) (209). Responsiveness to GH is negatively correlated with the dose of GC. In 14 severely growth-impaired children with rheumatoid arthritis (mean height SDS $= -4.0$, GH administered at a dose of $0.5\,mg/kg/wk$ resulted in an increase in mean growth velocity from 1.9 to $4.5\,cm/yr$ (210). Similarly, the response to GH treatment appears to be related not only to dose of concomitant GC but also to disease severity in juvenile idiopathic arthritis (211). While difficult to prove, preservation of height SDS most likely represents a beneficial therapeutic outcome for these children. Still, long-term GH-responsiveness and effects of GH therapy on final height in GC-treated children remain unknown, and GH treatment of GC-dependent children remains experimental.

Salutary effects of GH therapy ($0.05\,mg/kg/day$ administered daily) on the growth of children following renal transplantation are also reported. While GH treatment of growth disordered children who are postrenal transplant is not yet approved, growth rates of prepubertal posttransplant children have generally increased two-to-three fold during the first one to two years of GH therapy, decline subsequently but remain above baseline growth velocity (212). Gains in height SD scores approximate 1 SD following two to four years of therapy, and approximately one-half of the children achieve ''normal'' heights (i.e., within 2 SD

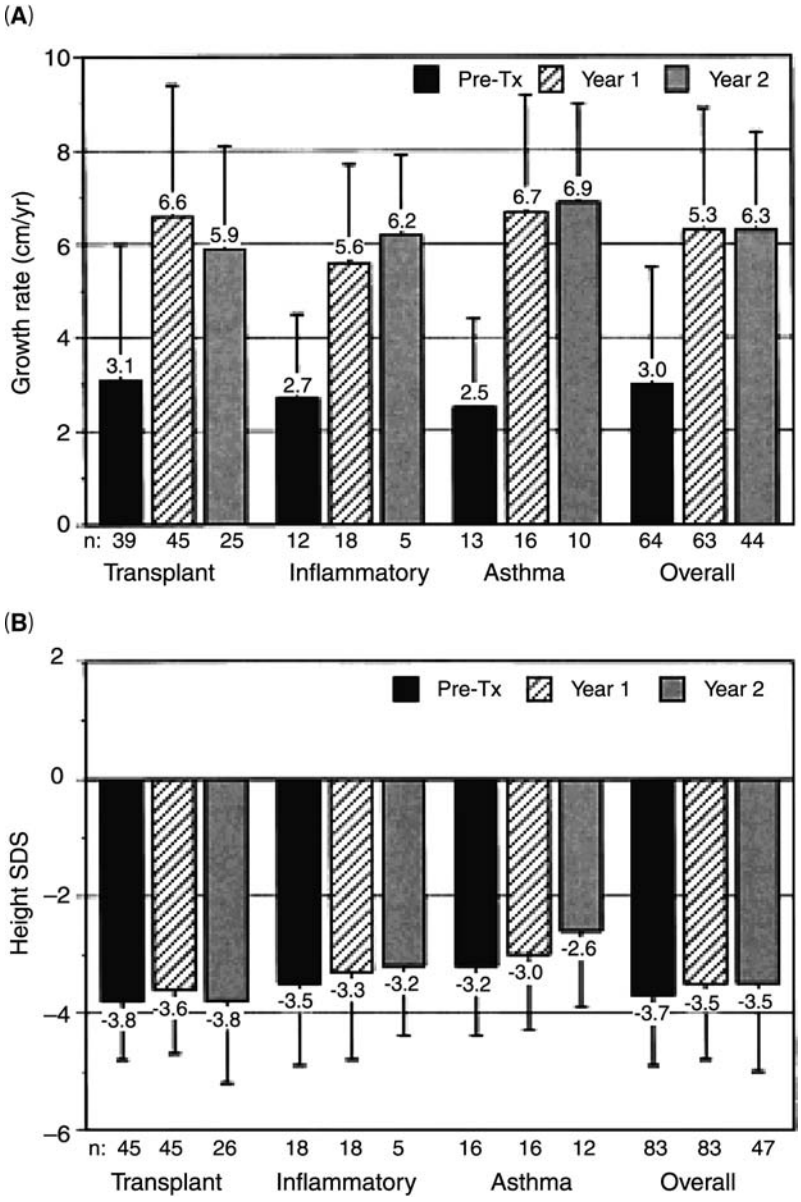

Figure 6 Effect of growth hormone therapy on growth in children treated with glucocorticoids. *Source:* From Ref. 209.

of the mean) (Table 2). Growth stimulation in pubertal children with renal allografts, while less consistent than that observed in younger patients, is still significant (213). Recent data indicate that recombinant human GH is effective in producing incremental growth and final adult height in pediatric renal allograft recipients without producing allograft dysfunction or increasing the incidence of targeted adverse effects (214).

In addition to restoring linear growth, GH therapy may counter some of the catabolic effects of GC. Studies using isotope tracer infusions suggest that GH, directly or through IGF-1, counteracts GC-induced protein catabolism through independent stimulation of protein synthesis without altering protein breakdown (215). Alternatively, accompanying hyperinsulinemia might contribute substantially to the observed protein anabolic effect by decreasing proteolysis. GH may also counteract the antianabolic effects of GC on bone. In a group of adults receiving chronic GC treatment, in whom GHRH-stimulated GH levels were suppressed, levels of osteocalcin and carboxy-terminal propeptide of type I procollagen rose with short term GH therapy (216). In children with juvenile rheumatoid arthritis, long-term GH-treated resulted in significant higher bone mineral content as well as total cross-sectional area (CSA), cortical CSA, and muscle CSA, and lower fat CSA compared to an untreated control group (217). Detailed studies of the metabolic effects of GH administration in children receiving long-term GC therapy have not yet been done.

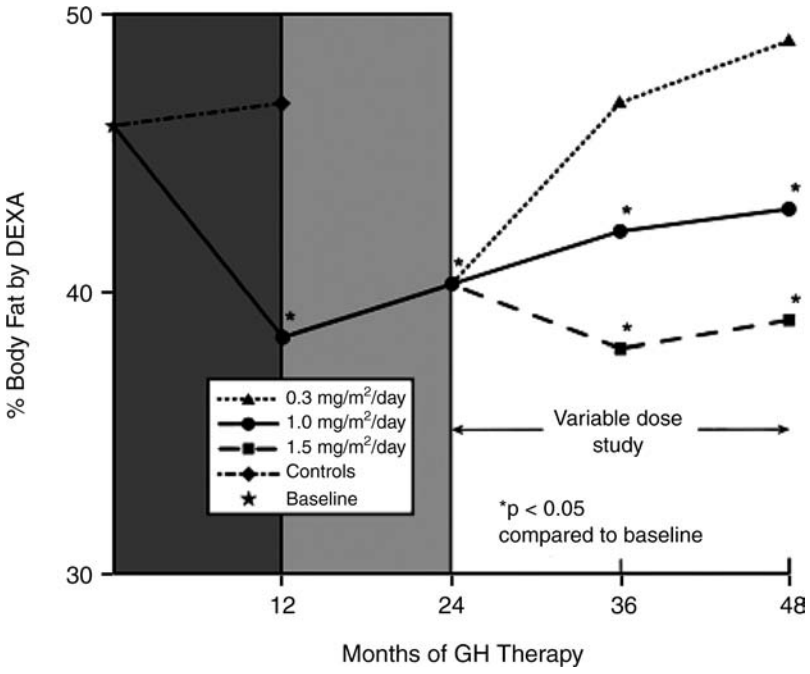

Figure 7 Effect of four years growth hormone therapy (variable dosing years three to four) on percent body fat in children with PWS compared to nontreated PWS control subjects. *Abbreviation*: PWS, Prader–Willi syndrome.

Potential adverse effects of combined GH/GC therapy in children with GC-dependent disorders include altered carbohydrate metabolism, stimulation of autoimmune disease activity, increased cancer risk, and, in transplant recipients, graft dysfunction or rejection. Elevated fasting and stimulated insulin levels have been observed in renal allograft patients receiving GH; however, these changes frequently predate institution of GH therapy, correlate with prednisone dosage, and are not affected by the addition of GH. Among all GH-treated GC-dependent children, detectable elevations in blood glucose concentrations have been rare. GH-induced exacerbations of chronic disease activity also appear to be very unusual, but the number of patient-years available for study of this question remains small. With regard to renal allograft function and survival, actions of GH on glomerular hemodynamics, glomerular morphology, and immune stimulation are theoretical reasons for concern. Nevertheless, most investigators report no difference between GH-treated and control renal allograft patients with regard to changes in GFR, effective plasma flow, other measures of renal function, and rates of allograft rejection.

Although preliminary analysis of one randomized prospective study suggested that GH might slightly increase allograft rejection rates, final analysis indicated that biopsy-proven acute rejection episodes were not significantly more frequent in the group receiving GH (218). Long-term and careful follow-up of children with renal transplants receiving GH therapy is still needed to resolve this important issue.

Prader–Willi Syndrome

Growth of children with Prader–Willi syndrome (PWS) is characterized by moderate intrauterine and postnatal growth retardation, followed by near-normal growth rates as caloric intake increases and obesity typically develops. Substantial evidence now supports a true GH deficient state in PWS: lack of nutrition-induced growth acceleration, relatively low IGF1 levels, diminished GH responses to GH provocation, and body composition abnormalities (i.e., reduced muscle mass, increased fat mass) resembling states of GHD. Administration of GH to children with PWS results in growth rate increases equivalent to those observed in severely GH-deficient children. In addition, GH therapy increases lean body mass, decreases fat mass, and increases bone mineral density (219,220). Reductions in fat mass result from increases in total body energy expenditure and preference for fat oxidation as an energy source (confirmed reductions in respiratory quotient). In addition to cognitive and behavioral disabilities, profound hypotonia severely limits the physical function of individuals with PWS. Treatment with GH improves measures of strength and agility in children with PWS, substantiating claims of ''real life'' benefits of GH for these children. Early institution of treatment during infancy improves (already) abnormal body composition and may accelerate the acquisition of motor skills (221). Changes in body composition and physical function are dose-dependent and attenuate, but do not regress during prolonged GH therapy (Fig. 7) (222).

Growth failure related to PWS is a recent FDA approved indication for GH treatment.

Concerns about increased occurrence of scoliosis and glucose intolerance have not been realized, but cases of sudden death in predominantly young and very obese children with PWS relatively early in the course of GH therapy have been reported. This observation is difficult to reconcile with studies showing improvement in respiratory function as a result of GH therapy (223). While the role of GH therapy in these deaths remains uncertain, most endocrinologists now require sleep studies, correction of airway obstruction, weight reduction if needed prior to institution of GH therapy, and careful observation for changes in airway function when treatment is underway (224).

Other Syndromes and Defects Associated with Short Stature

Short stature is a component of more than 100 syndromes. Subnormal secretion of GH has been reported in some children with Down syndrome (225). Preliminary treatment trials of these children show short-term responses to GH similar to those of Russell-Silver syndrome and TS. Short stature is also a common problem in children with neural tube defects such as spina bifida or myelomeningocele, who may have spinal and/or lower abnormalities. Secretion of GH is normal unless accompanying hydrocephalus impairs hypothalamic-pituitary function. In a small group of patients with neural tube defects and subnormal GH secretion, GH treatment over 36 months significantly improved growth rates of body length and arm span. However, the increase in length SD score was not significant (226).

GH may also serve as a useful adjunct for other disorders that result in short stature due to overproduction of sex steroids. A recent trial of GH and GnRH agonist therapy in children with congenital adrenal hyperplasia and a poor height prognosis has shown promise for improving growth rates and adult height outcomes (227). Similarly, a subset of children with idiopathic central precocious puberty treated with adjunctive GH may achieve greater adult heights (228).

The expansion of GH therapy into populations affected by mental disability or with limited ambulatory capability raises complex ethical questions by focusing attention on the expectation that successful GH therapy ought to improve the quality of life, rather than merely the height, of treated individuals (229). For each of these groups of patients, analysis of larger, longer prospective trials, conducted within a GH investigational protocol, are needed before recommendations about efficacy can be made. When this information is available, treatment decisions can be made for individual patients (rather than diagnostic groups) based upon degree of disability and likelihood of long-term enhancement in quality of life.

Adults with Growth Hormone Deficiency and the Elderly

The availability of unlimited supplies of human GH has been accompanied by a wealth of new information regarding the consequences of GHD in adults and the benefits of replacement treatment (Vol. 2; Chap. 3). Lack of GH in adulthood can lead to contraction of lean body mass and water, expansion of fat mass (particularly central abdominal fat), diminution of bone mineral content, increased concentrations of total and LDL cholesterol, increased atherothrombotic propensity, impaired cardiac function, impaired psychological well-being, and decreased life expectancy (230). Placebo-controlled studies of GH therapy in adults with complete GHD have revealed a marked increase in muscle mass, decrease in fat mass (231,232), and improvements in exercise performance (233). Subjective improvements in ''quality of life'' indicators such as vigor, ambition, and sense of well-being are also reported (234). Consequently, treatment of adult GHD is now an FDA-approved indication for GH therapy. Selection of appropriate candidates (i.e., those with severe GHD) remains challenging in the absence of multiple pituitary hormone deficiencies. Although responses to the insulin tolerance test remain the gold standard (115), arbitrary cut-offs for ''normal'' GH responses and IGF-1 levels cloud the ability to establish a diagnosis (235). For virtually all patients with isolated GHD, a period of observation off GH treatment is recommended to confirm the diagnosis, and does not appear to be harmful (114).

Adverse effects due to fluid retention (e.g., edema, carpal tunnel syndrome) are fairly common, but reverse with dose reduction or continuation of same dose. Doses used are markedly lower than those used per kilogram during childhood: the Growth Hormone Research Society recommends that dosage not be weight-based. Patients are to commenced on a low dose (0.15–0.3 mg/day) and then gradually increased in accordance with clinical and biochemical responses. Maintenance dosage varies considerably, and is influenced by gender and age, but rarely exceed 1 mg/day. Women usually require higher doses than men, while the elderly require lower doses (236). When appropriate dosage adjustments are made to achieve IGF-1 levels in the normal range, GH replacement has beneficial effects on cardiac and exercise performance and atherosclerosis in both women and men with severe GHD.

The normal decline in the activity of the GH-IGF-1 axis that occurs with advancing age appears to contribute to the decrease in lean body mass, increase in adipose tissue mass, and possibly loss of energy. Administration of GH to non–GH-deficient men over 60-years-old caused significant increases in lean body mass, bone density, and skin thickness, with concomitant reductions in fat mass (237). Following GH therapy, deterioration of these parameters

resume in an age-appropriate fashion. While the implications of such therapy are enticing, the risk-benefit ratio and cost-effectiveness of GH therapy in the general aging but healthy population have not been established. Administration of GH has also been used by younger adults in conjunction with heavy resistance exercise training in an effort to maximize skeletal muscle protein anabolism and strength. However, in a placebo-controlled study of young normal men, resistance training supplemented with GH did not further enhance muscle anabolism and function (238). These results suggest that increased fat-free mass seen with GH supplementation was probably due to an increase in lean tissue other than skeletal muscle.

Catabolic States/Acquired Immune Deficiency Syndrome Wasting Syndrome

Well-documented salutary effects of GH on nitrogen retention and protein synthesis rates have prompted investigation of GH therapy in various catabolic, regenerative, or reparative states. Nitrogen balance is improved by GH in postoperative patients under hypo-caloric conditions (239), and GH partially reverses nitrogen wasting in obese humans made catabolic by dietary restriction (240). GH also improves the efficiency of parenteral nutrient utilization in patients requiring total parenteral nutrition. Improvements in weight and lean tissue mass have also been documented in pediatric burn patients (241). However, results of controlled trials of GH treatment in acute care settings have not all been favorable. One placebo-controlled trial in normal adults showed that full-thickness wound healing was significantly delayed by GH therapy (242). A large randomized controlled trial of high-dose GH treatment of adult patients during a severe catabolic illness in the intensive care setting showed higher mortality in GH-treated patients (243). Although perhaps influenced substantially by the high doses of GH administered, these data have nevertheless created substantial concern about use of GH in the critical care setting. Most physicians currently discontinue GH in acutely ill patients in the hospital who are taking GH chronically. Whether GH treatment of overwhelmingly ill patients induces the release or potentiates the action of harmful cytokines is currently under investigation.

Anabolic effects of GH therapy have also created interest in its use as ancillary therapy for several chronic illnesses. GH has been approved in the use of AIDS wasting syndrome, characterized by excessive lean muscle mass loss and cachexia in the advanced stages of disease. GH has also been shown to improve the lipodystrophy associated with HIV infection in adults and recently was demonstrated to have similar effects in adolescent HIV patients with lipodystrophy (244).

Improved lean tissue mass, height and weight gain, and decreased protein catabolism have been reported in children with Crohn's disease and chronic ulcerative colitis (245). In children with cystic fibrosis, a randomized controlled trial showed significant improvements in height and weight gain, lean tissue mass, and pulmonary function, accompanied by decreased hospitalization time (246). Another randomized control trial demonstrated that the growth response of prepubertal CF children receiving enteral nutritional supplementation is improved by GH therapy (247). During the next decade, we can look forward to considerable new knowledge about the anabolic effects of GH in these and related settings.

ADVERSE EFFECTS OF GROWTH HORMONE TREATMENT

Recombinant biosynthetic GH preparations are highly purified and free of contaminants. The possibility of viral transmission through GH has been virtually eliminated. However, surveillance of patients who received pituitary-derived GH for development of Creutzfeld–Jacob remains important. Antigenicity of GH preparations is also low (248), although GH antibodies can be detected in 10% to 30% of treated children. With rare exceptions (>0.1%), these antibodies do not impede effects of GH.

GH administration to healthy subjects acutely increases serum T3 and reciprocally decreases free thyroxine (T4). Similarly, laboratory indications of hypothyroidism may be seen in as many as 25% of GH-deficient children treated with GH, with declines in serum T4 levels reflecting increased peripheral conversion of T4 to T3 (249). GHD patients, who display subnormal nocturnal TSH surges, signifying a preexisting central hypothyroidism, are more likely to display subnormal T4 and free T4 levels during GH therapy, and to benefit from thyroid replacement (250). Recent data suggest that GH treatment "unmasks" central hypothyroidism in patients with organic hypopituitarism at risk for multiple hormone deficiencies, but it does not appear to cause the same problem in idiopathic, isolated GHD (251). In general, most studies indicate that children with normal thyroid function before treatment do not develop significant perturbations in thyroid hormone metabolism during GH therapy.

GHD is associated with reductions in lean body mass and total body water, with accompanying reductions in renal plasma flow and GFRs. Edema and sodium retention rarely occurs early in the course of GH therapy (particularly in older, heavier children and adolescents), attributable to an antinatriuretic effect on the renal tubule of GH and/or IGF-1. Minor elevations in plasma renin activity and aldosterone observed in the first three days of treatment resolve within a week or two (252). Occasionally, fluid shifts within the central nervous system are sufficient to cause benign intracranial hypertension (pseudotumor cerebri) and its symptoms of headache, visual loss, vomiting, and papilledema. It is speculated that

direct fluid retaining properties of GH and/or action of locally produced IGF-1 on CSF production are causative. Most instances have occurred during early (though not invariably) treatment of patients with severe GHD or other risk factors for this condition (e.g., chronic renal insufficiency, PWS). Cessation of GH therapy has reversed the symptoms in reported cases, and some patients experience spontaneous resolution of symptoms in spite of continued GH treatment (253). Resumption of GH treatment has been successfully accomplished with reinitiation at a lower dose and gradual return to the initial dose. It is recommended that fundoscopic examination be performed on all patients before initiation of GH therapy and periodically thereafter (254).

Until recently, GH had been used primarily as replacement therapy for GHD. Adverse effects of GH therapy become more likely as higher dosages are used for pharmacological GH augmentation therapy. Recommendations for GH dosage, derived largely from growth response data, are in excess of calculated estimates of normal GH production in a prepubertal child, and dosing guidelines for adolescents are increasing further. IGF-1 levels in some GH-treated girls with TS approach those found in acromegaly (255), and anecdotal reports of development of acromegaloid features (e.g., large hands and feet) during higher-than-conventional dose GH therapy have appeared. GH excess reduces insulin sensitivity, and given the trend toward higher dosages, it is important to assess a GH dose effect on carbohydrate metabolism. Nearly all studies of GH therapy in children and adults show an increase in fasting and postprandial insulin levels. However, glucose homeostasis is generally not impaired (256). Further, normal levels of blood glucose and glycosylated hemoglobin may be preserved by hyperinsulinemia, which when persistent can be associated with atherosclerosis and hypertension. One study of long-term high dose GH therapy found normal glucose metabolism and higher levels of insulin (which did not correlate closely with dose) that returned to normal when GH therapy was discontinued (257). Another more recent study showed no increase in the incidence of type 2 diabetes among a large group of GHD children treated with GH for a mean of six years (258). Given these reassuring data, a recent report of an increased frequency of type 2 diabetes in childhood GH recipients were surprising. Less than half of affected subjects were obese or had other risk factors for diabetes, two-thirds were in puberty, and diabetes persisted in all patients after GH discontinuation (259). Other pharmacoepidemiological databases have not reported in increase in diabetes incidence. Careful prospective follow-up of individuals during and after GH therapy is needed to resolve this issue.

Perhaps the greatest concern regarding GH therapy is the theoretical possibility that GH could facilitate the development of cancers. Importantly, a recent study revealed hypopituitary adults not undergoing GH replacement therapy had an increased rate of malignancies, with a predominance of colorectal cancer (260). On the other hand, GH raises serum concentrations of IGF-I, which is mitogenic and antiapoptotic. In animals, there is evidence of a cause and effect relation between supra-physiological doses of GH and development of leukemia (261). There are three clinical settings which have raised concern: (i) GH-treatment induced new malignancy; (ii) GH-treatment induced recurrent malignancy; (iii) GH-treatment induced second malignancy in those already treated for one tumor. In 1988, reports from Japan describing leukemia in GH-treated children raised concern about new malignancy (262). An extended follow-up study of 6284 recipients of pituitary-derived GH revealed a relative risk of leukemia in recipients of GH of 2.6 (90% confidence interval, 1.2–5.2). Five of six subjects had antecedent cranial tumors as the cause of GHD, and four had received radiotherapy (263). Subsequent analyses, based on much larger total patient-years of GH treatment, indicate that the rates of new leukemia in (non-Japanese) patients without preexisting risk factors who are treated with GH is no greater than expected for the general population (264). This lack of increased risk in children without preexisting risk factors was recently confirmed in the Japanese population (265). Any possible increased incidence of leukemia appears limited to those patients with known risk factors, and due to the small numbers of events in such patients, it remains impossible to determine any contribution of GH therapy.

With regard to nonleukemia cancers, a retrospective analysis of large postmarketing database found no evidence of an increased risk of developing an extra-cranial, nonleukemia neoplasm in GH-treated patients (266). In one epidemiologic study of solid cancers in 1848 U.K. patients treated with pituitary-derived GH during childhood and early adulthood between 1959 and 1985, there were two deaths each from colon cancer and Hodgkin's disease, leading to a significantly elevated odds ratio 07.9, which persisted even after eliminating high risk patients (267). While the number of occurrences in this study was very small and the levels of IGF-1 were not known in the study population, concern about possible adverse effects of elevated IGF-1 levels has prompted ongoing debate about how best to monitor and respond to IGF-1 and IGF-BP3 levels (268,269).

With regard to recurrent malignancy, no report has associated GH therapy with an increased incidence of tumor recurrence. A lack of reliable knowledge about the natural history of recurrence of these tumors, however, supports a cautious interpretation of such data. Most recurrences occur within the first two years of treatment, and most endocrinologists defer institution of treatment until a year of stable remission has passed. While this may result in some lost growth for the child, it also avoids the inevitable association of GH therapy and tumor recurrence during this high-risk period.

With regard to second malignancy, important data from the Childhood Cancer Survivor Study and its over 13,000 childhood cancer survivors (minimum of five years) revealed that among the 361 children treated with GH, the relative risk for development of secondary solid tumors was 3.21 (95% CI 1.88–5.46), with the primary risk occurring in survivors of acute leukemia (RR ~5). It is important to note that IGF-1 levels were not reported in this study and that all but one of the secondary tumors occurred at a site previously exposed to external radiation (270). These results are in opposition to the experience at one center which recently reported no evidence for increased risk of relapse or secondary malignancy in a smaller group of GH treated ALL survivors (271).

In humans, GH and IGF-1 have been shown to affect numerous immune functions, but these effects do not appear to translate into clinically relevant problems in GH-treated children. The possibility that GH might influence the growth rate of melanocytic nevi has been raised. However, a recent study failed to show any relationship between melanocytic nevi count and duration of GH therapy in girls with TS, and the melanocyte count in children with GHD was not different from controls and was not influenced by GH therapy (272). GH treatment causes an increase in both the proliferative and hypertrophied zones of the growth plate that may reduce the force needed to shear it. An increased frequency of slipped capital femoral epiphysis has been reported during treatment with GH (273). This rare complication is more common in children with organic causes of severe GHD, who require close monitoring for limp, and hip or knee pain (274). Progression of scoliosis may be more rapid during GH therapy, but available data does not suggest an increased frequency of scoliosis as a result of GH exposure. Nevertheless, some children more likely to receive GH therapy (e.g., TS, PWS) already have an increased frequency of scoliosis, and require close monitoring. Concerns raised regarding other potential adverse effects of GH therapy (e.g., reduced testicular volume, gynecomastia, deterioration of renal function in CRF patients, cardiac ventricular hypertrophy in Turner and NS) have, to date, either not been realized or considered to be of sufficient clinical significance to alter prescribing (275).

In summary, nearly two decades of experience with recombinant GH has proven this therapy to be remarkably safe when used in conventional substitution doses for GHD. Higher-dose therapy for other causes of growth retardation also appears to be safe, but continued surveillance of its metabolic effects is indicated. There appear to be very few medical contraindications to GH therapy. Nevertheless, the experience of transmission of Creutzfeld–Jakob via pituitary GH is a poignant reminder that a farsighted view must be taken of the potential ramifications of long-term hormonal therapy.

ETHICAL ISSUES OF GROWTH HORMONE TREATMENT

Limited availability of GH once provided a barrier to expanding its use beyond children who were unequivocally GH deficient. Today, increased supply of GH has been matched by increased demand. Ten years ago in the United States, there was one approved indication for GH, two GH manufacturers, and approximately 10,000 children receiving treatment at a cost ranging between $5000 and $40,000 per year. Today, we have seven FDA-approved indications for GH, multiple U.S. and international manufacturers, and well over 30,000 active patients treated. Nevertheless, GH therapy remains expensive. Relaxed diagnostic criteria have obliterated any clear boundary between GHD and sufficiency, allowing many partially affected children access to treatment. Future goals of GH therapy appear likely to shift further toward supplementing and enhancing individuals' well being rather than merely returning them to some physiologic baseline. Careful longitudinal studies, many already in progress, will be required to determine the efficacy of GH in accomplishing these goals.

But what can be done with GH is not necessarily what should be done. Ethical justification for these goals, not merely the efficacy of GH therapy in accomplishing them, also deserves careful scrutiny. New uses of GH raise complex philosophic, psychological, and economic, as well as medical questions that physician-scientists alone cannot answer (276). Even though GH has been is shown to increase the height of non–GH-deficient children without apparent toxicity, additional considerations are needed to responsibly assess the long-term value of the added height increment, and to balance expected benefit with issues of resource allocation and fairness (277).

Widespread distribution of GH has been partially deterred by high drug costs. Paradoxically, while the source of GH is no longer limited, the resources with which to pay for it are becoming increasingly so. Prescribing GH requires a difficult and often uncomfortable balancing of responsible use of medical resources with an obligation to do the best for each individual patient. But is taller really better for each patient (278)? Concern about psychological harm is invoked as a primary rationale for treating short stature yet data confirming the efficacy of GH therapy in alleviating the psychosocial consequences of short stature are scarce. Moreover, consideration for the potential psychological harm of an implied message of disease state or lack of parental acceptance in treating children without GHD may be as important. If the ultimate goal of GH therapy is not tall stature but, rather, an improved quality of life, documentation of psychosocial impairment due to stature prior to therapy and improvement following GH therapy ought to play an important role in the initiation of GH therapy and evaluation of its efficacy (279). To date, however,

growth rate and final adult height remain the measures by which therapeutic success is judged.

Responsiveness to GH, described above in many groups of short-statured individuals, is a necessary but not sufficient indication for treatment. Because of enormous expense and concerns that such widespread access would not ameliorate disadvantages of short stature, it seems appropriate to restrict access within this group based upon the presence of significant functional or psychological disability related to stature and the likelihood of improvement with treatment. Most short children will not satisfy these additional criteria. Children with severe GHD are likely to be both more disabled and responsive than non–GH-deficient children, justifying their preferential treatment. Is it justified to restrict access of others to GH based on the laboratory diagnosis of GHD? Some have argued that equally short children share a similar disability, regardless of whether stimulated GH levels fall just above or just below an arbitrary threshold. On the other hand, the recent FDA decision to approve GH therapy for very short non-GHD children has validated the notion advanced by Allen and Fost in 1990 that, if non-GHD children are GH responsive and truly disabled by height, there would be little ethical justification for treating only the child with lower GH test results (280).

Alleviating the disability of short stature, rather than normalization of GH levels, has traditionally been the primary goal of GH therapy. Determining an appropriate end-point for GH therapy remains controversial, since height is but a surrogate measure for the true hoped-for therapeutic benefit (i.e., improved psychosocial function), which has thus far eluded documentation. Attainment of genetic potential for height, a realistic possibility with optimal diagnosis and treatment, remains a goal for many (281). Consistent adherence to the goal of alleviating disabling short stature, on the other hand, implies that GH therapy be discontinued when each treated child reaches an adult height no longer considered a disability. While no policy regarding GH therapy will ever eliminate the first percentile, the second strategy has as its goal bringing children into the normal opportunity range for height without further enhancing those who have achieved a height within the normal adult distribution. By adhering to the treatment of disabling short stature, and resisting the enhancement of normal stature, physicians treating children with GH would minimize their contribution to society's heightist perception that to be taller is to be better (282).

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6

The Skeletal Dysplasias

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INTRODUCTION

The human skeletal dysplasias are a heterogeneous group of disorders, which result in disproportionate short stature. Although most disorders are rare, these developmental defects of the skeleton cause a significant proportion of the cases of moderate to severe short stature (1).

Before 1970, the diagnosis for most patients with disproportionate shortening of the limbs was ''achondroplasia,'' and those with short trunks were thought to have ''Morquio syndrome.'' The understanding and appreciation of the heterogeneity within the skeletal dysplasias led to a systematic description of well over 200 different forms, which have been primarily classified on the basis of clinical and radiographic changes. With the recent explosion of knowledge in the biochemistry and molecular biology of connective tissue, rapid progress has occurred in the delineation of the basic defect in these disorders. It is important to make a specific diagnosis in each case so that an accurate prognosis can be given and proper genetic counseling provided. Furthermore, each of these disorders is associated with a variety of skeletal or nonskeletal complications, which an accurate diagnosis allows one to anticipate, treat promptly, or prevent.

DIFFERENTIATION OF THE SKELETAL DYSPLASIAS

The current nomenclature for these disorders is confusing. The specific name for a given condition usually describes the skeletal segment involved (e.g., the epiphyseal dysplasias and the metaphyseal dysplasias), or uses a descriptive Greek term [e.g., thanatophoric (death bringing) dysplasia, metatrophic (changing) dysplasia, and diastrophic (twisted) dysplasia]. Some disorders are eponyms (e.g., Kniest dysplasia and Ellis–van Creveld syndrome). Occasionally, the name was derived to describe the pathogenesis (osteogenesis imperfecta), but usually inaccurately (e.g., achondroplasia and achondrogenesis).

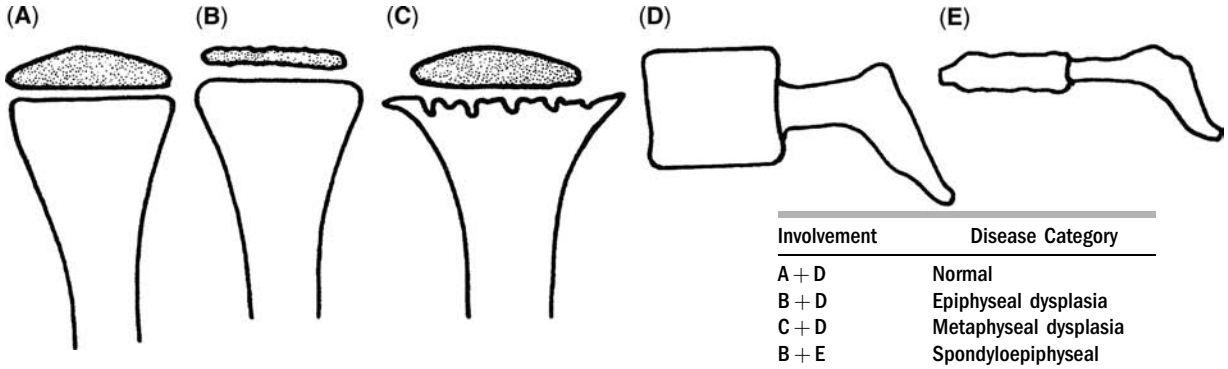
The extent of the heterogeneity in these disorders and the variety of methods used for their classification has resulted in further confusion. Clinical classifications have divided the skeletal dysplasias into two types namely those with short-limbed dwarfism and those with short-trunk dwarfism. Age of onset of the disease and associated clinical abnormalities has also been used in subdividing these disorders. Although many other disorders have been classified on the basis of their apparent mode of inheritance, for example, the dominant and the X-linked varieties of spondyloepiphyseal dysplasia (SED).

The most widely used method for differentiating the skeletal dysplasias has been the detection of skeletal radiographic abnormalities. Radiographic classifications are based on different parts of the long bones that are abnormal (epiphyses, metaphyses, or diaphyses; Figs. 1 and 2). Thus, there are epiphyseal and metaphyseal dysplasias, which can be further divided depending on whether the spine is also involved (SEDs and spondylometaphyseal dysplasias). Furthermore, each of these classes can be divided into several distinct disorders based on a variety of other clinical and radiographic differences.

As the morphology, pathogenesis, and especially the basic biochemical and molecular defect in each of these disorders are unraveled, this nomenclature is being changed to refer to the specific pathogenetic or metabolic defect. The etiologic or pathogenetic nomenclature is now being used for certain skeletal dysplasias, such as the mucopolysaccharidoses, mucolipidoses (e.g., β-glucuronidase deficiency, fucosidosis), type II collagenopathies, and disorders of mineralization (hypophosphatasia).

International Classification and Nomenclature

In an attempt to develop a uniform nomenclature for these syndromes, an international classification and nomenclature for the skeletal dysplasias were proposed in 1969 and updated in 1977, 1983, 1991, 1997,



Involvement	Disease Category
A + D	Normal
B + D	Epiphyseal dysplasia
C + D	Metaphyseal dysplasia
B + E	Spondyloepiphyseal dysplasia
B + C + E	Spondyloepimetaphyseal dysplasia

Figure 1 Classification of chondrodysplasias based on radiologic involvement of long bones (**A**–**C**) and vertebrae (**D** and **E**).

2001, and 2005 (2,3). The most recently updated classification can be found at (4).

The term ''dwarfism'' is no longer used and the disorders have been referred to as ''dysplasias'' or ''dysostoses.'' The international classification originally divided the skeletal dysplasias into five major groups:

1. Osteochondrodysplasias: Abnormalities of cartilage or bone growth and development.
2. Dysostoses: Malformation of individual bones, singly or in combination (does not reflect a generalized disorder of the skeleton).
3. Idiopathic osteolyses: A group of disorders associated with multifocal resorption of bone.
4. Skeletal disorders associated with chromosomal aberrations.
5. Skeletal disorders associated with primary metabolic disorders.

In the differential diagnosis of short stature, osteochondrodysplasias are of major importance. These are a complex group of diseases that are caused by primary abnormalities of cartilage or of bone growth or development. This group was divided into the following:

1. Defects of growth of tubular bones or spine, or both (further referred to as chondrodysplasias).
2. Abnormalities in the amount, density, and remodeling of bone (includes disorders with a decrease or increase in bone and disorders of mineralization and mineral metabolism).
3. Disorders involving disorganized development of cartilage and fibrous connective tissue.

Recent advances in molecular genetics have made possible the identification of the basic defects in many common skeletal disorders. The Fifth International Nomenclature Committee, which met in Los Angeles in 1997, not only updated the nomenclature with the addition of a number of newly described syndromes, but also completely revised the organization of these disorders into a clinically and pathogenetically based classification, which was continued in the 2001 and 2005 nomenclatures (Table 1). Thus, disorders that share clinical, radiographic, morphologic, or biochemical features, suggesting that they share common pathogenetic mechanisms, were grouped together. This classification will certainly undergo constant revision because the basic defect in each of these disorders is discovered.

DIAGNOSIS AND ASSESSMENT

The diagnosis of skeletal dysplasias is based on the clinical, radiographic, pathologic, and, increasingly, biochemical and molecular studies. Table 1 summarizes the clinical, genetic, and radiographic features of the common chondrodysplasias and those with abnormalities in the amount, density, and remodeling of bone.

History

An accurate medical and family history may be of major importance in arriving at a diagnosis. A complete family history, details of stillborn children, and parental consanguinity should be obtained. Parents should always be closely examined, looking for evidence of a dysplasia in a partially expressed form. Because each of the skeletal dysplasias most frequently appears as a sporadic case in the family, an isolated instance of a skeletal dysplasia in a family cannot provide information on the mode of inheritance of the particular disorder. However, the type of familial aggregation, when it occurs, can be helpful. For example, if two dwarfed siblings are born to normal parents, then achondroplasia, which is an autosomal dominant trait, is unlikely, and one should suspect an autosomal recessive disorder. If two achondroplastic parents produce a severely affected offspring, it is most likely homozygous achondroplasia, rather than thanatophoric dysplasia. However,

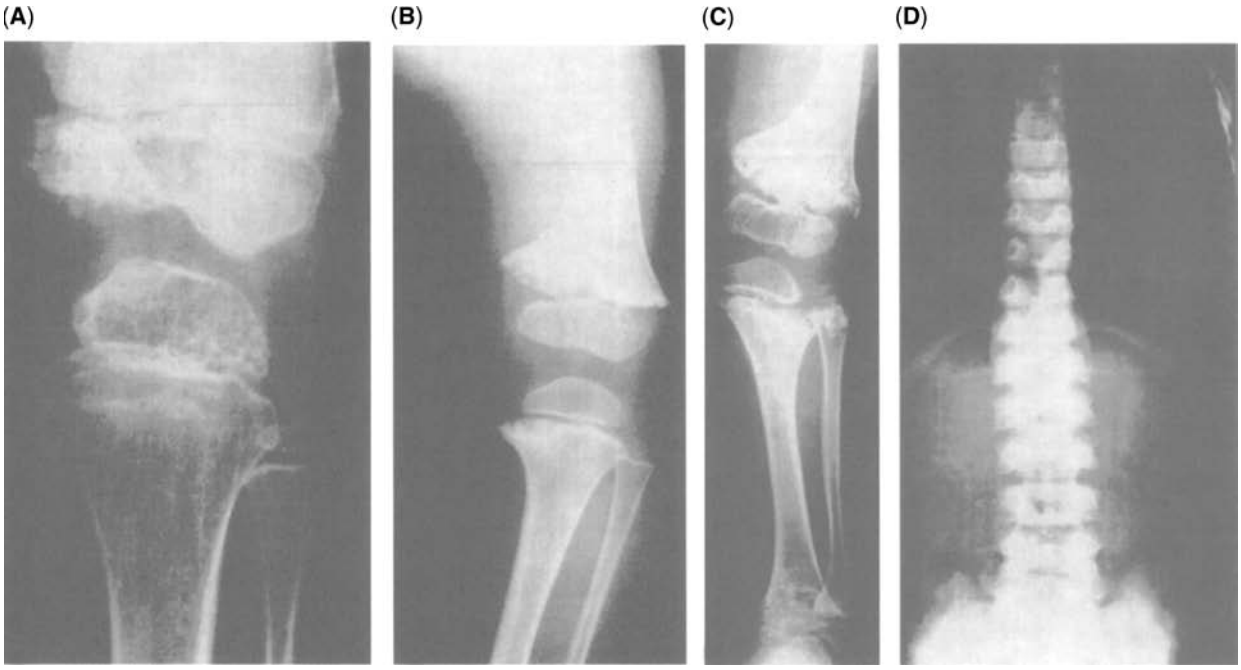


Figure 2 Radiographs of knee (**A–C**) and spine (**D**) from patients with a variety of chondrodysplasias. (**A**) Epiphyseal dysplasia: note the small irregular epiphyses and normal metaphyses from a patient with spondyloepiphyseal dysplasia congenital. (**B**) Metaphyseal dysplasia: note the irregular and widened metaphyses with normal epiphyses from a patient with metaphyseal dysplasia, Schmid type. (**C**) epimetaphyseal dysplasia: note the abnormal epiphyses and metaphyses from a patient with spondyloepimetaphyseal dysplasia, Strudwick type. (**D**) Platyspondyly: note the flat and irregular vertebrae from a patient with spondylometaphyseal dysplasia, Kozlowski type.

different modes of inheritance have been observed in disorders that resemble each other clinically, such as the X-linked and certain autosomal forms of SED. On the other hand, in some dominant disorders, a high incidence of gonadal mosaicism has been shown to account for recurrent cases in the same family. For example, in osteogenesis imperfecta type II, gonadal mosaicism may result in a recurrence risk of 6%.

Because the skeletal dysplasias become apparent at various ages, it is helpful to obtain accurate measurements from infancy onward and, especially, to know whether the shortening was evident at birth. Although in certain disorders marked variability in expression is seen with both pre- and postnatal onset of the disease in the same family (e.g., osteogenesis imperfecta type I), this information may help to limit the diagnosis to a small number of disorders. Thus, a child who was normal until two years of age and then developed disproportionate short-limbed dwarfism is more likely to have pseudoachondroplasia or multiple epiphyseal dysplasia rather than achondroplasia or SED congenita. In some disorders, growth may be normal for several years. For instance, in the X-linked form of SED tarda, growth retardation is not apparent until between 5 and 10 years of age. Relative body proportions may change with age in some disorders, such as metatrophic dysplasia, in which only the limbs are short at birth; because of progressive kyphoscoliosis, such patients become short trunked during childhood.

Physical Examination

A detailed physical examination may disclose the correct diagnosis or point to the likely diagnostic category (Vol. 2; Chap. 1). It is essential to determine whether the shortening is proportional. In general, patients with disproportionate short stature have skeletal dysplasias, whereas those with relatively normal body proportions have endocrine, nutritional, prenatal, or other nonskeletal defects. There are exceptions to these rules: cretinism can lead to disproportionate short stature, and a variety of skeletal dysplasias, such as osteogenesis imperfecta and hypophosphatasia, may result in normal body proportions.

A disproportionate body habitus may not be readily apparent on casual physical examination. Measurements that are essential for determining whether an abnormally short individual is disproportionate include the following:

1. Upper/lower segment ratio (U/L ratio): Although sitting height is a more accurate measure of the head and trunk length, it requires special equipment for consistent accuracy. U/L segment ratio, on the other hand, provides a fairly accurate measure of body proportions

(*Text continued on p. 156*)

Table 1 Clinical, Genetic, and Radiographic Features in the Chondrodysplasias

Dysplasias	Clinical Features	Radiographic Features
Achondroplasia group (the disorders in this group have similar radiographic changes but range from severe neonatally lethal thanatophoric dysplasia through achondroplasia to mild hypochondroplasia)		
Achondroplasia	AD; 80% represent new mutation; the most common skeletal dysplasia (1:15,000 births); rhizomelic shortening of limbs (recognizable at birth); final height averages 135 cm in men and 125 cm in women, with wide variability; hands are short and broad, wedge-shaped gap between third and fourth fingers (trident); lumbar gibbus in infancy usually replaced by prominent lumbar lordosis; limbs with skin folds in children; the mean head circumference follows a curve above the 97th percentile for normal individuals; achondroplasia specific curves are valuable to recognize hydrocephalus; prominent frontal bossing, hypoplasia of maxilla, mandibular prognathism; hypotonia is frequent during infancy; mental development is normal; except for patients with severe complications or sudden infant death, life span is normal; recurrent otitis media and chronic serous otitis are common and lead to conductive hearing loss in adults; overgrowth of fibula and joint laxity cause progressive genu varum; nerve root compression and spinal claudication are common complications in adults; (FGFR3 mutation)	Large calvaria, short base of skull, small foramen magnum (computed tomographic scan norms are available); ribs are short, cupped anteriorly; decreased lumbosacral interpedicular distance, squared off ilia, small sacrosciatic notches; limbs are short and broad; oval radiolucency in proximal femur and humerus in infancy; overgrowth of fibula
Hypochondroplasia	AD; recognized from 2–3 yr of age; short-limbed (rhizomelic) short stature; there is wide variability in severity and much overlap in appearance with achondroplasia; this type of skeletal dysplasia is probably very common and easily undiagnosed in mildly short individuals; head is normal; patients are stocky and muscular; hands and feet are short and broad; mild genu varum and mild lumbar lordosis; mild mental retardation has been reported in some cases (FGFR3 mutations)	Mild achondroplastic changes: skull normal or mildly enlarged; ribs are normal or slightly flared; distal lumbosacral interpedicular narrowing; long bones: rhizomelia; short wide bones; elongated fibula; prominent deltoid tubercles
Thanatophoric dysplasia	AD; the most common lethal type; markedly short limbs; large bulging forehead ± cloverleaf skull; prominent eyes; small, narrowed, pear-shaped thorax; ± congenital heart and CNS defects (FGFR3 mutations)	The long bones are short and bowed; metaphyseal flaring with medial spikes; severe platyspondyly; the vertebrae are hypoplastic, U-shaped on AP view; cupped and short ribs; large calvaria

(*Continued*)

Table 1 Clinical, Genetic, and Radiographic Features in the Chondrodysplasias (*Continued*)

Dysplasias	Clinical Features	Radiographic Features
Spondylodysplasic and other perinatally lethal conditions group (distinct lethal types of skeletal dysplasia)		
Achondrogenesis type IA	AR; lethal; very short limbs; short and barrel-shaped chest; extremely soft skull; round or oval face	Characterized by poor ossification of the spine, more extreme shortening of femora and other long bones; long bones have concave ends and spurs in the middle shaft; type IA (Houston-Harris) is differentiated from Achondregenesis IB (Fraccaro) by the lack of rib fractures, appearance of the long bone, and cartilage histology
Metatropic dysplasia group		
Metatropic dysplasia	AD; normal to long in length at birth, short limbed in infancy, becoming short trunked later with progressive kyphoscoliosis; prominent joints; tail-like sacral appendage, large head with ventriculomegaly may be present; C1/C2 subluxation is frequent and requires surgical fusion; severe cases die in infancy of RDS	Extreme platyspondyly with flattening of vertebrae and relatively large intervertebral spaces; long bones: irregularly expanded metaphyses giving barbell-like appearance; flattened and irregular epiphyses; short and broad tubular bones in hands; marked flaring of iliac crests (halberd appearance)
SRD group with or without polydactyly		
Short-rib polydactyly syndrome (I, II, III)	AR; lethal, hydropic appearance, narrow thorax, severe RDS, polydactyly; in type I (Saldino Noonan) high frequency of cloacal abnormalities and postaxial polydactyly; type III is probably the mild end of type I disease; type IV is likely the mild end of type II (Majewski) which has high frequency of cleft lip and palate, multiple internal anomalies, and pre- and postaxial polydactyly	Extremely short horizontal ribs; the pelvis is small and hypoplastic in type I, whereas in II the pelvis is normal; in type I the long bones are very short with metaphyseal spurs; in type II the long bones have a more rounded appearance, especially the middle segment
ATD (Jeune)	AR; long narrow thorax and RDS with variable severity; ±postaxial polydactyly; progressive nephropathy; cystic changes in kidney, liver and pancreas	Ribs are short, cupped anteriorly; square short ilia; flat acetabulum with spurs at ends (trident appearance)
Chondroectodermal dysplasia (Ellis–van Creveld)	AR; narrowed thorax, short limbs, and polydactyly; often with congenital cardiac anomalies (ASD, single atrium, PDA) and ectodermal abnormalities; hypoplastic nails, natal teeth, multiple frenula, cleft lip and palate, epispadias	Ribs and pelvis are similar to ATD; acromesomelic shortening of limbs; hamate-capitate fusion
Atelosteogenesis and omodysplasia group (characterized by hypoplastic humeri or femora, absence of ossification of several bones, or ossification of what should remain cartilage)	Atelosteogenesis types I and III are due to Fibrillin B mutations, as are spondylocarpotarsal syndrome and Larsen syndrome	
Otopalatodigital syndrome type II	X-linked; characterized by distinct facies, short and broad distal segments of thumbs and toes; proportional short stature and sometimes mental retardation; facies have prominent forehead, flat nasal root, flattening of the midface and small jaw; ±hearing defect (conductive); dislocation of radial heads and/or hips may be present (Fibrillin A mutations)	Small ilia; hypoplastic distal radius results in dislocations; wide lumbar interpedicular distance; small mandible; radiographic changes may not become apparent until later in infancy

(*Continued*)

Table 1 Clinical, Genetic, and Radiographic Features in the Chondrodysplasias (*Continued*)

Dysplasias	Clinical Features	Radiographic Features
Diastrophic dysplasia group		
Diastrophic dysplasia	AR; acute swelling of pinnae of ears in infancy; cauliflower ears; laryngomalacia; short limbed (rhizomelic), severe clubfeet, joint contractures, proximally placed abducted thumb; progressive scoliosis; there is wide variability in expression even within the same family; sulfate transporter defect DTDST. More severe DTDST deficiency in atelosteogenesis II and achondrogenesis IB and less severe in autosomal recessive MED	Hypoplasia of epiphyses and flaring metaphyses in long bones; extracarpal bones; short and wide metacarpals and phalanges; lumbar interpedicular narrowing; C2/C3 dislocation; peritracheal, ear pinnae, and precocious costal cartilage ossification
Type II collagenopathies group (all the disorders in this group share defects in type II collagen secondary to mutations in the COL2A1 gene		
Kniest dysplasia	AD; short trunk, progressive kyphoscoliosis; joint limitation; face is flat and round; myopia and cleft palate are common; Swiss cheese cartilage	Coronal clefts in flattened vertebrae, small ilia, increased acetabular angles; barbell-like femora as a result of broad metaphyses, delayed ossification of femoral heads, cloud effect in epiphyseal region; squared-off metacarpals and phalanges
Stickler syndrome	AD; marfanoid habitus, myopia, retinal detachment, conductive hearing loss, hyperextension of joints, may lead to joint pains and morning stiffness; cleft palate and mandibular hypoplasia. In some families, a type XI collagen defect has been found, and in a few, the disorder does not segregate with either COL2 or COL11. Stickler phenotype associated with congenital deafness is caused by an autosomal recessive defect of COL9	Mild epiphyseal dysplasia (especially proximal femur and distal tibia), degenerative arthrosis (hips), wedging of thoracic vertebrae, and Schmorl's disease of spine
SED congenita (Spranger-Wiedeman)	AD; evident at birth; variable severity; rhizomelic shortening of limbs, but these appear long relative to trunk; hands and feet are normal in size; clubfeet; neck is extremely short; it is important to rule out C1/C2 subluxation, which may lead to dislocation; broad barrel chest, lordosis; severe myopia, joint laxity, cleft palate, genu valgum or varum, waddling gait	Platyspondyly and epiphyseal dysplasia; delayed ossification of epiphyseal centers and the epiphyses appear irregular, fragmented, and flattened (especially femoral); coxa vara; vertebrae are ovoid in childhood but later become flat, irregular, with narrowed disk space; odontoid hypoplasia with C1/C2 subluxation
Achondrogenesis II (Langer-Saldino) and hypochondrogenesis	AD; lethal; very short limbs; short and barrel-shaped chest; extremely soft skull; round or oval face; deficient type II and the presence of type I collagen in cartilage	In contrast to achondrogenesis type I, the long bones are straighter, relatively longer with cupping of their ends, with milder cases known as hypochondrogenesis
Spondlyoepimeta-pashyseal dysplasias (SEMD; Strudwick type)	AD; resembles SED congenita at birth; SEMD is distinguished from SED by radiologic evidence of metaphyseal changes; Strudwick type is characterized by specific radiologic changes such as peripheral ''popcornlike'' ossification of the femoral epiphyses, pectus carinatum, and genu valgum; type II collagen abnormalities have been documented	Delayed epiphyseal ossification, club-shaped femora (1st yr), metaphyseal changes (>3 yr), multiple epiphyseal centers in femoral heads, greater involvement of fibular and ulna than tibia and radius; platyspondyly; pear-shaped vertebrae; C1/C2 subluxation

(*Continued*)

Table 1 Clinical, Genetic, and Radiographic Features in the Chondrodysplasias (*Continued*)

Dysplasias	Clinical Features	Radiographic Features
Other spondyloepiphyseal (meta) physeal dysplasias group		
SED tarda	X-linked recessive; short stature develops in midchildhood; short limbs and short trunk; mild to severe kyphoscoliosis; large chest capacity; hands and feet are normal in size; early onset osteoarthritis in back and hips; (mutations in sedlin)	Flat vertebrae with hump-shaped center; hypoplastic iliac wings; epiphyseal hypoplasia of large bones; premature osteoarthrosis of hips
Dyggve-Melchior-Clausen dysplasia	AR; short trunk, short stature with barrel chest, lumbar lordosis, restricted joint mobility, and waddling gait; mental retardation in most cases. Those with normal mentation have been called Smith-McCort (mutations in *DYMECLIN*)	Changes similar to those in SED; anterior beaking of vertebral bodies; a fine lace-like ossification above the iliac crest and irregular small carpal and metacarpal bones
Multiple epiphyseal dysplasia and pseudoachondroplasia group		
Pseudoachondroplastic dysplasia	Epiphyses and metaphyses of the tubular bones are involved, with platyspondyly and anterior tonguing of the vertebral bodies; acetabular irregularity, hypoplastic ischium and pubis; striking hand involvement with shortening of tubular bones, irregular metaphyses, and small round epiphyses	
Multiple epiphyseal dysplasias (Fairbanks and Ribbing types)	AD; (except for rMED) mild short stature; pain and stiffness in knees, hips, and ankles; waddling gait is common in severe Fairbanks type; osteoarthropathy of hips in mild Ribbing type—mutations in COMP, COL9A1, 2 and 3, matrilin 3 and DTDST	Characterized by flattened, fragmented, or irregular epiphyses (all areas, including hands and feet in Fairbanks; primarily the hips in Ribbing); earliest features may be delay in epiphyseal ossification; no metaphyseal or vertebral changes are seen; Schmorl's nodes are common
Chondrodysplasia punctata group (stippled epiphyses)		
Chondrodysplasia punctata (punctate epiphyseal dysplasia)	Several dysplasias (AR, rhizomelic type; XLD, Conradi-Hunerman; and XLR forms); laryngomalacia and upper airway obstruction; limbs are proximally shortened in the rhizomelic type and asymmetrically shortened in the XLD types; cataract, ichthyosis, and contractures are common; rhizomelic type is caused by a peroxisomal defect, whereas the Conradi-Hunerman form is due to a sterol metabolic defect; the XLR form is due to a mutation in arylsulfatase E	Stippled calcification of epiphyses, periarticular tissues, and growth plate zones; stippling of laryngeal cartilage; coronal clefts of vertebrae
Metaphyseal dysplasia group		
Metaphyseal dysplasias		
Jansen	AD; severe short stature; recognizable in early infancy; rhizomelic shortening; severe leg bowing, mandibular hypoplasia; joints are large with contractures; arms less affected than legs; mutations in PTHRP receptor	All types are characterized by metaphyseal involvement; all metaphyses including hands and feet are severely affected but improve with age
Schmid	AD; mild to moderate short stature (130–160 cm) and bowing of legs; enlarged wrists and flaring of rib cage; coxa vara; mutations in Type X collagen	Most prominent changes are in hips, shoulders, knees, ankles, and wrists

(*Continued*)

Table 1 Clinical, Genetic, and Radiographic Features in the Chondrodysplasias (*Continued*)

Dysplasias	Clinical Features	Radiographic Features
McKusick type (cartilage-hair hypoplasia)	AR; severe to moderate postnatal growth deficiency, short broad hands and loose joints; genu varum; fine, light, sparse hair and light complexion; increased susceptibility to severe varicella infection; mutations in *RMRP*	Knees especially are involved, in contrast to Schmid; proximal femoral metaphyses are normal to mildly involved; fibula is long relative to tibia; ribs are short with anterior cupping
Others	Combination of metaphyseal abnormalities and immune deficiency can also be found in Schwachman syndrome (AR), associated with pancreatic insufficiency and chronic neutropenia; metaphyseal chondrodysplasia—thymic alymphopenia syndrome (AR); and in adenosine deaminase deficiency (AR)	
Spondylometaphyseal dysplasia group (association of vertebral changes along with metaphyseal abnormalities in the long bones		
Spondylometaphyseal dysplasia (SMD) Kozlowski type	AD; growth retardation is usually apparent after 1–2 yr; short trunk, short stature, and waddling gait develop; pectus carinatum, kyphoscoliosis, and precocious osteoarthritis; numerous other less well defined types of SMD have been described	Platyspondyly and general metaphyseal irregularities in the tubular bones; ''open staircase'' appearance to vertebrae on AP films; marked retardation of carpal ossification
Mesomelic dysplasia group	Heterogeneous group characterized predominantly by shortening of the middle segments of the limbs	In all types, the bones of the forearms and shins are disproportionately shortened
Dyschondrosteosis	AD, the common type; mesomelic short stature (mild to moderate); Madelung deformity of the wrist. Heterozygous mutations in SHOX gene	Hypoplasia of the distal ulna; $\pm$ radial head dislocation (Madelung deformity)
Langer type	AR, rare, represents the homozygous form of dyschondrosteosis; severe short stature, mandibular hypoplasia Homozygous for SHOX mutation	Limb bones are short and thick; hypoplastic fibula and distal ulna
Robinow	AD; flat facial profile, mesomelic shortening, and genital hypoplasia; hypoplastic mandible and hypertelorism, flat nose, and hypoplastic nails	Madelung deformity; posterior osseous fusion of vertebrae; hemivertebrae
Nievergelt type	AD; brachydactyly and clubfeet	Rhomboid-shaped radius, ulna, tibia, fibula; radioulnar and tarsal synostosis
Rheinhardt	AD; radial bowing of hands and lateral bowing of legs	Short radius and ulna; hypoplasia of distal ulna and proximal fibula
Acromelic and acromesomelic dysplasia group (shortening of the limbs, primarily affecting the hands and feet)		
Acromesomelic dysplasia	AR; several distinct skeletal dysplasias characterized by disproportionate shortening, predominantly affecting forearms, hands, feet, and legs; recognizable at birth; trunk slightly shortened (mutations in NPR2)	Mild epiphyseal ossification delay; brachydactyly with cone epiphyses; hypoplasia of iliac base and irregular acetabulum; wedging of vertebrae in adults

(*Continued*)

Table 1 Clinical, Genetic, and Radiographic Features in the Chondrodysplasias (*Continued*)

Dysplasias	Clinical Features	Radiographic Features	
TRP dysplasia	AD; mild disproportionate short stature, sparse hair, pear-shaped nose, medial accentuation of the eyebrows; short stubby hands; multiple joint contractures; severe genu valgum or varum; TRP type 1 involves, mutations or a smaller deletion in 8q24.12 than in the Langer–Giedion syndrome, which has exostoses as well as developmental delay	Numerous phalangeal cone-shaped epiphyses of the hands; Legg-Pertheslike changes occasionally occur in the hips	
Albright's Osteodysptrophy (Pseudohypoparathyroidism) Type E Brachydactyly	AD, mild short stature, marked short IV metacarpal in hand (± feet); *GNAS1* mutations in Albright's	Short IV metacarpals and metatarsals	
Dysplasia with significant membranous bone involvement group			
Cleidocranial dysplasia	AD; variable expressivity; large prominent forehead, wide persistent open fontanelles, drooping shoulders, narrow chest, abnormal dentition, coxa vara and joint laxity, short and squared fingers; proportionate short stature may occur. Transcription factor defect (CFBAI)	Varying degree of hypoplasia of membranous bones; absent or hypoplastic clavicles; narrowed and high pelvis; delayed closure of the anterior fontanelle with wormian bones are characteristic	
Bent-bone dysplasia group			
Campomelic dysplasia	AR; bending of long bones; cutaneous dimples at the site of bend; large head, 46 XY sex reversal in phenotypic females is common; some with severe RDS because of small thorax, hypoplastic tracheal rings, and other anomalies; SOX 9 mutations. Various types of short-limb bent bone dysplasias have been described (Kyphomelic dysplasia) that must be differentiated	Slender bent femur and tibia; enlarged dolichocephalic skull with shallow orbits; pelvis is tall and narrow; hypoplastic ischiopubic rami, hypoplastic scapulae	
Multiple dislocations with dysplasia group			
Larsen syndrome	AD (AR?); marked hyperlaxity and multiple dislocations (especially hips, knees, elbows) are characteristic; prominent forehead, low nasal bridge, hypertelorism, and cleft uvula are common features; disproportionate short stature; the associated skeletal abnormalities and craniofacial features help to distinguish Larsen syndrome from Ehlers-Danlos syndrome (mutations in *Filamin B*)	Multiple joint dislocations with secondary epiphyseal deformities; supernumerary carpal and tarsal ossification centers develop; premature fusion of the epiphyses and shaft of the first distal phalanges	
Dysplasias with decreased bone density group			
Osteogenesis imperfecta (defects in type I collagen as a result of mutations in either COL2A1 gene or COL1A2 gene			
Type 1	Excessive bone fragility, blue sclerae, conductive hearing loss in adolescence, hyperlaxity of ligaments, nonprogressive aortic root dilatation (12%), most have late-onset short stature, some families with opalescent teeth	General osteopenia (especially vertebral bodies), angulation at site of previous fractures, wormian bones in skull	AD

(*Continued*)

Table 1 Clinical, Genetic, and Radiographic Features in the Chondrodysplasias (*Continued*)

Dysplasias	Clinical Features	Radiographic Features	
Type 2	Lethal, low birth weight and short birth length, soft skull, beaking of the nose, hypotelorism, short and deformed limbs, thin and fragile skin; prenatal diagnosis by ultrasound, biochemical and molecular genetic studies	Extreme beading of ribs, crumpled appearance of long bones (femora), diffuse osteopenia of skull	Almost all are AD; gonadal mosaicism
Type 3	Nonlethal severe bone fragility leading to progressive deformity and marked short stature, sclerae may be blue at birth but become less blue with age, most with opalescent dentin, cardiorespiratory complications may lead to death	General osteopenia and marked deformity of bones; fractures may be present at birth, bowed long bones, progressive platyspondyly (codfish vertebrae) and kyphoscoliosis, wormian bones in skull	AD
Type 4	As type 1 but with white sclerae (may be blue at birth), some families with opalescent teeth	Osteopenia, variability in severity and age of onset of fractures, multiple wormian bones of skull	AD
Disorders with Defective mineralization			
Hyperphosphatasia-with osteoectasia (Juvenile Paget's disease)	Onset 2–3 yr, progressive painful skeletal deformity, fractures, short stature, large skull; elevation of alkaline phosphatase	Dense areas interspersed with lucent areas, generalized demineralization	AR
Hypophosphatasia Congenital lethal	Disproportionate short stature at birth, bowing deformity, thin skull vault, death from respiratory distress; low serum alkaline phosphatase; *ALPL* mutations	Generalized poor ossification, thin ribs, hypoplastic vertebrae, splayed and frayed metaphyses	AR
Tarda	Milder, onset in childhood, bowing of legs, premature loss of teeth; reduced serum alkaline phosphatase, elevated phosphoethanolamine in the urine; *ALPL* mutations	As in congenital type but milder changes	AD
Hypophosphatemic rickets	X-linked hypophosphatemic rickets; bowing of legs and short stature, late dentition; low serum phosphate; *PHEX* mutations; *FGF23* mutations in the adult AD form	Radiographic changes are those of rickets	X-linked dominant
Pseudo vitamin D deficiency rickets (VDD)			
Type 1	Defective 1-hydroxylation of 25-hydroxyvitamin D	As in rickets	AR
Type 2	Impaired target organ responsiveness to vitamin D		AR
Increased bone volume or density			
Osteopetrosis			
Precocious form	Onset in early infancy, failure to thrive, malignant hypocalcemia, anemia, thrombocytopenia, hepatosplenomegaly, optic atrophy leading to blindness, impaired bone resorption as a result of defect in maturation of osteoclasts; early bone marrow transplantation may be successful; Mutations in *TC1RG1, CLCN7,* and *OSTM1*	Generalized hyperostosis at birth, "bone in bone" appearance (vertebrae), crowded marrow cavity, dense base of skull	AR
Tarda	Onset in childhood; may go undetected until adulthood; excessive fractures, mild craniofacial disproportion, mild anemia, osteonecrosis of bones (especially mandible) may develop; a distinct form with renal tubular	Generalized increased density, defective metaphyseal modeling, dense base of skull	AD or AR

(*Continued*)

Table 1 Clinical, Genetic, and Radiographic Features in the Chondrodysplasias (*Continued*)

Dysplasias	Clinical Features	Radiographic Features	
	acidosis and mental retardation has been found to be caused by a deficiency of carbonic anhydrase type 2; chromosome location: 8q22		
Pycnodysostosis	Short limbs, short stature from infancy; wide anterior fontanelle, large cranium with open fontanelle, small chin, short hands and feet, increased fractures, sclerae may be blue, mutations in Cathepsin K	General hyperostosis, hypoplasia of distal phalanges in hands, wide sutures, and wormian bones	AR
Dysosteosclerosis	Postnatal onset of short stature, severe hypodontia and early loss of teeth, fractures, visual and hearing loss; SHOX mutations or deletions	General hyperostosis, platyspondyly	AR
Osteopoikilosis	Commonly asymptomatic, joint pains	Numerous small osteodense foci in epiphyses and carpal centers or tubular bones	AD
Craniotubular dysplasias			
Craniometaphyseal dysplasia	Broad osseous prominence of nasal root, bony encroachment on cranial foramina and nasal passages; *ANKH mutations*	Hyperostosis of skull, mandible, nasal and maxillary bones; lack of modeling of metaphyses of long bones (Ehrlenmeyer flask appearance)	AD and AR
Diaphyseal dysplasia (Camurati Engelmann)	Failure to thrive and fatigability, onset at age 4–10 yr, progressive increased pain in the legs, encroachment on cranial nerves; syndactyly, enamel hypoplasia; *TGFB1* mutations	Symmetric fusiform enlargement of the diaphyses, normal metaphyses and epiphyses, sclerosis of anterior base of skull	AD
Craniodiaphyseal dysplasia	Flattening of nasal root in early infancy, with increasing hypertelorism, marked encroachment of cranial nerves in foramina, normal stature	Massive hyperostosis and sclerosis of the skull and face with widened shafts of tubular bones	AR
Endosteal hyperostosis and sclerosteosis	Progressive mandibular enlargement from childhood; in adults sclerotic encroachment of optic and acoustic nerves	Marked accretion of osseous tissue at the endosteal surface, fusion between carpal bones	AR and AD
Tubular stenosis (medullary stenosis)	Hypocalcemia, delayed closure of fontanelle, and early-onset myopia	Narrowing of medullary cavity caused by widening diaphyseal cortex	AD
Pachydermoperiostosis	Progressive thickening of the skin, clubbing of fingers, easy fatigability, joint pain, blepharitis, sensory hearing loss	Subperiosteal thickening of tubular bones	AD
Frontometaphyseal dysplasia	Pronounced supraorbital ridge; *FNMA* mutations	Prominent frontum, $\pm$ large frontal sinuses; no hyperostosis of rest of skull; mild metaphyseal changes in long bones	X-linked dominant
Osteodysplasty (Melnick-Needles)	Abnormal gait and bowing of extremities, dislocation of hip, delayed closure of fontanelle, usually normal stature, exophthalmos, protruding cheeks, micrognathia, incurving of the distal segment of the thumbs; *FNMA* mutations	Uneven thickening of cortex bones, metaphyseal modeling defect, wavy ribs, narrowed iliac wings	AD (AR rare)

Abbreviations: AR, autosomal recessive; AD, autosomal dominant; XLR, X-linked recessive; XLD, X-linked dominant; ASD, atrial septal defect; PDA, patent ductus arteriosus; CNS, central nervous system; AP, anteroposterior; RDS, respiratory distress syndrome; SRD, short-rib dysplasia; ATD, asphyxiating thoracic dysplasia; VDD, vitamin D deficiency; SED, spondyloepiphyseal dysplasia; DTDST, diastrophic dysplasia sulfate transporter; FGFR3, fibroblast growth factor receptor 3; SMD, Spondylometaphyseal dysplasia; SHOX; short stature homeobox; TRP, Trichorhinophalangeal. For a complete listing of Skeletal Dysplasias, see The International Nomenclature of Constitutional Disorders of Bone, 2005.
Source: From Ref. 4.

and can be easily obtained. The lower segment measure is taken from the symphysis pubis to the floor at the inside of the heel, and the upper segment is obtained by subtracting the lower segment value from the total height. McKusick has published standard U/L curves for both white and black Americans that are quite useful for rapid assessment of proportion (1). For example, a normal white infant has an U/L segment ratio of approximately 1.7; it decreases to 1.0 at approximately 7 to 10 years of age and then falls to an average U/L of 0.95 as an adult. Blacks, on the other hand, have relatively long limbs and have an U/L of approximately 0.85 as adults.

2. Arm span: Another index of limb versus trunk length, and this measurement usually falls within a few centimeters of total height.

These measurements must be obtained before the possibility of a mild skeletal dysplasia, such as hypochondroplasia or multiple epiphyseal dysplasia, can be excluded. Short-limbed dwarfs have an abnormally high U/L ratio and an arm span that is considerably less than the height.

If a child has short-limbed dwarfism, it is important to determine whether all segments of the limb are equally shortened or whether the shortening primarily affects the proximal (rhizomelic), middle (mesomelic), or distal (acromelic) segment (Fig. 3). It is also important to assess the size and shape of the fingers and determine if there is brachymetacarpia (Vol. 2; Chap. 1).

The presence or absence of extraskeletal manifestations may be helpful in making a diagnosis. During the examination, attention should be given to the head size, facial appearance, and specific physical findings, such as myopia, cleft palate, clubfoot, hearing, joint laxity, and bone deformity. In the older child or adult, the complications associated with specific disorders may provide additional information for making the diagnosis. For example, spinal stenosis with spinal cord claudication is characteristic of achondroplasia; odontoid hypoplasia and C1/C2 subluxation are frequently found in Morquio syndrome, SED, and metatrophic dysplasia; and fibular overgrowth (and genu varum) is seen in achondroplasia and cartilage hair hypoplasia.

Skeletal Radiographs

A full series of skeletal views is usually required. These views include anteroposterior (AP), lateral, and Towne views of the skull, AP and lateral views of the spine, and AP views of the pelvis and extremities, with separate AP views of hands and feet. Lateral views of the foot are particularly helpful in identifying punctate calcifications of the calcaneus, which may be a clue to the diagnosis of the milder forms

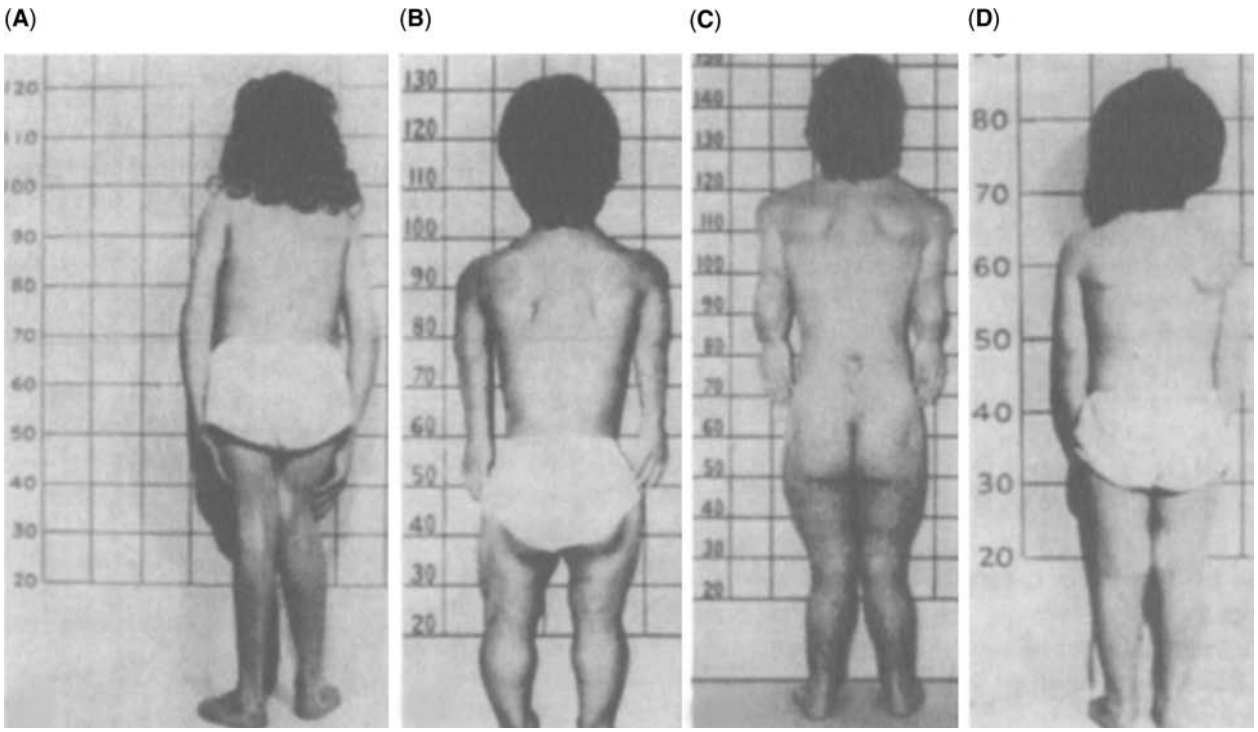


Figure 3 Different forms of disproportionate dwarfism: (**A**) short-trunk dwarfism in a girl with Dyggve–Melchior–Clausen syndrome, (**B**) short-limb dwarfism of the rhizomelic type in a boy with achondroplasia, (**C**) short-limb dwarfism of the mesomelic type in a boy with mesomelic dysplasia, Langer type, (**D**) short-limb dwarfism of the acromelic type in a girl with peripheral dysostosis.

of chondrodysplasia punctata, confirming the delayed ossification of the calcaneus and talus in newborns with SED congenita, and in delineating the double ossification centers of the calcaneus in the Larsen syndrome. Lateral views of the knee may reveal the double patellar ossification centers in recessive multiple epiphyseal dysplasia due to diastrophic dysplasia sulfate transporter (DTDST) mutations.

Attention should be paid to the specific parts of the skeleton that are involved (spine, limbs, pelvis, and skull) and, within each, where the abnormality is located (epiphysis, metaphysis, diaphysis, or combination). Because the skeletal radiographic features in many of these disorders change with age, reviewing radiographs taken at different ages is helpful (3,5). Moreover, epiphyseal closure, which occurs after puberty, frequently obliterates the specific abnormalities that would have permitted a specific diagnosis to be made had the films been taken before puberty. Nevertheless, skeletal radiographs alone are often sufficient to make the diagnosis because the classification of these disorders has been based primarily on their radiographic features.

Apart from the changes in the epiphyses, diaphyses, and metaphyses, some radiographic features characterize certain disorders:

- ''Dumbbell-shaped'' femur in the newborn period: Metatropic dysplasia and Kniest dysplasia.
- Bending of long bones (campomelia): Common in campomelic dysplasia, kyphomelic dysplasias, osteogenesis imperfecta, congenital hypophosphatasia, and thanatophoric dysplasia.
- Calcified projections or spikes on lateral borders of the metaphyses of the femur: Thanatophoric dysplasia, achondrogenesis, and short-rib polydactyly syndrome types I and III.
- Fractures of long bones in the newborn: Osteogenesis imperfecta, congenital osteopetrosis, and severe hypophosphatasia. In the older individual, fractures may also be seen in a variety of osteopetrotic syndromes, including dysosteosclerosis and pyknodysostosis.
- Marked delay in epiphyseal center ossification: SED congenita, Kniest dysplasia, and other SED and multiple epiphyseal dysplasias.
- Stippled epiphyses: The chondrodysplasia punctatas, cerebrohepatorenal syndrome, warfarin-related embryopathy, and, occasionally, with chromosomal trisomy, lysosomal storage diseases, diphenylhydantoin-induced embryopathy, the Smith–Lemli–Opitz syndrome, and congenital infections.
- Severely shortened ribs: Short-rib polydactyly syndromes, asphyxiating thoracic dysplasia (ATD), chondroectodermal dysplasia, thanatophoric dysplasia, and metatropic dysplasia.
- Decreased ossification of the vertebral bodies: Most severe in the achondrogenesis syndromes.
- Severe platyspondyly: Metatropic dysplasia, thanatophoric dysplasia (U-shaped in thoracic spine and inverted U-shape in the lumbar spine), osteogenesis imperfecta type II, congenital hypophosphatasia, Morquio syndrome, spondylometaphyseal dysplasia, brachyolmia, and others.
- Coronal clefts of the vertebra: Kniest dysplasia, Rolland–Desbuquois syndrome, Weisenbach-Zweymuller syndrome, chondrodysplasia punctata, and atelosteogenesis.
- Oval translucent appearance of the proximal femora and humeri in infants: Achondroplasia, thanatophoric dysplasia, and hypochondroplasia.

These examples are representative of only a few of the many typical radiographic features seen in the skeletal dysplasias (Table 1). Furthermore, other radiographic differences within what is now considered a given skeletal dysplasia may be found as the complete heterogeneity of this group of disorders is delineated by the molecular and biochemical studies.

Microscopic Evaluation

Histologic examination of the chondro-osseous tissue can be useful in making an accurate diagnosis of several specific skeletal disorders, especially the lethal neonatal types (6). In certain other conditions, the pathologic examination is useful in ruling out a diagnosis (A protocol for the collection of skeletal tissues is described in Section V).

On morphologic grounds, the chondrodysplasias can be broadly divided into the following disorders:

1. Minimal or no qualitative abnormality in endochondral ossification: Achondroplasia and hypochondroplasia (in which abnormalities in the height and arrangement of proliferative columns, particularly in the center of the large growth plates, are the only changes).
2. Abnormalities mainly in cellular morphology: large chondrocytes containing prominent inclusions; for example, achondrogenesis IA, pseudoachondroplasia, and certain SEDs; sparse matrix with collagen rings around the chondrocytes as in diastrophic dysplasia and achondrogenesis IB. Dilatation of the chondrocyte rough endoplasmic reticulum (RER); for example, the SEDs, pseudoachondroplasia, and Kniest dysplasia. Thus, dilatation of the RER is not a diagnostic finding, although it suggests defective synthesis or abnormal processing of a matrix protein in these conditions.
3. Abnormalities in matrix morphology: Areas of cell degeneration with wide collagen fibrils, scar formation, and intracartilaginous ossification (diastrophic dysplasia). ''Swiss-cheese'' appearance of cartilage (Kniest dysplasia). Large lacunae containing numerous chondrocytes (Dyggve–Melchior–Clausen syndrome). Areas of dystrophic ossification, fibrous dysplasia, and fat deposition in the reserve zone cartilage of the matrix (chondrodysplasia punctata). Wide interwoven connective septa in epiphyseal cartilage and basal zone (fibrochondrogenesis).
4. Abnormalities primarily localized to the area of chondro-osseous transformation (reduced and disorganized columnization, thanatophoric dysplasia, short-rib polydactyly syndromes). Broad matrix septa surrounding clusters of hypertrophic cells (the metaphyseal dysplasias and opsismodysplasia).

Biochemical Studies

Great progress has been made in recent years about the biochemical defect involved in certain of the skeletal dysplasias. These include not only the metabolic defects associated with the lysosomal storage disorders and disorders of mineralization, but also a whole host of defects uncovered through molecular and pathogenetic studies, which are outlined below. These findings may help us to understand the basic biology of the normal bone and provide us with new means for prenatal diagnosis and treatment.

Prenatal Identification

Many of the skeletal dysplasias manifest in the prenatal period and can be identified by prenatal ultrasound. The great majority of cases, however, occurs for the first time in a family and is thus unsuspected and picked up by routine ultrasonography by measurement of femur length. This is true for both dominant disorders such as thanatophoric dysplasia and SED congenita and for recessive disorders such as diastrophic dysplasia and cartilage hair hypoplasia. Many of these disorders will manifest shortening of the extremities by as early as 13 to 16 weeks, but in achondroplasia femoral shortening may not be evident until the late second trimester. Of course, those disorders that do not present in the newborn period cannot be detected by prenatal ultrasound.

Retrospective analysis (7) in 250 cases revealed that the accuracy in diagnosis in 250 cases referred to the International Skeletal Dysplasia Registry at Cedars-Sinai Medical Center (8) was approximately 30%. Further retrospective analysis of accuracy of prenatal diagnosis in 1000 cases referred to the Registry (1990–2000) indicated that a correct referring diagnosis was made in 37% of the cases, but in over 60% of the cases either an incorrect diagnosis or no diagnosis was made (9). Analysis of this 1000 case cohort showed that the most common diagnoses were osteogenesis imperfecta type II (20%), thanatophoric (11%), and achondrogenesis II (8.2%). Another 37% represented a number of other specific skeletal dysplasias, 12% were dysmorphic syndromes, and 5% were otherwise normal and probably represented early onset intrauterine growth retardation. In 4.5% of the referred cases, radiographs or histology could not make a specific diagnosis. With the increasing use of prenatal ultrasound, increasing numbers of skeletal dysplasia cases are recognized by ultrasound. However, the above data show that prenatal ultrasound parameters need to be defined for a more accurate prenatal diagnosis of the skeletal dysplasias.

With the explosion of knowledge concerning the basic molecular defect in the skeletal dysplasias, prenatal diagnosis by mutation detection in amniocytes or chorionic villous cells can be accomplished if the exact mutation is known in a previously affected child, because most of these disorders are due to private mutations in the same gene. Linkage analysis can be used in families with multiple affected members, even if the specific mutation is not known. In achondroplasia, where almost all cases share the same mutation, molecular diagnosis can be readily accomplished by sequence analysis or restriction fragment polymorphism. This is especially valuable when both parents are achondroplasts and want to rule out the 25% possibility of the fetus being a lethal homozygote.

MOLECULAR STUDIES AND MECHANISM OF SHORT STATURE IN SKELETAL DYSPLASIAS

Significant developments in the field of molecular genetics have allowed mapping and identification of the genes causing many of the skeletal dysplasias (10–12). The specific gene defects that produce skeletal dysplasias can be classified into several distinct pathogenetic categories. These include (i) defects in extracellular (matrix) structural proteins; (ii) defects in metabolic pathways (including enzymes, ion channels, and transporters); (iii) defects in folding, processing, transport, and degradation of macromolecules; (iv) defects in hormones, growth factors, receptors, and signal transduction; (v) defects in nuclear proteins (transcription factors and homeobox genes); (vi) defects in ribonucleic acid processing and metabolism; and (vii) defects in cytoskeletal proteins (Table 1).

The emerging data of the last few years outlining the molecular basis of skeletal dysplasias has been instructive in several respects. For example, phenotypically distinct entities have been found to be allelic variants, with mutations in the same gene (e.g., achondroplasia, hypochondroplasia, thanatophoric dysplasia, and certain craniosynostotic syndromes). On the other hand, certain dysplasias have been found to be due to mutations in different genes [e.g., multiple epiphyseal dysplasia with mutations in cartilage oligomeric matrix protein, the type IX collagens (matrilin 3 and DTDST)]; and Stickler syndrome, with mutations in types II and XI collagen and recently also in collagen IX. In most of the conditions in which the molecular basis has been defined, there are numerous other mutations described. Consequently, diagnosis by deoxyribonucleic acid analysis is difficult, unless the distinct mutation in the family has been defined, or expensive sequencing of the entire gene is performed. Chip technology may eventually solve this problem. Achondroplasia is unusual in that over 98% of the cases are due to a single mutation, making an inexpensive molecular diagnostic test possible.

Genes that Influence the Adult Height in Normal as well as in Specific Skeletal Dysplasias

The Short Stature Homeobox Factor in Idiopathic Short Stature and Specific Skeletal Dysplasias

Linear growth is a multifactorial trait that is influenced and regulated by a combination of environmental and internal factors. Among the intrinsic determinants

of final body height, genetic factors have become more and more prominent, and the list of genes involved in growth-related processes has been extended accordingly (Vol. 2; Chaps. 1, 2 and 3). One of the most exciting additions to this list is represented by the discovery of the pseudoautosomal gene, that is, short stature homeobox (SHOX) (13).

SHOX gene is located on the short arm of the human X-chromosomes as well as Y-chromosomes and small deletions in these regions are consistently associated with short stature suggesting that a growth-promoting gene resides within the pseudoautosomal region 1 (PAR1) of the sex chromosomes, and that haploinsufficiency of this pseudoautosomal growth gene causes short stature. The gene escapes X inactivation and is highly expressed in bone marrow stromal fibroblasts. Although the chromosomal localization and its expression pattern certainly rendered it an attractive candidate, final evidence for the growth-related function of SHOX was provided by the discovery of a C→T transition generating a premature stop codon in exon 5 within a pool of 91 idiopathic short-stature patients.

A major extension to the growth-related function of SHOX initially established in idiopathic short stature has been provided by the analysis of Léri–Weill syndrome patients. Léri–Weill syndrome (dyschondrosteosis) represents a short stature condition characterized by a symmetric shortening of the forearms and lower legs, and a bilateral shortening and bowing of the radius with a dorsal subluxation of the distal ulna (Madelung deformity). According to the clinical description, Langer's type of mesomelic dysplasia has been suggested to represent the homozygous form of Léri–Weill dyschondrosteosis. Despite a prevalence of affected women, the syndrome was originally suggested to be inherited in an autosomal dominant manner. Recent genetic analyses performed in Léri–Weill syndrome families for SHOX mutations reported SHOX deletions in 50% and point mutations in 6–10%. Recently, it was found that in another 6% of Léri–Weill syndrome there is a deletion of a segment outside the SHOX gene on its centromeric side (still in the PAR) (14). In the remaining (34%) the genetic basis is yet unknown, which argue for diverse genetic etiologies of Léri–Weill dyschondrosteosis. Interestingly, one of the nonsense mutations found in Léri–Weill dyschondrosteosis is identical to the C→T transition originally observed in idiopathic short-stature patients. This finding was the first direct evidence that identical SHOX mutations can lead to different clinical phenotypes. All groups reported that a homozygous loss of SHOX causes Langer's type of mesomelic dysplasia.

As Léri–Weill dyschondrosteosis shares several phenotypic characteristics with Turner syndrome; it seemed conceivable that SHOX mutations could also account for additional somatic features in Turner syndrome. Further evidence for SHOX as the Turner short stature gene comes from XY females with interstitial Yp deletions. Some of these patients exhibit Turner stigmata but are of normal size. The Turner growth gene, therefore, has to reside either on Yq (and its respective homolog on the X-chromosome) or within the PAR of the sex chromosomes that is present in two copies in these females. The only gene within the PAR involved in the determination of final height is SHOX.

Expression analyzes on human embryos have recently been carried out by antisense in situ hybridization. These studies not only explains the observed short stature and Léri–Weill dyschondrosteosis phenotype, including Madelung deformity, but also suggests an involvement of SHOX-related growth impairment in the expression of additional somatic Turner syndrome stigmata including high-arched palate, abnormal auricular development, cubitus valgus, genu valgum, and short. These results strongly suggest that SHOX mutations, besides short stature, can cause the skeletal defects seen in patients with Turner syndrome.

The Filamin Mutations in Skeletogenesis

The filamins are cytoplasmic proteins that regulate the structure and activity of the cytoskeleton by cross-linking actin into three-dimensional networks, linking the cell membrane to the cytoskeleton and serving as scaffolds on which intracellular signaling and protein trafficking pathways are organized.

Filamins regulate the organization of cytoskeletal F-actin into either parallel bundles or orthogonal gel networks and also mediate interactions between subcortical actin networks and transmembrane receptors to modulate cell–cell, cell–matrix, and intracytoplasmic signal transduction. Mammals have three filamin genes, namely, FLNA, FLNB, and FLNC (15,16). FLNA and FLNB seem to be ubiquitously expressed; FLNC is predominantly expressed in muscle. The filamin monomer comprises an N-terminal actin-binding domain followed by a series of 24 β-sheet repeats that collectively bind many cytoplasmic and transmembrane proteins. Filamins exist in vivo as dimers. Dimerization, leading to homo- and possibly heterodimer formation, is mediated by interactions between C-terminal sequences.

Mutations in FLNA produce a spectrum of X-linked malformation and osteochondrodysplasia syndromes. FLNA loss-of-function mutations are usually embryonically lethal in males and underlie a neuronal migration disorder in females. Mutations producing structural changes in the protein lead to numerous developmental anomalies in the brain, skeleton, and viscera.

More recently, mutations in the gene encoding filamin B has been identified in four human skeletal disorders: (i) autosomal recessive spondylocarpotarsal syndrome (SCT, OMIM 272460); (ii) autosomal dominant Larsen syndrome (OMIM 150250); (iii) autosomal

dominant perinatal lethal atelosteogenesis I (AOI, OMIM 108720); (iv) autosomal dominant lethal atelosteogenesis III (AOIII, OMIM 108721). Filamin B is expressed in human growth plate chondrocytes and in the developing vertebral bodies in the mouse. These data indicate an unexpected role in vertebral segmentation, joint formation, and endochondral ossification for this ubiquitously expressed cytoskeletal protein.

The presence of short stature, epiphyseal delay, and disharmonious bone mineralization in the group of disorders associated with mutations in FLNB suggest a role for filamin B within the epiphyseal growth plate. Some mutations in FLNA also affect stature and skeletal mineralization. Overall expression of filamin A and filamin B in the growth plate was studied and it was found that filamin A is primarily detected in hypertrophic chondrocytes, whereas filamin B is distributed in all chondrocytes of the growth plate.

The demonstration that mutations in FLNB are associated with a spectrum of autosomal dominant and recessive skeletal dysplasias indicates Larsen syndrome, AOI and AOIII are genetically related conditions and adds SCT to the allelic series. Like mutations in FLNA, mutations in FLNB produce a diversity of phenotypes, depending on the nature and location of the mutation. The premature termination mutations underlying SCT could lead to nonsense-mediated decay and absence of filamin B, but production of a stable truncated protein lacking a dimerization domain cannot be ruled out. Cell lines from the families with SCT were not available, and so these hypotheses could not be distinguished. Dominant missense mutations in FLNB underlie Larsen syndrome, AOI and AOIII disorders characterized by joint dislocation, implicating filamin B in joint morphogenesis.

The presence of the same mutation (604A→G) in individuals with AOI and AOIII, conditions previously separated on grounds of radiology and chondro-osseus histology, suggests that such distinctions do not reflect differences in their primary molecular pathogenesis. Female carriers of null mutations in FLNA manifest a neuronal migration disorder, periventricular nodular heterotopia. Although both FLNA and FLNB are expressed in the periventricular region of the brain, individuals with SCT do not have seizures or other evidence for anomalous neuronal migration, indicating that presumptive loss-of-function mutations in FLNB do not have the same biological consequence as similar mutations in FLNA. Understanding the specific mechanisms by which FLNB mutations produce defects in skeletal morphogenesis and abnormalities in other organ systems will be facilitated by the findings described here. This will include the identification of specific filamin B-binding partners and the pathways and downstream signal transduction targets that interact with this cytoskeletal protein during development.

MANAGEMENT

Effective management requires (i) precise diagnosis, (ii) prompt recognition of specific skeletal and non-skeletal complications, (iii) appropriate orthopedic and rehabilitative care, (iv) emotional support and psychosocial counseling, and (v) genetic counseling. There is no specific cure for any of these conditions.

Growth Hormone

The use of recombinant growth hormone therapy for achondroplasia has been evaluated by several centers in relatively short-term trials (Vol. 2; Chap. 5). In most of the trials, there is a statistically significant increase in predicted growth rate in the first year of treatment (17). However, this trend of increased growth velocity decreases in the second year of treatment and thereafter. If the data is extrapolated for a prolonged period of treatment, it appears that the increase in final height in these patients will at most be only a few centimeters above predicted, and will not result in a significant increase in final height. There are no studies that have pursued multiyear trials. Furthermore, several studies have shown great individual variability in responsiveness in these patients. We have conducted a long-term follow-up on our two years hGH-treated achondroplasia patients, who were then left untreated for up to eight years. Although these patients demonstrated an increase in growth velocity during treatment (years), long-term evaluation showed no significant change in height from pretreatment predicted height. Multiyear trials must be conducted to document whether any long-term benefits could take place. The data suggesting a decrease in responsiveness to hGH over time brings up the question of whether performing pulsatile therapy; for example, treatment for two years, off for a year and whether continuation of this cycle will replicate the increase in growth velocity seen in the first year of treatment. The pertinent issue is whether this treatment can produce a clinically relevant increase in height. Only a few studies with a limited number of patients have been performed in hypochondroplasia with variable results. Furthermore, these studies were not performed on patients with a documented molecular defect in fibroblast growth factor receptor 3 (FGFR3), which may complicate the interpretation of the responsiveness, because hypochondroplasia is genetically heterogeneous. Growth hormone trials on only a few patients with other less common skeletal dysplasias have been conducted, and there is no sufficient available data to conclude whether there is a treatment benefit. However, in view of the increased predisposition to malignancy in cartilage-hair hypoplasia, we believe that growth hormone therapy should not be attempted in this condition.

Limb Lengthening

Orthopedic management aims at maximizing mobility and correcting deformity; if deformities in the lower

limbs are left uncorrected beyond puberty, early onset of osteoarthritis may lead to mechanically unsound joints. Early recognition of spinal deformity and its early treatment with bracing or surgical intervention may reduce morbidity (from scoliosis) in adult life.

Extended limb lengthening was first developed for the treatment of leg length discrepancy and later became utilized bilaterally for increasing the height of dwarfed individuals. The original techniques involved osteotomies involved cutting the periosteum and distraction of the broken ends, the gap being filled in with bone grafts and stabilized with hardware, which was subsequently removed. Serious complications including osteomyelitis, hypertension, nerve, and vascular damage frequently followed. It was not until the Siberian orthopedist, Ilizarov developed a new technique, which involved percutaneous breaks in the bone with the periosteum remaining intact. He developed a new circular fixator–distractor, but the secret to his success was the subperiosteal break, leaving nerve and blood supply intact and the extremely slow rate of distraction. Up to 6 in. of new bone could be achieved in each of the limb segments, allowing many dwarfs to enter the normal growth curves.

Over the last 20 years a number of other techniques have been developed, which all involve percutaneous osteotomies of the metaphyses or diaphyses of the long bones, the insertion of screws and wires above and below the fracture line and the attachment of an external telescoping fixator. The devise is then turned several times a day, extending the fracture gap by about 1 cm/day. When the desired increase in length is attained, the distraction is stopped and the callus is allowed to consolidate. When consolidation is complete, the device and pins are removed. There is a substantial recovery time with active physical therapy because the muscles become stretched and weak. The different techniques vary in the type of distractor and whether pins or wires are used, which bones are done simultaneously and in what sequence. If using the large circular Ilizarov distractors, both femurs cannot be done simultaneously, and either both segments of one leg or a contralateral femur and tibia are done simultaneously. If one has to stop before the entire process is completed, one can end up with significant asymmetry. If one uses a linear fixator and pins, both tibias can be done simultaneously, followed by both femurs. The humeri are frequently lengthened by 4 in. in between the two leg segments.

All patients develop one or more complications during this prolonged lengthening process, including pin-tract infections, malunion, delayed consolidation, varus or valgus deviations, joint contractures or dislocations and muscle, nerve and vascular damage. We have used the Vilarrubias technique, which utilizes a linear Wagner fixator with two large screws on each side of the break and simultaneous lengthening of the tibias, followed by the humeri and then the femurs. Tendonotomies of the Achilles tendon and flexors and the hips are also performed, allowing plantar flexion of the foot and a marked decrease in the lumbar lordosis. We believe that the latter will significantly increase spinal canal volume, hopefully preventing or decreasing the spinal claudication that most achondroplastic dwarfs experience.

By using a team approach of orthopedists, geneticists, psychologists, physical therapists, etc., and carefully monitoring nerve conduction and blood flow, one can minimize the complications. We do not start limb lengthening in dwarfed children until they are at least 13 years of age, because we believe that it should be the child and not the parents who make the decision to undergo this prolonged, inconvenient, and complication-ridden procedure. Achondroplastic dwarfs are excellent candidates for ELL as only their limbs are short and they have excessive soft tissues, with joint laxity and tortuous vessels and nerves. The procedure has also been done in a variety of other forms of short-limb dwarfism and the tibias alone have been done in patients with Turner syndrome. It is not recommended for patients with short trunks or proportionate short stature.

Collection of Skeletal Tissues

A variety of histologic, histochemical, immunohistochemical, ultrastructural, biochemical, and molecular studies of chondro-osseous tissue and skin can be performed. Specimens can be sent to one of the laboratories that specialize in processing and interpreting tissue from the skeletal dysplasias (e.g., The International Skeletal Dysplasia Registry, Cedars-Sinai Medical Center 8700 Beverly Blvd, Suite 665W, Los Angeles, CA 90048, U.S.A., Tel.: +310–423–9915). Protocols for collection of samples and directions for obtaining informed consent and shipping can be found in its (8).

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Overgrowth Syndromes: Evaluation and Management of the Child with Excessive Linear Growth

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INTRODUCTION

Growth is the process by which cells increase in number, size, and functional capacity, and individual systems and organs differentiate, enlarge, and mature operationally for the purpose of achieving maximal somatic size and optimal function in keeping with the "genetic" or "intrinsic" potential of the individual and species. The unusually tall and rapidly growing child and adolescent may be one who is manifesting his or her endogenous genetic potential for growth, is achieving his or her growth capacity at an earlier than usual age, or is displaying the effect of a genetic error or hormonal-secretory abnormality (Table 1) (1). The study of children with overgrowth syndromes is important not only for the evaluation, diagnosis, and clinical management of the affected patient and family, but also for the insight into the regulation of normal growth that derives from identifying the primary pathogenetic mechanisms responsible for these aberrations. Before considering the various causes of tall stature and their management, we will review genetic and hormonal factors that regulate normal growth of the fetus, infant, child, and adolescent.

GROWTH EPOCHS

Growth begins immediately after conception and continues at varying rates throughout gestation, infancy, childhood, and adolescence. The rate of fetal growth is the most rapid experienced. Growth velocity remains accelerated but at an ever slowing rate during the first two years of life. Growth rate remains steady or slows slightly during childhood and preadolescence; it then rapidly accelerates during early puberty to the point of peak height velocity after which it steadily decelerates until adult stature has been achieved.

Prenatal Growth

The embryonic period begins two weeks after fertilization and extends over the next eight weeks to the start of the fetal period. By the 10th week of gestation, organogenesis is nearly complete and the approximate fetal crown-rump length is 3 to 4 cm (2,3). Fetal growth velocity accelerates rapidly in the second trimester, peaking in mid-gestation when the crown-rump length velocity approximates 2.5 cm/wk (4). Linear growth rate during this interval reflects the effects of maternal well-being and nutrition upon placental size and function and its ability to transfer oxygen and nutrients to the fetus and upon endogenous fetal growth-regulatory processes. Linear growth velocity declines in the last half of gestation because of progressive constraint exercised by a relatively restricted uterine size. As fetal length velocity slows, the rate of weight gain accelerates, reaching maximum accretion at about 34 weeks gestation. At term, birth length approximates 51 cm in both boys and girls, while birth weight averages 3400 g with male body weight exceeding female by approximately 100 g (5).

Fetal pituitary and serum levels of growth hormone (GH) increase at mid-gestation and then decline toward term as central inhibitory controls mature. GH receptors (GHR) are widely dispersed in fetal tissues but present in low abundance in early gestation, increasing in late gestation and after delivery. Serum concentrations of GH binding protein (GHBP), the extracellular domain of the GHR, are low in fetal blood (6). These findings suggest that fetal growth is relatively GH-independent. Nevertheless, the length of neonates with isolated GH deficiency is approximately −0.8 SD below the normal mean birth length, and the length of infants with GH insensitivity due to inactivating mutations in the gene encoding the GHR is

Table 1 Causes of Excessive Growth

Intrinsic tall stature
Constitutional (familial, genetic) tall stature
Short stature Homeo SHOX excess: Triple X syndrome; 47XYY; 47XXY; 48XXYY
Acquired tall stature
Growth hormone excess
Pituitary growth hormone-secreting tumor
Multiple endocrine neoplasia type I
McCune-Albright syndrome (GNAS)
Neurofibromatosis type 1 (NF1)
Carney complex (PRKAR1A)
Ectopic growth hormone secreting tumors
Ectopic growth hormone releasing hormone-secreting tumors
Disorders of pubertal development
Sexual precocity
Isosexual: Central precocious puberty; Pseudoisosexual; Incomplete
Heterosexual
Female: Congenital adrenal hyperplasia (CYP21, CYP11B1, 3BHSD), androgen secreting tumors of the ovary (arrhenoblastoma, gonadoblastoma) or adrenal, cortisone reductase deficiency (HSD11B1, H6PG), cortisol resistance (glucocorticoid receptor), aromatase deficiency (CYP19A1), exposure to exogenous androgens
Male: Aromatase excess: calcifying Sertoli cell tumor of the testis—Peutz-Jeghers syndrome; chorioepithelioma, feminizing tumor of the adrenal or testis; exposure to exogenous estrogens
Hypogonadism
Primary: 47XXY, 48XXYY
Secondary: Isolated gonadotropin deficiency—Kallmann syndrome (KAL1, FGFR1)
Androgen insensitivity syndrome (androgen receptor)
Estrogen deficiency
Aromatase deficiency (CYP19A1)
Estrogen insensitivity (estrogen receptor)
Familial glucocorticoid deficiency (Melanocortin-2 receptor, MRAP, ''Achalasia–Addisonianism–Alacrima'' syndrome)
Hyperthyroidism
Hyperinsulinism
Obesity
Type I diabetes mellitus
Infant of a diabetic mother
Persistent hyperinsulinemic hypoglycemia of infancy (ABCC8, KCNJ11)
Lipodystrophic syndromes
Syndromic: Beckwith–Wiedemann, Nevo
Syndromic overgrowth[a]
Sotos syndrome—Cerebral gigantism [Nuclear receptor SET domain-containing protein 1(NSD1)]
Weaver syndrome (NSD1)
Beckwith–Wiedemann syndrome (Imprinting chromosome 11p15.5, H19, CDKN1C, NSD1)
Perlman syndrome
Simpson–Golabi–Behemel, type 1 (GPC3)
Phosphatase and tensin homologue (PTEN) hamartoma-tumor syndrome (Bannayan–Riley–Ruvacalba/Cowden)
Proteus
Nevo
Marfan
Homocystinuria (CBS)
NF1
Fragile (X) site mental retardation 1

[Associated gene(s)]

[a]Selected—For comprehensive list of syndromes associated with overgrowth please see Table 16 in Cohen (1).

approximately −1.6 SD below the mean (7). By contrast, transgenic mice expressing high levels of pituitary GH or GH-releasing hormone (GHRH) are not large at birth, but overgrow by 14 to 21 days of age (8). The secretion of maternal pituitary GH is suppressed in the second half of pregnancy by secretion of a placental GH variant that binds to both fetal and maternal GHR. The amino acid (aa) sequence of mature placental GH differs from that of pituitary derived GH by 13 aa; it is detectable in maternal blood at 10 to 12 weeks gestation, and its concentrations increase by the third trimester (9). Secretion of placental GH is tonic and independent of hypothalamic GHRH. Placental GH enhances maternal production of insulin-like growth factor (IGF) -I and that, in turn, stimulates uterine and breast growth (10). Human placental lactogen is synthesized in the syncytiotrophoblast, secreted differentially into both maternal and fetal circulations, and regulates, in part, the synthesis of fetal IGF-I and -II (11). It has been estimated that GH-related factors may account for up to 40% of the variance in birth weight (12).

Interactions of nutrition, glucose, insulin, and the IGFs play primary roles in regulation of fetal growth (13). IGF-I is expressed in the placenta and in all fetal tissues in the early embryo with levels rising 400% between 25 and 31 weeks and term, while cord blood levels of IGF-I are positively correlated with birth size. Although fetal IGF-II concentrations remain relatively constant throughout gestation, it too is important for fetal growth, perhaps functioning as a constitutive growth factor in early gestation (vide infra) (13,14). The fetal growth-promoting actions of IGF-I and -II are mediated by the IGF-I receptor (IGF1R) (15). Growth factors, nutrient flux, glucose availability, and circulating insulin concentrations interact to regulate fetal IGF production. Experimentally, fetal pancreatectomy reduces IGF-I levels with resultant compromise of fetal growth, while fetal glucose or insulin infusion augments fetal IGF-I values and growth (16). Mice in which either IGF-I or IGF-II has been eliminated (''knocked out'') are 40% smaller at birth than wild type (wt) animals, whereas, mice with loss of both IGFs are 70% smaller than their wt counterparts at birth (17). In the Cre/Lox P conditional ''knock-out'' mouse with selective absence of liver-generated IGF-I resulting in a 75% reduction in circulating IGF-I values, in utero and postnatal growth are normal suggesting that locally synthesized IGF-I acting through paracrine or intracrine mechanisms is effective in maintaining growth in this species (18). Loss-of-function mutations in the gene encoding the IGF1R have been associated with intrauterine growth retardation in humans (19,20). Transgenic mice with an extra copy of *IGF2*, the gene encoding IGF-II, demonstrate fetal overgrowth (21). The cation-independent mannose-6-phophate receptor internalizes and degrades circulating IGF-II; experimental loss of this receptor results in an overgrown newborn mouse that is 40% larger than wt indicating that prolongation of

IGF-II activity in utero also promotes fetal growth (22). The insulin receptor too mediates some of the fetal growth promoting actions of IGF-II (17). In man, *IGF2* is sited on chromosome 11p15.5 and is expressed only by the paternal allele. Immediately downstream of *IGF2* is *H19*, a gene that normally is expressed only by the maternal allele; *H19* represses maternal *IGF2* transcription by controlling its pattern of promoter methylation and thereby its expression. Deletion of *H19* in mice results in biallelic expression of *IGF2* and a 30% increase in birth weight (23). In patients with the Beckwith–Weidemann syndrome (BWS) (vide infra), the maternal *H19* allele may itself by inactivated by hypermethylation, thereby permitting biallelic expression of *IGF2* leading to fetal overgrowth due to increased IGF-II production (15). Mice in which the gene encoding insulin has been "knocked out" are 10% to 20% smaller than wt animals at birth, indicating that insulin also plays a role in the regulation of intrauterine growth (17). Although hyperinsulinism has long been associated with fetal overgrowth, the increased weight of the infant of a woman with gestational diabetes is due primarily to accumulation of adipose tissue and glycogen stores rather than bone and muscle (15).

Growth in the Neonate, Infant, and Child

Infantile growth is rapid; by one year of age relative to birth length and weight length has increased approximately 50% and weight has tripled (24). By one year of age, the weight gain of breast fed infants is usually less than that of those who are formula fed, although length and head circumference do not differ (25). Linear growth velocity declines from more than 20 cm/yr at two to three months of age to 8 to 9 cm/yr at age 24 to 36 months. Linear growth occurs in a series of "stepwise (saltatory) .... jumps separated by variable intervals of no change" (26). Weekly and semi-weekly increases of 0.5 to 2.5 cm take place during single days separated by 3 to 60 day intervals of paused growth that are preceded or accompanied by incremental weight gain (27). There may be substantial change in length and weight channels in the first one to two years of life as infants experience "catch up" or "catch down" growth (28). These differing growth patterns likely represent compensatory responses to intrauterine enhancement or restraint of fetal growth (29). Approximately 50% of healthy infants cross height-for-age growth channels during the first 24 months of life, and shifts in linear growth channels continue through five years of age, but with decreasing frequency (28,30). Once a youngster has established his/her growth channel, he/she remains within it with great consistency. Eighty percent of small but appropriate for gestational age preterm infants "catch up" in height to their chronologic age peers in the first 6 to 24 months of life (4). Infants who are small or thin at birth and experience rapid, early postnatal catch-up growth between birth and two years are at risk for development of childhood obesity (29,31). In mid-childhood linear growth rate averages 5 to 6 cm/yr in both genders with gradual decline to levels as low as 2 to 3 cm/yr immediately prior to the pubertal growth spurt.

Growth in Puberty

More than 20% of adult stature is attained, and 50% of adult bone mass is accrued during the pubertal growth spurt. Following the prepubertal "dip" in linear growth velocity, there is rapid acceleration of growth rate that is maximal (peak height velocity) at 12 years in girls and at 14 years in boys. Growth rate then declines until near adult height is achieved with fusion of the epiphyseal growth plates. An accelerated rate of growth is often demonstrable in girls even prior to the first physical sign of puberty (thelarche most commonly), while peak height velocity occurs coincident with Tanner breast stage III and several months prior to menarche (average age 12:4 years). In boys, linear growth is relatively slow in early puberty, and maximal growth rate is deferred coinciding with Tanner male genital stage IV. It is the delay in reaching the pubertal peak height velocity that results in the greater adult stature of males relative to females. As a consequence of increase in vertebral height, adult stature rises approximately one to 2 cm in the third decade of life.

Genetic background, nutritional environment, socioeconomic status, and adverse events such as injuries and illnesses determine postnatal growth patterns and adult stature. In the absence of intrinsic and environmental insults, growth (GH) and thyroid hormones direct these processes. Concentrations of serum GH are high in early infancy and then decline, while IGF-I values vary substantially but are generally low relative to values in older children. Deficiencies of both growth (GH) and thyroid hormones inhibit while excess production of these hormones enhance linear growth during infancy, childhood, and adolescence. GH and sex hormones contribute to the accelerated growth rate of puberty (32). The production rate of GH increases 2 to 3 fold during adolescence as a consequence of increased basal GH secretion and its pulsatile release—enhanced primarily by estrogens. Entrained upon augmented GH secretion as well as increased androgen and estrogen production, the synthesis and secretion of hepatic and chondrocyte IGF-I rise resulting in the rapid rate of chondrocyte proliferation that is the hallmark of the pubertal growth spurt.

THE CELL CYCLE

Somatic growth of the body and its organs is dependent upon increase in both cell number and cell size. Cell number increases by cell division at the completion of the cell cycle, the process that leads to

DNA replication and mitosis (33). Classically, there are four phases to the cell cycle: G1 (''gap1'')—an early resting phase (G0) is followed by a mid-to-late phase during which the process of cell replication begins as functional components of the succeeding S phase are transcribed; S (''synthetic'')—the phase in which DNA replication occurs; G2 (''gap2'') phase during which DNA is replicated; M (''mitotic'')—the metaphase of mitosis during which chromosomes condense and align on the spindle; under the direction of an anaphase-promoting complex, the chromosomes then segregate by cytokinesis during anaphase; later, the nuclear envelope is reconstructed and daughter cells separate during telophase. Upon completion of the cycle the cell returns to the resting early G0 phase and the process resumes when directed to do so. Errors at any checkpoint in this process may lead to arrest of cellular division. When newly synthesized DNA is damaged, regulatory proteins P53 and phosphatase and tensin homologue (PTEN) are activated by phosphorylation and interrupt the cell cycle by inhibiting G1-to-S progression.

The four phases of cell replication are directed by the interaction of cyclin-dependent kinases (CDKs), phosphorylating enzymes that are members of the serine-threonine protein kinase (PK) family, and by nuclear cyclins that serve as stimulatory subunits for the catalytic CDK subunits. Cyclins and CDKs heterodimerize to form a holoenzyme (33). Cyclins are synthesized in response to specific transcription factors and then degraded by ubiquitination in a tightly choreographed pattern as the cell moves through its successive stages. CDK activity is negatively regulated by specific CDK inhibitors. The retinoblastoma tumor-suppressor protein (pRb—p105) and related proteins (p107, p130) serve as inhibitory ''gatekeepers'' maintaining the cell in the resting, non-replicative G0 state by repressing the function of a number of stimulatory transcription factors (vide infra). pRb does so by recruiting histone deacetylases to the chromatin sites of target genes. Histone acetyltransferases and deacetylases are enzymes with opposing functions that, respectively, add or remove acetyl groups from lysine residues in the amino terminal regions of histone proteins about which DNA is wound. By modifying the three-dimensional structure of chromatin, they regulate gene transcription (34). CDKs phosphorylate pRb (p105) and related proteins thereby removing the inhibiting effects of these proteins and permitting transcription of growth factors that then stimulate the cell cycle to begin and progress.

Cyclin D1 (CCND1, OMIM 168461, chromosome 11q13) and its related proteins and functional partners—cyclins D2 and D3 and CDK4 (CDK4—OMIM 123829, chromosome 12q14) and CDK6 (CDK6—OMIM 603368, chromosome 7q21–q22)—function in the early to midpoint of G1 by phosphorylating and inactivating pRb; in concert with cyclin E (CCNE1—OMIM 123837, chromosome 19q13.1) and CDK2 (CDK2—OMIM 116953, chromosome 12q13), the D cyclins permit the cell to transition from G1 to S (35). CDK2 also facilitates progression of the S phase. Cyclin A (CCNA2—OMIM 123835, chromosome 4q27) links with CDK2 during the S phase. Both cyclins A and B (CCNB1—OMIM 123836, chromosome 5q12) interact with CDK1 (CDC2—OMIM 116940, chromosome 10q21.1) during G2 and M phases with cyclin B playing a pivotal role in the late G2 phase of the cell cycle (33).

Transcription of CCND1 is induced by IGF-I and -II, androgens, amino acids, retinoic acid, parathyroid hormone related protein (PTHrP), and transforming growth factor (TGF)β. In addition to associating with several catalytic CDK subunits, cyclin D1 binds to both histone acetyltransferases and deacetylases and thus removes the factor(s) that mediate the repressive effect of pRb. Independently of its effects on the cell cycle, cyclin D1 binds also to a number of transcription factors exerting either inhibitory or stimulatory effects thereon. Cyclin D1 enhances the binding of estrogen receptor (ER)α to its response element and thereby increases its transcriptional activity. It suppresses androgen receptor (AR) activity by competing for needed AR coactivators or by recruiting AR corepressors. Cyclin D1 blocks the differentiation of adipocytes stimulated by peroxisome proliferator-activated receptor-γ. It also inhibits the transcriptional activity of intracellular signal transducer and activator of transcription (STAT) 3 in response to stimulation by Janus kinase (JAK) 1, a tyrosine kinase excited by binding to the intracellular domain of a number of cytokine receptors including those for GH and prolactin. CDKs 8 and 9 participate in the regulation of RNA Polymerase II activity during the S phase of the cycle.

Thus, the process of cell replication is initiated by its reception of a mitogenic signal that stimulates transcription of cyclins D1, D2, D3, and E and increases nuclear levels of cyclin D-CDK 4/6 and cyclin E-CDK2 complexes. In turn, these heterodimers phosphorylate pRb causing it to dissociate from E2F, a family of transcription factors that activates several genes whose products are essential for normal progression of the cell cycle including those encoding cyclins A and E. In response to transcription factors of the E2F family (E2F1, OMIM 189971, chromosome 20q11.2), nuclear levels of cyclin E and its associated CDK2 peak in late G1, catalyze the transition to the S phase, are essential for promoting DNA synthesis and replication of the cell's genome during the S phase, and are rate-limiting for cellular proliferation. During and after completion of the cell cycle, the mitotic cyclins are polyubiquitinated and degraded through the proteasomal pathway (36,37). Excessive synthesis or impaired catabolism of cyclins and CDKs are associated with a variety of human neoplasms (33).

More than 13 CDKs and 25 cyclins have now been recognized (33). Approximately, 60 other PKs

interact with and regulate the CDKs themselves or otherwise influence the progression of the cell cycle (38). These include several of the JAKs that interact with CDK4, mitogen activated protein kinases (MAPK) that regulate cyclin D expression, receptors with intrinsic tyrosine kinase activity such as that for platelet derived and vascular endothelial growth factors that appear to act at the S and M phases of the cell cycle, and IGF-I that signals through the MAPK pathway. Polo-like (serine/threonine) kinases (PLK3, OMIM 602913, chromosome 8p21) are important regulators of centrosome maturation and the transition from metaphase to anaphase. While most of the extracellular signals that influence cell growth and replication act in the G1 phase of the cycle, data suggest that cells in the S phase may autoregulate division through growth factor signaling pathways that act during phase G2 (38).

MECHANISMS OF LONGITUDINAL GROWTH

Chondroblasts and osteoblasts, as well as myotubes and adipocytes, develop from a common mesenchymal precursor (39). Bone morphogenetic proteins (BMP), primarily BMP-2 and -7—members of the TGFβ superfamily, direct this pluripotent cell into the pathway that leads to formation of chondroblasts and osteoblasts acting through specific cell membrane receptors and the SMAD (mothers against decapentaplegic) intracellular signal-transduction pathway (40). Linear growth occurs as chondrocytes in the cartilage growth plate proliferate and enlarge and terminates as they die and the growth plate undergoes endochondral calcification and fusion of the distal epiphyseal and central metaphyseal regions of a typical long bone (41). Indeed, an individual's adult stature is the result of the extent of the proliferative and hypertrophic phases of the chondrocyte cycle. The growth plate is composed of horizontal zones of chondrocytes arranged in longitudinal columns in progressive stages of maturation (Fig. 1) (42). At its epiphyseal end is the germinal (also termed stem cell, resting, or reserve) zone of chondrocytes (or chondroblasts) medial to which is the proliferating zone of chondrocytes. In this region, chondrocytes enter the cell cycle, divide mitotically (proliferate), and synthesize matrix composed of collagen types II and IX and sulfated proteoglycans [glypican (GPC), chondroitin], and other products. When the stimulus to chondrocyte division is lost, the cells differentiate further, enlarge, and enter the prehypertrophic or transitional zone before transforming into rounded hypertrophic chondrocytes that are characterized by expression of collagen type X (43). Matrix vesicles discharged from these large chondrocytes release calcium phosphate and matrix metalloproteinases leading to calcification of matrix and cellular apoptosis resulting in a low oxygen environment that invites invasion by blood vessels from the underlying metaphyseal primary spongiosium. Chondroclasts, osteoclasts, and osteoblasts accompany blood vessel invasion, resorb calcified matrix and apoptotic chondrocytes, and initiate the process of endochondral bone formation by developing an ossification center that slowly moves toward the distal ends of the long bones. The growth plate is surrounded by the perichondrium with progenitor cells that eventually form osteoblasts and then the periosteum (43).

Anauxetic dysplasia (OMIM 607095) is a rare osteochondrodystrophy manifested by extreme short stature (adult height < 85 cm), hypodontia, mild developmental delay, and characteristic radiographic abnormalities including hypoplasia of the femoral capital epiphyses and necks and iliac bodies and

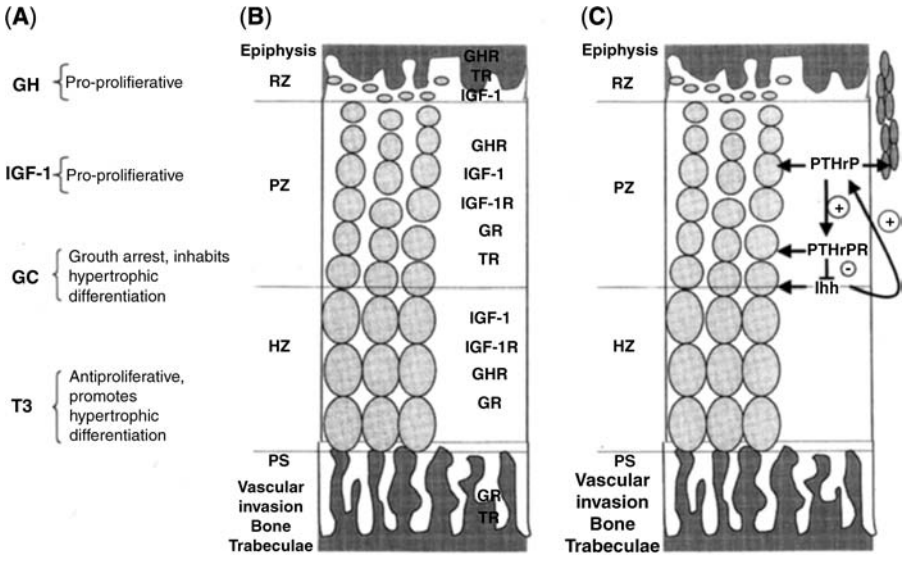

Figure 1 The cartilage growth plate depicting (**A**) the biologic activities and sites of action of GH, IGF-I, GC, and T3; (**B**) sites of expression of the GH, thyroid, IGF-I, and GC receptors; and (**C**) the interaction of IHH and PTHrP. *Abbreviations*: GH, growth hormone; IGF, insulin-like growth factor; GC, glucocorticoids; T3, triiodothyronine; IHH, Indian hedgehog; PTHrP, parathyroid hormone related protein RZ, resting or reserve zone; PZ, proliferative zone; HZ, hypertrophic zone; PS, primary spongiosum. *Source:* Reproduced from Ref. 42.

vertebral malformation (44). Histologic examination of the cartilaginous growth plate in these patients reveals greatly decreased numbers of chondrocytes in the germinal and proliferative zones and diminution of the hypertrophic zone. This disorder has been linked to loss-of-function mutations in a mitochondrial RNA-processing endoribonuclease (RMRP—OMIM 157660, chromosome 9p21–p12). The RNA component of this ribonucleoprotein is an untranslated RNA gene product. RMRP is essential for ribosome construction, synthesis of RNA primers for mitochondrial DNA generation, and catabolism of cell cycle-related mRNA. In addition to anauxetic dysplasia, loss-of-function mutations in RMRP lead to two other clinically distinct osteochondrodystrophies: cartilage hair hypoplasia (OMIM 250250) and metaphyseal dysplasia without hypotrichosis (OMIM 250460). Homozygous or compound heterozygous mutations in RMRP associated with anauxetic dysplasia (+14G→A, +90_91AG→GC, 111–112(ins14N), +254C→G) result in impaired ribosomal assembly that severely curtails protein synthesis and hence cell growth (44). Mutations in different segments of RMRP have been found in patients with cartilage hair hypoplasia and metaphyseal dysplasia without hypotrichosis and presumably account for the less severe foreshortening of these osteochondrodystrophies and perhaps for other forms of short stature. Collectively, these data confirm the critically fundamental roles that RMRP and ribosomal protein synthesis play in cell division and growth.

In utero, insulin, pituitary GH, and IGF-I and-II are among the most important hormonal determinants of fetal growth. Deficiency of fetal insulin (leprechaunism) or IGF-I results in substantial intrauterine growth retardation, while insulin or IGF-II excess (infant of diabetic mother or neonate with the BWS, respectively) leads to fetal overgrowth; GH deficiency or resistance leads to modest foreshortening (45). Postnatally, GH, IGF-I, and thyroid hormones are the primary hormonal stimuli to growth of the infant and child, and their effects are enhanced and augmented by sex hormones during puberty (41). Pituitary GH directly stimulates division of resting chondrocytes in the reserve zone of the cartilage growth plate and their secretion of IGF-I while also increasing chondrocyte levels of the IGF1R. Expression of the GH receptor in the germinal chondrocyte is enhanced by GH, thyroid hormones, and glucocorticoids. Locally synthesized IGF-I then acts in a paracrine manner to augment clonal expansion of chondrocytes in the proliferative zone and to enhance their differentiation and progression into the hypertrophic zone. Circulating IGF-I of hepatic origin is likely also to be necessary for optimal chondrocyte multiplication (46). [Because GH and IGF1R are expressed in resting, proliferative, and hypertrophic chondrocytes in varying proportions, the function(s) of GH and IGF-I may not be so clearly demarcated as described above (47).] Both GH and IGF-I are necessary for maximal linear growth; both hormones decrease the duration of the cell cycles of germinal and proliferating chondrocytes and the length of the hypertrophic differentiation stage (42,48). In addition, IGF-II probably also plays a role in growth plate dynamics, as in the mouse in whom IGF-I has been ''knocked-out,'' there is an increase in expression of IGF-II in the germinal and proliferative zones of the growth plate (48). The major intracellular GH signal transduction system in chondrocytes is mediated by JAK-2 and STAT 5b. Many IGF binding proteins (IGFBP) that either enhance or repress the action of GH are also found in and about chondrocytes, adding another layer of complexity to the regulation of chondrocyte growth and maturation by GH and the IGFs. GH and IGF-I impact not only differentiation, proliferation, and maturation of chondrocytes but also their death (47).

Fibroblast growth factors (FGF) comprise a family of at least 25 polypeptides with a high affinity for heparin and a shared homologous central core domain of 120 aa that affect cell differentiation, growth, migration, and survival (49). They interact with a family of four FGF receptors (FGFR) with three immunoglobulin-like regions in their extracellular domains and intrinsic tyrosine kinase activity within their intracellular domains. FGFs regulate growth and development of endochondral bone and the longitudinal growth of long bones by accelerating maturation and fusion of the growth plate (43). *FGFR2* (OMIM 176943, chromosome 10q26) is expressed by the earliest germinal chondrocytes and induces the expression of *SOX9* (OMIM 608160, chromosome 17q24.3–q25.1), a transcription factor essential for differentiation of chondrocytes (and male genital formation); inactivating mutations in *SOX9* lead to abnormalities of cartilage formation and genital development (campomelic dysplasia, OMIM 114290). *FGFR3* (OMIM 134934, chromosome 4p16.3) is expressed in both reserve and proliferating chondrocytes; when activated, it stimulates proliferation of very immature cells while limiting division of proliferating chondrocytes (43). Gain-of-function mutations in *FGFR3* are pathogentically related to achondroplasia (OMIM 100800) and hypochondroplasia (OMIM 146000). In mice in which *Fgfr3* has been ''knocked out,'' there is skeletal overgrowth with elongated growth plate zones of proliferating and hypertrophic chondrocytes (41). *FGFR1* (OMIM 136350, chromosome 8p11.2–p11.1) is expressed in hypertrophic chondrocytes and may play a role in their maturation, senescence, and death. *FGF2* (OMIM 134920, chromosome 4q25–q27), *FGF9* (600921, chromosome 13q11–q12), and *FGF 18* (OMIM 603726, chromosome 5q34) may serve as important ligands for the FGFRs in developing cartilage.

Ghrelin, a GH secretagogue and orexigenic protein produced primarily by oxyntic cells of the gastric fundus, is also expressed, synthesized, and secreted by proliferative and prehypertrophic human, mouse,

and rat chondrocytes; in these cells, there is a unique ghrelin receptor that differs from the GH secretagogue receptor previously characterized (50). Ghrelin does not enhance proliferation of chondrocytes but may affect their basal metabolism, maturation, and life span. Leptin, an anorexigenic product of white fat cells, has been identified in hypertrophic chondrocytes near invading capillaries from underlying spongiosa; it enhances endothelial cell proliferation and migration, suggesting that leptin may regulate early stages of endochondral ossification (51). In vitro, leptin receptors are expressed by chondrocytes, and leptin stimulates intracellular signal transduction via the STAT pathway (52). In vivo, leptin induces chondrocyte synthesis of IGF-I, but may also be linked to inflammatory processes within cartilage (53).

Thyroid hormones, primarily triiodothyronine (T3), are essential for the progressive differentiation of germinal to proliferative chondrocytes acting in concert with GH and IGF-I, hypertrophy of chondrocytes, and invasion of the growth plate by blood vessels from the metaphyseal spongiosum (42). They act, in part, by halting DNA synthesis within chondrocytes by induction of inhibitors of cyclin/CDK activity ($p21^{Cip1\ Waf1}$, $p27^{Kip1}$), thus terminating progression of the cell cycle and hastening terminal differentiation (54). Glucocorticoid receptors (GCCRs) are expressed in proliferating and hypertrophic chondrocytes where glucocorticoids enhance their differentiation, inhibit their proliferation, and accelerate their apoptosis (42). They do so both by acting directly on the chondrocyte and by reducing expression of the genes encoding the GH receptor, IGF-I, and the IGF1R. They also impede IGF-I mediated DNA synthesis by altering IGFBP synthesis and decreasing intracellular conversion of thyroxine to T3 (41). At the same time, glucocorticoids enhance recruitment of chondrocyte precursors, in part by increasing expression of *SOX9*, and protect their potential for proliferation (42). Familial glucocorticoid deficiency (FGD) (vide infra) is a cause of tall stature clinically, while experimentally steroid deprivation enhances growth in insects by disinhibiting insulin signaling (55).

Estrogen is the sex hormone that is primarily responsible for the adolescent growth spurt and fusion of the cartilage growth plate (41). In males with loss-of-function mutations in *CYP19A1* (OMIM 107910, chromosome 15q21.1) encoding aromatase or ESRα (OMIM 133430, chromosome 6q25.1) encoding the major nuclear ERα, there is continuous growth but no pubertal acceleration leading to tall stature; the growth plates of subjects with aromatase deficiency fuse only after they are exposed to estrogen (56,57). The normal adolescent growth spurt is likely the result of the rise in secretion of pituitary GH evoked by the early pubertal increase in estrogen production, while decline in linear growth and growth plate fusion are consequences of higher levels of estrogens. At low levels, estrogens enhance the growth of chondrocytes in the proliferative zone. At higher levels, estrogens decrease chondrocyte proliferation and accelerate their maturation, senescence, and apoptosis; they also enhance osteoblastic invasion of the cartilaginous growth plate (58). Estrogens act through both ERα and ERβ expressed primarily in hypertrophic chondrocytes but present also in the resting and proliferative zones (59,60). Growth plate fusion may be mediated primarily through ERα (61). Chondrocytes also express P450 aromatase permitting local conversion of gonadal and adrenal androgens to estrogens (56). Furthermore, estrogens enhance local synthesis of IGF-I and its receptor.

Androgens influence chondrocyte maturation primarily by their conversion to estrogens (62). However, resting and hypertrophic chondrocytes also express the nuclear AR (60,63). Non-aromatizable androgens (e.g., dihydrotestosterone) increase linear growth experimentally implying that they too are able to influence chondrocyte growth directly (41). Dihydrotestosterone stimulates chondrocyte proliferation in vitro by increasing IGF-I production and expression of the *IGF1R* and acts synergistically with calcitriol to enhance chondrocyte maturation (64). Calcitriol also stimulates chondrocyte proliferation in vitro acting through IGF-I and its receptor and perhaps by increasing local expression of AR.

Prehypertrophic chondrocytes synthesize and secrete Indian hedgehog (IHH), a member of a family of proteins important for embryonic patterning and differentiation (41). IHH coordinates and enhances the proliferation and differentiation of chondrocytes, the differentiation of osteoblasts, and links chondrogenesis to bone formation. IHH production is self-regulated through its stimulation of PTHrP secretion from periarticular perichondrium (Fig. 1). Reciprocally, PTHrP acting through its PTH/PTHrP receptor-1 expressed in late proliferating and early hypertrophic chondrocytes inhibits BMP-6 stimulated expression of chondrocyte IHH and thereby decreases the rate of chondrocyte maturation, thus prolonging the stage of chondrocyte proliferation and increasing bone length (65). Gain-of-function mutations in *PTHR1* result in Jansen metaphyseal chondrodysplasia (OMIM 156400, chromosome 3p22–p21.1), a chondrodystrophy characterized by slowing of chondrocyte maturation causing disorganized metaphyses leading to skeletal deformities and impaired linear growth as well as hypercalcemia. Loss-of-function mutations in *PTHR1* lead to Bloomstrand chondrodysplasia (OMIM 215045) in which chondrocyte maturation is hastened, the growth plates fuse prematurely, and linear growth is stunted.

Seventy to ninety percent of adult stature is genetically determined and heritable; nutritional intake, environmental conditions, severity and duration of illness, and other socioeconomic factors influence an individual's ability to achieve his/her full genetically programmed growth potential (66). In a genome wide assessment of 1816 adult American Caucasians of European origin, the heritability of

height was 79% (SE ± 2.9%). Ten percent of height variability was linked to chromosome 9q22.32. Other loci that influenced height were also identified on chromosomes 1p13.1–q21.3 (Cathepsin K—CTSK), 2q24.3, 4p16.3 (*FGFR3*), 7p14.2 (*GHRHR*), 9q34.3, 14q23.2, 17q22-q23.3 (GH1, COL1A1), and Xq24. Several short stature syndromes have been mapped to the region of Xq24 implying the presence of a height regulating gene(s) in this area as well as at the opposite end of the X chromosome where *SHOX* is found (vide infra). A syndrome of mental retardation, hypoplasia of the hypothalamic infundibulum and anterior pituitary lobe, and GH deficiency has been linked to Xq26.3 and has been associated with hemizygous loss-of-function mutations in *SOX*3 (SRY-BOX 3—OMIM 313430) (67,68). There are polymorphic variations of many genes that influence adult height by either impeding or enhancing growth; for example.

GH1 (Human GH—OMIM 139250, chromosome 17q23.3); deletion or loss-of-function mutations in GH1 are well associated with GH deficiency and growth retardation. A subtle variation of GH1 has been reported in which the nucleotide adenosine is substituted for thymidine at N:1663 in intron 4; this results in a GH product with slightly decreased biologic activity as manifested by lower IGF-I concentrations and shorter stature during childhood and an adult height in females that is 2 cm less than that of women with thymidine at N:1663 (69,70).

Growth hormone receptor (GHR—OMIM 600946, chromosome 5p13–p12)—exon 2 of the 9 coding exons for the GHR encodes the signal peptide; exons 3 to 7 encode the extracellular domain of 246 aa; much of exon 8 encodes the transmembrane domain, and the remainder of exon 8 together with exons 9 and 10 encode the intracellular domain. There are two isoforms of the GHR—one full-length and a second in which the 22 aa encoded by exon 3 are omitted (d3-GHR) by homologous recombination, a process that mimics alternative splicing. Although the GHR genotype (*GHR/GHR*, *GHR/d3-GHR*, *d3-GHR/d3-GHR*) does not affect basal growth rate, when non-GH deficient children with one or two *d3-GHR* allele(s) are treated with rhGH, they grow more rapidly than do those with the *GHR/GHR* genotype (71). Similarly, GH deficient patients with one or two *d3-GHR* allele(s) grow more rapidly when treated with rhGH than do GH deficient children with two GHR alleles (72). When the GHR and *d3-GHR* isoforms are expressed in HEK fibroblasts in vitro, in response to GH the transcriptional activity of the luciferase reporter gene is approximately 30% greater in cells with *d3-GHR* than with GHR. Heterozygous loss-of function mutations in the extracellular, ligand-binding domain of GHR have also been associated with idiopathic short stature (73,74).

IGF1/IGF1R—Mutations that lead to loss-of-function of IGF-I (OMIM 147440, chromosome 12q22–q24.1) or its receptor (OMIM 147370, chromosome 15q25–q26) result in restricted fetal and postnatal growth (19,75,76). However, a seemingly ''minor'' polymorphic dinucleotide repeat intronic variant of *IGF1* results in lower levels of IGF-I and decreased prenatal and postnatal growth—particularly of head circumference (77). A polymorphic variant of decreased type I deiodinase activity (DIO1—OMIM 147892, chromosome 1p33–p32) that is associated with higher concentrations of free IGF-I and increased muscle strength in elderly men has been identified, suggesting the possibility that this variant might also be related to stature (78). Selective expression of IGF-I in muscle is associated with increased muscle bulk and strength and intra-articular injection of IGF-I increases growth plate width, suggesting the possibility that enhanced expression of endogenous IGF-I in an individual's growth plate might similarly increase linear growth in humans (79). It seems likely that various isoforms of IGF1 and its receptor, the interaction of several integral membrane proteins (e.g., $\alpha V\beta 3$ integrin) that coordinate *IGF1R* function and their ligands, and the overlapping intracellular signal transduction systems that mediate cellular function and target gene transcription may be associated with determination of adult stature (80).

The SHOX gene (SHOX—OMIM 312865, chromosome Xpter-p22.32) is a growth regulating gene located within the pseudoautosomal region (PAR) of the short arms of the X and Y chromosomes and is expressed by both sex chromosomes. In girls with Turner syndrome due to monosomy X (45X) or its variants in which the PAR of the second X chromosome is completely [46X/45Xdel p, $46XXq_i$] or partially absent (46XX/45X), their short stature and skeletal anomalies are attributed in part to haploinsufficiency of *SHOX* (81). Loss-of-function mutations in one *SHOX* allele or its isolated deletion have been associated with idiopathic short stature and Leri–Weill dyschondrosteosis (OMIM 127300), while loss of both *SHOX* alleles leads to Langer mesomelic dysplasia (OMIM 24970). On the other hand, overdosage of *SHOX* as in girls with the triple X syndrome leads to very tall stature that is first noted in mid-childhood (82). At birth the lengths of 80% of triple X females are within the normal range and a substantial number are quite small (55% less than the 10th percentile and some with intrauterine growth retardation); these children grow rapidly between 4 and 8 years, and by 14 years, the height of 67% of these adolescents is above the 90th percentile. In part, their pubertal growth pattern may also reflect subnormal estrogen secretion, because many 47XXX girls ultimately manifest ovarian dysfunction. Other findings in 47XXX girls include dysmorphic facial features and developmental delay. The tall stature of individuals with 47XXY, 47XYY, and 48XXYY may in part be attributable to the expression of multiple copies of SHOX. In the human fetal growth plate, *SHOX* is expressed in the reserve, proliferative, and hypertrophic zones;

during childhood and adolescence, it is expressed primarily in hypertrophic chondrocytes (83,84). The protein product of *SHOX* is most likely a transcription factor that possibly regulates the growth and function of osteogenic precursors. Somewhat unexpectedly, expression of *SHOX* in human chondrocytes results in arrest of the cell cycle and chondrocyte death (83). Expression of mutant forms of *SHOX* associated with idiopathic short stature and Leri–Weill syndrome did not do so. SHOX arrests cell division in phases G1 and G2/M by increasing intracellular levels of the CDK inhibitor—$p21^{Cip1}$, one of the CDK inhibitors activated by thyroid hormone. The CDK inhibitors $p21^{Cip1}$ and $p27^{Kip1}$ are highly expressed in normal chondrocytes in terminal stages of differentiation. Although it seems paradoxical that *SHOX* inhibits chondrocyte proliferation and hastens apoptosis while increasing stature, it may be that SHOX is essential for their coordinate growth and maturation.

CYP191A (Aromatase—OMIM 107910, chromosome 15q21.1)—the number of tetranucleotide tandem repeats in intron 4 is positively related to adult height in men (85).

Vitamin D receptor (*VDR*—OMIM 601769, chromosome 12q12–q14)—single nucleotide polymorphic (SNP) variants of *VDR* have been significantly associated with adult stature. Two SNPs of *VDR* near the 3′ end of the gene are particularly so related: the mean adult height of females with a guanine (G) in place of adenosine (A) at a BsmI cleavage site in intron 8 and thymidine (T) instead of cytosine (C) at a TaqI cleavage site in exon 9 of *VDR* (haplotype GT) is 1.6 cm greater than is that of individuals with the opposite haplotype (AC) (86). Although the basic mechanism(s) through which these variations influence *VDR* function and subsequent growth is unknown at present, it has been hypothesized that these sites are surrogate markers for more functionally significant variants of *VDR* within its 3′ untranslated region that influence its transcription or the stability of its mRNA.

Melanocortin-4 receptor (MC4R—OMIM 155541, chromosome 18q22)—*MC4R* mediates the anorexigenic effects of α-melanocyte stimulating hormone within the complex of neurons that regulates feeding. Nucleotide 2745 of *MC4R* may be either C or T; the adult stature of subjects with homozygosity for T at N:2745 (T/T) is approximately 5 cm greater than that of subjects with the C/C genotype, while the adult height of those with the C/T genotype is intermediate (87). There was no significant difference in body composition between the polymorphic genotypes in this study. Other components of the system that regulates appetite and energy expenditure might also be expected to affect linear growth.

Catecholamine O-methyltransferase (COMT, OMIM 116790, chromosome 22q11.2)—estrogens are sequentially metabolized by a P450 hydroxylase to less biologically active catechol-estrogens and these are further degraded by COMT to inactive products. A polymorphism in which methionine is substituted for valine in codon 158 (Val158Met) results in two products with different biologic activities: that containing Val158 is 3 to 4 fold more biologically active than is that with Met158. Prepubertal and early pubertal 10 to 12 year old girls with the Val158 variant of COMT are taller, more pubertally advanced, have greater bone mineral content, and higher free estradiol concentrations than do subjects with the Met158 variant (88).

It seems possible that subtle variations in genes in which inactivating mutations have been associated with clinical impairment of growth might exert growth restraining or growth enhancing effects in the normal population. Some candidates for this role include:

Growth hormone releasing hormone receptor (GHRHR—OMIM 139191, chromosome 7p14.2); loss-of-function mutations in GHRHR result in GH deficiency and growth retardation (89).

STAT5b—The GHR signals through JAK2 phosphorylation of *STAT5b* (OMIM 604260, chromosome 17q11.2) that in turn relays the message to the nucleus where it stimulates or represses selected target genes. Loss-of-function mutations in *STAT5b* result in GH insensitivity (90).

Suppressor of cytokine signaling (*SOCS2*) (OMIM 605117) primarily exerts an inhibitory effect on GH signaling by binding to GHR and interrupting its ability to activate JAK2. ''Knock-out'' of *SOCS2* in mice leads to excessive growth of the mutant in response to endogenous or exogenous GH (91). Paradoxically, however, overexpression of *SOCS2* can also lead to increased growth indicating that *SOCS2* has dual effects depending on its quantitative level.

NUTRITIONAL EFFECTS ON GROWTH

The effects of nutrition on growth are well documented—suboptimal nutrition resulting in foreshortening is no more evident than in the adolescent with anorexia nervosa (92). On the other hand, obesity increases the rate of growth and accelerates skeletal maturation in males and females; pubertal onset is advanced in girls with obesity but often normal in obese males and occasionally significantly delayed. Many boys with constitutional delay in growth and sexual development (CDGD), a variant of the normal growth and maturational patterns, are modestly underweight for height and often do not achieve their predicted adult heights based on parental size by approximately 2 cm. On the other hand, obese males with CDGD have been reported to exceed their predicated adult heights by as much as 6 cm suggesting that over-nutrition might increase adult stature in some subjects (93). However, in general although over-nutrition during childhood often results in greater height during this epoch, it does not result in increased adult stature in most subjects, because bone age also matures more

rapidly and the onset of puberty is earlier than in normal weight or lean children (94–96).

INTRINSIC/CONSTITUTIONAL (FAMILIAL, GENETIC) TALL STATURE

Excessive growth may begin during fetal life and persist to adulthood (Constitutional tall stature, Bannayan–Riley–Ruvalcaba and Marfan syndromes) or wane during childhood and adolescence (Sotos and Beckwith–Wiedemann syndromes). Alternatively, rapid growth may begin during childhood due to benign (constitutional tall stature) or disease factors (hyperthyroidism, diabetes mellitus type I) and wane (sexual precocity) or persist (GH excess). Intrinsic tall stature connotes that the innate potential for linear growth of an individual exceeds that of the normal population and is defined as adult stature greater than two SD above mean height-for-age and gender but possibly within the broadest range of normal encompassing the 99.9th percentile (+4 SDs) (97). Intrinsically tall stature may be ''constitutional,'' a variant of normal, or it may be associated with a pathologic state (Table 1). The definition of constitutional tall stature is dependent on the society in which the subject resides; thus, the heights of Northern Europeans are greater than those of North Americans at comparable ages; e.g., the mean adult height of Dutch men (184.0 cm) approximates the 90th percentile (184.5 cm) for height of American males (98). The most common form of intrinsic/constitutional tall stature is familial genetic tall stature, i.e., tall children born to tall parents (99). While not necessarily long at delivery, the growth of the constitutionally tall infant is rapid and within the first several months and years of life his/her length upwardly traverses growth channels before assuming a genetically programmed channel within or above the high normal range (Fig. 2) (30). In all other respects the constitutionally tall subject is normal without stigmata of pathologic causes of excessive growth. Their pubertal maturation is normal or may even be delayed (100). Constitutionally tall children (and adults) may or may not have somewhat increased secretion of GH or higher levels of IGF-I and IGFBP-3 relative to shorter subjects. While in children of normal height, glucose suppresses and thyrotropin and gonadotropin releasing hormones (GnRH) do not affect GH secretion, in exceptionally tall children and adolescents, glucose and the releasing hormones often provoke GH secretion (101). Thus, the major pathologic state to exclude when considering the diagnosis of constitutional (familial, genetic) tall stature is GH excess (vide infra) (Fig. 3).

Although at one time tall stature may have been considered a handicap, it is no longer (102). Thus, intervention to foreshorten the tall youth is seldom undertaken in North America at present (103). A major problem in establishing the efficacy of therapy, i.e., the reduction in adult height that may be attributed to treatment, has been difficulty in accurately predicting adult height of the constitutionally tall child, because there are many discrepancies in such predictions dependent on methodology, gender, skeletal maturation, and other variables (104). Older data indicate that in girls with a bone age of less than 13 years, adult height might be foreshortened by approximately 5 cm (relative to pretreatment predicted height) when ethinyl estradiol 0.1 mg daily is administered until the bone age approximates 15 years (105). Overall, reported mean height reductions in constitutionally tall females treated with high doses of estrogen have ranged from 1.1 to 10.0 cm and in

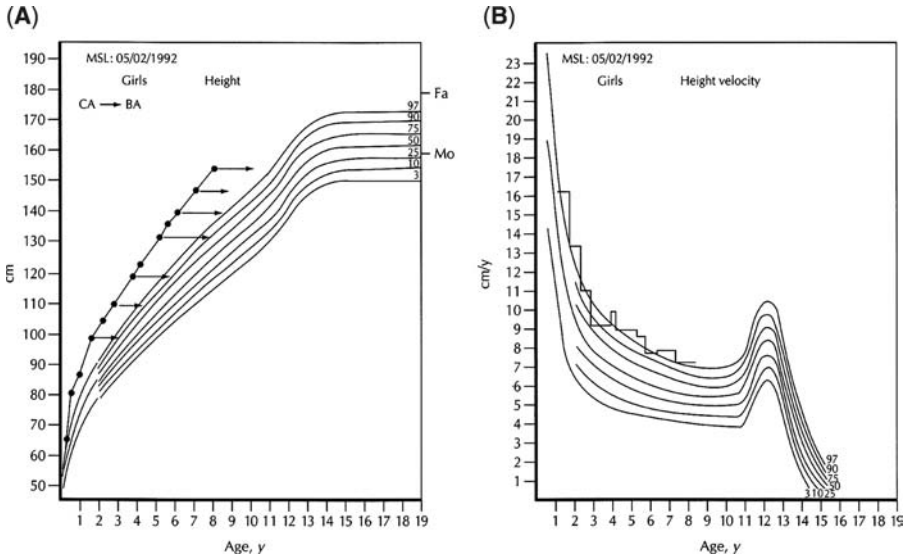

Figure 2 (**A**) Linear growth and growth rate of the normally tall subject parallels the normal curve. (**B**) Growth rate of the normally tall subject. *Source*: Reproduced with permission from Ref. 99.

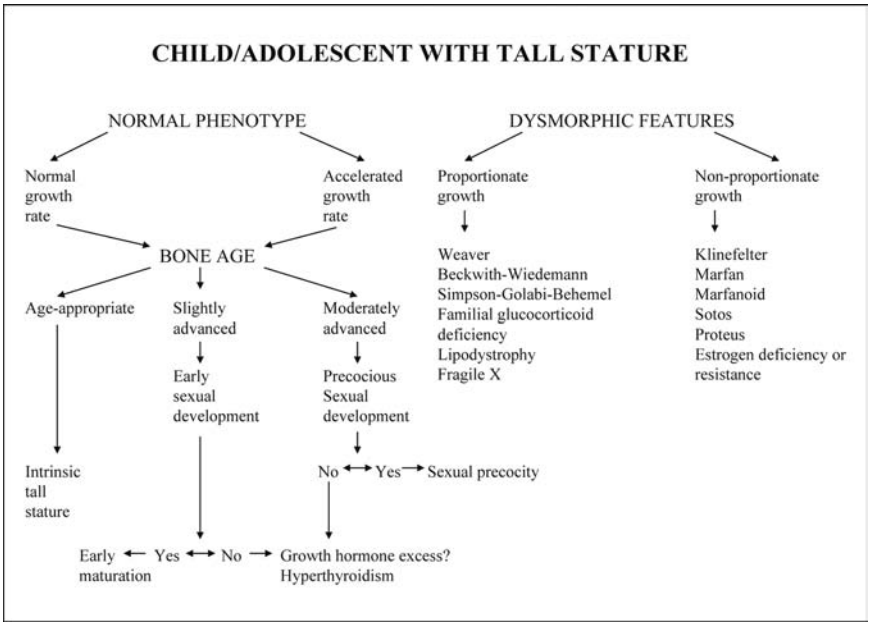

Figure 3 Evaluation of the excessively tall child or adolescent. When there are no dysmorphic characteristics, the differential diagnosis includes the child with constitutional tall stature, early sexual maturation or isosexual precocity, and growth hormone excess. Specific features guide the diagnostic evaluation of the tall patient with dysmorphic features. *Source:* Adapted from Ref. 102.

constitutionally tall males from 4.7 to 9.6 cm (104). The more immature the bone age when treatment is begun, the greater has been the calculated reduction in adult height. Although initially considered to be without complications, estrogen therapy for tall stature in girls has been associated with reversible protein S deficiency and hence a pre-thrombotic state, although few subjects are known to have had thromboembolic complications (106,107). Perhaps more meaningful is the report that estrogen treatment of tall female children and adolescents leads to impaired fertility in later life; as adults, these women are ''40% less likely to conceive in any given cycle of unprotected intercourse'' and have an increased need to employ fertility enhancing agents to conceive (108). In normally tall boys, it is even less necessary to consider intervention. High doses of testosterone are of variable and often limited effectiveness and carry the risk of severe acne (109,110). The use of a somatostatin analogue designed to suppress endogenous GH secretion has been reported to decrease adult stature marginally in tall subjects (111). One might envision the use of a GH receptor blocker (pegvisomant—designed to decrease GH activity in patients with hypersecretion of GH) for a similar purpose (112). One might design an ER agonist targeted to ERs of the chondrocyte that would accelerate the rates of chondrocyte maturation and growth plate fusion and foreshorten the tall subject in a manner similar to the current uses of ER antagonists and aromatase inhibitors to increase the (predicted adult) height of short children (113,114). Surgical approaches to limit growth include arthrodesis of the growth plates of the long bones of the legs and resection of the long bones themselves. Hopefully, these agents and procedures will be employed rarely and with great discrimination. Furthermore, there are no data to suggest that tall stature is a significant psychological or social handicap in the majority of subjects (115). Although the cancer and mortality rates of taller subjects may be greater than that of shorter persons, the rate of suicide is less in tall than in normally sized individuals (116,117).

GROWTH HORMONE EXCESS

In children and adolescents, excessive secretion of GH is suggested clinically by an accelerated rate of linear growth that is first noted during early childhood, with stature greater than that anticipated for mid-parental height, and continued growth when deceleration might be anticipated often as a consequence of gonadotropin deficiency and failure of sex hormone-induced maturation and fusion of the cartilaginous growth plate. With increasing duration of GH hypersecretion, other clinical manifestations of GH excess appear such as coarsening of facial features and mandibular prominence due to acral overgrowth and enlargement of the hands and feet. Headaches, diaphoresis, loss of visual acuity and narrowing of visual fields, and visceromegaly may develop over time.

Excessive GH secretion is most commonly due to a pituitary tumor composed of eosinophilic GH secreting cells derived from a monoclonal line of pituitary somatotrophs or somatomammotrophs that often display a somatic gain-of-function mutation in *GNAS* (Arg 201Cys, Gln227Arg). *GNAS* (OMIM 139320, chromosome 20q13.2) encodes the α_s subunit of the G-protein coupled receptor. Activating

mutations lead to loss of the intrinsic GTPase activity of $G\alpha_s$ and hence prolongation of the signal transduction system that stimulates adenylyl cyclase activity, cyclic AMP formation, PKA, and transcription of its target–*GH1*, the gene encoding human pituitary GH. In patients with the McCune–Albright syndrome (OMIM 174800) of large and confluent, irregularly bordered, ''coast of Maine'' café-au-lait spots, polyostotic fibrous dysplasia, and endocrinologic abnormalities including gonadotropin-independent sexual precocity, pituitary gigantism due to excessive GH secretion, thyrotoxicosis, and hyperadrenocorticism, there is a germline heterozygous mutation in *GNAS* (Arg201Cys) that is expressed in a mosaic pattern (118–120). In patients with the Carney complex (OMIM 160980) of cutaneous and cardiac myxomas, melanotic skin pigmentations, Sertoli cell tumors, hyperadrenocorticism, and GH hypersecretion, there are heterozygous, loss-of-function germline mutations in *PRKAR1A* (OMIM 188830, chromosome 17q23–q24) encoding the type I α regulatory subunit of cyclic AMP-dependent PKA thus permitting enhanced function of its catalytic subunit(s) in response to cyclic AMP. Patients with multiple endocrine neoplasia type I and an inactivating mutation in *MEN1* (OMIM 131100, chromosome 11q13) encoding the 613 aa tumor suppressor menin also develop GH secreting tumors. Neurofibromatosis type I (OMIM 162200, chromosome 17q11.2) is an autosomal dominantly transmitted disorder characterized clinically by multiple smoothly edged, ''coast of California'' café-au-lait spots, axillary freckling, cutaneous neurofibromas, and a variety of endocrinopathies including excess GH secretion in association with an optic glioma (vide infra). The tumor leads to hypothalamic dysfunction and decreased somatostatin-mediated inhibition of GH production (119,121).

Excessive central production of GHRH or its ectopic secretion by tumors leads to increased GH secretion and somatic overgrowth (122,123). First identified in a 20-year-old woman with 45X Turner syndrome and symptoms and signs of GH excess (increase in foot and hand sizes and in the numbers of skin tags), elevated levels of somatomedin C (IGF-I), and enlarged sellar volume due to somatotroph hyperplasia, GHRH may be secreted by pancreatic and carcinoid tumors, gangiocytomas, and neurocytomas (124). Tumors that secrete GH ectopically are quite unusual (125).

In patients with GH hypersecretion, GH concentrations may be normal or elevated in randomly obtained serum specimens, but concentrations of IGF-I and IGFBP-3 are almost always high for age and gender. Serial measurement of serum GH levels within a 24 hour interval may reveal ''normal'' values that vary little over time in place of the usually episodic pattern of pulsatile GH release interspersed with basal intervals of very little GH secretion and low GH concentrations. In response to an oral glucose load in normal subjects, GH secretion is suppressed because hyperglycemia evokes insulin secretion that in turn lowers IGFBP-1 values and increases free IGF-I concentrations that reciprocally suppress GH release (97). By contrast, in patients with GH-secreting tumors, after glucose ingestion serum GH concentrations are not suppressed as anticipated and may even rise. GH secretion in these patients may also increase paradoxically in response to gonadotropin and thyrotropin-releasing hormones, because tumor cell membranes often inappropriately express receptors for these hypothalamic factors. [Normal tall adolescents and patients with chronic hepatic cirrhosis or chronic renal insufficiency may also fail to suppress or display a paradoxical GH secretory response to hyperglycemia and/or thyrotropin releasing hormone (101).] In patients with ectopic secretion of GHRH, serum concentrations of this peptide are usually elevated. Serum concentrations of prolactin are also often high in subjects with GH secreting pituitary tumors, either because the tumor is secreting both peptides or because the lesion has interrupted hypothalamic-pituitary blood flow thus lowering dopaminergic inhibitory control of lactotroph function. The pituitary tumor is usually easily documented by cranial imaging studies while site specific imaging may localize tumors that are producing GHRH.

The primary management of the child with an isolated GH secreting pituitary tumor is transsphenoidal neurosurgical removal of the neoplasm. When this procedure is unsuccessful or cannot be attempted because of tumor characteristics (e.g., involvement of the optic chiasm or nerves, local invasion), radiation of the tumor may be helpful. Children with GH secreting lesions that are unresponsive to these modalities of treatment have been managed with dopamine agonists (cabergoline) and long-acting somatostatin analogues with variable success (118,126). The use of pegvisomant, a GH receptor antagonist that prevents dimerization of the GH receptor, has been quite useful in the management of adults with acromegaly (127). Although experience with this agent in children is limited, a 12-year-old girl with an inoperable pituitary GH-secreting tumor responded well to tumor site radiation and pegvisomant with reduction in serum GH and IGF-I concentrations, decline in growth rate, and symptomatic improvement (128). In a series of 26 adults with acromegaly unresponsive to long-acting somatostatin analogue alone, addition of pegvisomant to the therapeutic regimen led to normalization of IGF-I concentrations in 95% of patients (129).

While most adults with untreated panhypopituitarism are short in stature, of interest is the report of an adult with untreated panhypopituitarism associated with the pituitary stalk interruption syndrome whose height at 18 years of age was 147 cm but who continued to grow at the rate of 2 cm/yr over the next 25 years and at 43 years of age had a height of 193 cm and a bone age of 16 years (130). A number of patients with panhypopituitarism due to septo-optic dysplasia

or following resection of hypothalamic tumors or craniopharyngiomas or of unknown origin have achieved normal or even very tall stature without GH administration (131–133). It has been suggested that the enhanced growth of these subjects in the absence of GH is partly due to estrogen deficiency and failure of growth plate fusion. (A similar mechanism has been invoked to account for the tall stature of patients with a deficiency of aromatase activity or a loss-of-function mutation in the ER rendering the subject estrogen resistant.) In addition, many of these subjects are obese and hyperinsulinemic (probably a secondary event); leptin has also been suggested as a growth promoting agent in these patients as has prolactin which values have been variable but frequently normal; it is likely that as yet unidentified growth factors are present in these individuals as well. The growth pattern of such a patient is depicted in Figure 4.

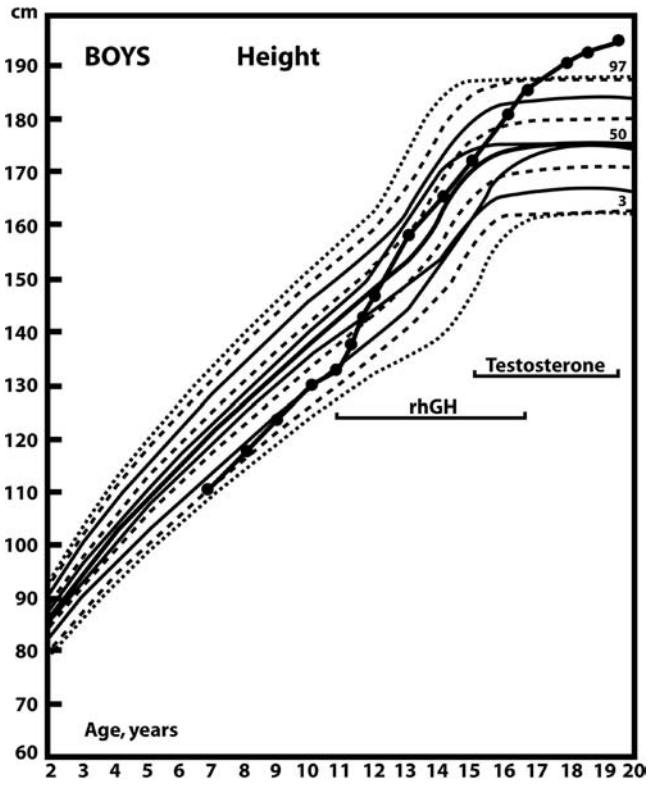

Figure 4 Growth without growth hormone (GH): At seven years of age this patient had a hypoglycemic episode; further evaluation revealed panhypopituitarism associated with septo-optic dysplasia. While receiving thyroxine and cortisol, he grew in height at the Tanner 10th percentile despite documented GH deficiency and low levels of insulin-like growth factor (IGF)-I and IGF-II. Administration of rhGH between 11 and 16.5 years of age resulted in acceleration of growth that continued with testosterone administration alone through 19 years of age. He had many acromegaloid features including large hands and feet, increased head circumference, and prognathism.

DISORDERS OF PUBERTAL DEVELOPMENT

Children and adolescents with either early or delayed pubertal development may be exceptionally tall. In the former, in response to endogenous sex hormones and secondarily increased secretion of GH, children with both complete and pseudoisosexual precocity grow rapidly and exceed statural norms by early or mid-childhood (134,135). However, in these children the rate of growth plate maturation exceeds the increase in height age resulting in early epiphyseal closure and growth arrest at an inappropriately young age leading to short stature as an adult. By contrast, children with accelerated growth in early childhood associated with only modest advance in bone maturation and pubertal onset within the early range of normal achieve their familial growth potential (Fig. 5A and B) (136). Precocious puberty may be isosexual or heterosexual (Table 1). Isosexual precocity in children may be (i) central in origin (i.e., the result of decline in inhibitory neurotransmitter input into hypothalamic neurons that secrete GnRH due to unknown factors or to congenital malformations of or acquired inflammatory, infiltrative, traumatic, or neoplastic insults to the central nervous system); (ii) pseudoisosexual—i.e., independent of central GnRH regulation and due to primary abnormalities of gonadal or adrenal sex hormone secretion or to exposure to exogenous sex hormones; (iii) incomplete—in which self-limited and non-progressive aspects of sexual development appear, e.g., premature thelarche, menarche, or pubarche. Children with heterosexual precocious puberty—e.g., virilization in a female and feminization in a male—also grow rapidly but fuse their epiphyses prematurely. A girl is considered to have sexual precocity if signs of pubertal development appear before eight years of age; in boys, onset of adolescent characteristics before 9 to 9.5 years of age is precocious (134,135). The evaluation and management of the sexually precocious child is discussed elsewhere (Vol. 2; Chap. 11).

The tall stature of the lad with Klinefelter syndrome (47XXY, 48XXYY) is likely due to both lack of growth plate fusion due to primary hypogonadism and to the presence of three or four copies of *SHOX* (vide supra). Adolescents and adults with primary gonadal insufficiency of various causes (but not that associated with Turner syndrome) are tall with eunuchoidal habitus, as growth plate fusion fails to occur. Similarly, patients with isolated deficiency of gonadotropin secretion (e.g., Kallmann syndrome—OMIM 308700) may be large.

HYPERINSULINISM

Infants born to women with poorly controlled diabetes mellitus during gestation are hyperinsulinemic due to the need to metabolize the large glucose load transmitted to the fetus by the mother, and

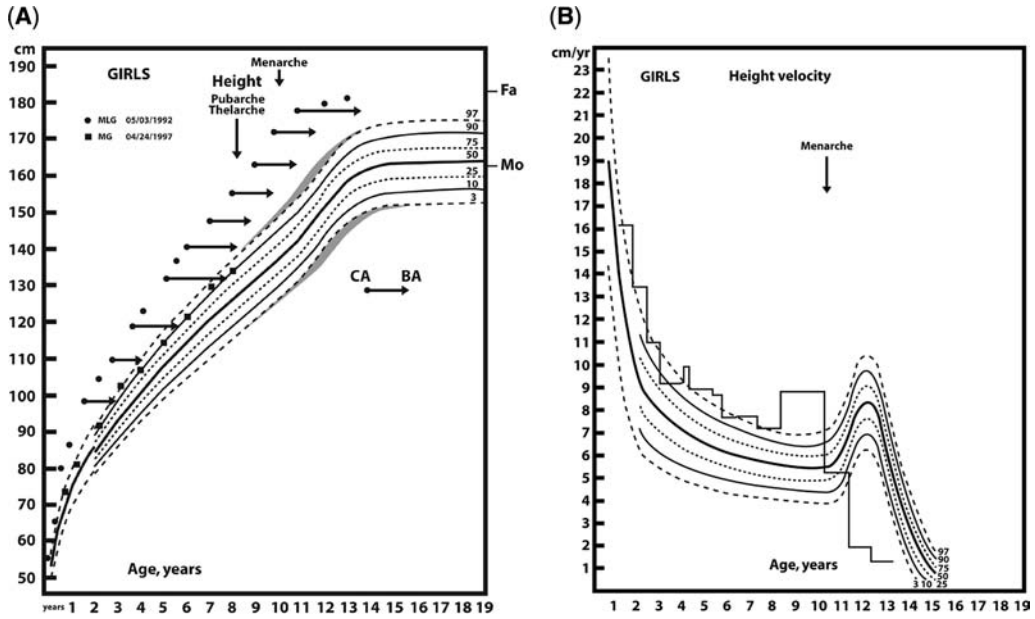

Figure 5 (**A**) Growth of sisters—one with accelerated childhood growth and early puberty (circles) and her prepubertal sister (squares); arrow head—height relative to bone age. (**B**) Growth rate of the child with tall stature and early puberty depicted in Figure 5A.

consequently they are excessively large at birth, primarily due to the accumulation of fat. Neonates with persistent hyperinsulinemic hypoglycemia of infancy (PHHI—OMIM 601820) have localized or diffuse pancreatic beta cell hyperplasia and are overgrown at birth. In this disorder, the inhibitory effect of a low blood glucose concentration upon insulin secretion is faulty due to autosomal recessive loss-of-function mutations in components of the inwardly rectifying potassium channels that regulate glucose-mediated insulin release. In many but not all neonates with PHHI, mutations in *ABCC8* (ATP-Binding Cassette, Subfamily C, Member 8—OMIM 600509, chromosome 11p15.1—also designated the sulfonylurea receptor or SUR) or *KCNJ11* (Potassium Channel, Inwardly Rectifying, Subfamily J, Member 11—OMIM 600937, chromosome 11p15.1—also designated KIR 6.2) have been identified (137). *ABCC8* encodes an ATP-sensitive protein with multiple membrane-spanning domains that is the regulatory subunit of a beta cell inwardly rectifying potassium channel (*KCNJ11*). When channel function is disrupted, potassium currents decline leading to accumulation of intracellular potassium, decreased polarization of the beta cell membrane, and opening of a voltage-gate calcium channel that results in increased levels of intracellular calcium thereby prompting insulin release and secondary loss of glucose-mediated control of insulin release. ABCC8 is the targeted receptor for sulfonylurea drugs. Neonates with the BWS are also hyperinsulinemic, but the somatic and visceral overgrowth of these patients is due also to excessive production of IGF-II, and alteration in other growth regulating factors (vide infra). Paradoxically, children with insulin-dependent diabetes mellitus are often tall when they initially present. This has been attributed to an interval of hyperinsulinism preceding exhaustion of the pancreatic beta cell. However, over time the growth of children with poorly controlled type I diabetes mellitus may be subnormal.

Obesity is the most common cause of tall stature in children. Obese children not only grow more rapidly and are taller than non-obese peers, but also their skeletal maturation is advanced prior to pubertal onset. In a study of Italian children, the growth velocity of obese boys and girls four to nine years of age was significantly greater than that of age and gender matched normal weight subjects; obese males ages 3 to 13 years and females ages 3 to 11.5 years were taller than non-obese peers (138). In this population, because of acceleration of ''physiologic maturity'' as reflected by an advanced bone age, their pubertal growth spurt was earlier but attenuated and their near-adult height was lower than that of normally weighted individuals. However, in general obese subjects achieve an adult stature that is within or above that expected for their parental heights.

The rate of growth of obese children is relatively rapid despite low serum concentrations of GH measured serially in sequentially collected specimens over 24 hours or after pharmacologic stimulation, reflecting in part the increased clearance of GH characteristic of obesity (139). Serum concentrations of IGF-I are variable in obese children but are usually within the normal range for age and gender as are levels of IGFBP-3 and acid label subunit (140,141).

Reduced levels of IGFBP-2 and a lower IGFBP-2/IGF-I ratio in obese children suggest increased bioavailability of IGF-I (142). Concentrations of GHBP are higher in obese children compared to normal weight controls, implying possible ''up-regulation'' of GHR and augmented GH sensitivity in obese subjects (143). Of importance also in the pathophysiology of the accelerated growth of obese children is their hyperinsulinism.

Although childhood obesity is most often exogenous (i.e., caloric intake exceeds caloric utilization) and not associated with underlying disease, when combined with developmental delay and other neurologic abnormalities, the possibility of a mitochondrial disorder in energy generation should be considered; two patients with normal in utero and postnatal growth, tall stature in childhood and near adult height greater than +2.5 SD above normal and a mutation in the mitochondrial ATPase 6 gene (*MTATP6*—OMIM 516060) have been described (144). Obesity does not necessarily lead to increased growth because it may be associated with GH deficiency and growth retardation due to congenital or acquired hypothalamic insults—e.g., Prader–Willi syndrome (OMIM 176270, chromosome 15q12), radiation, trauma, or tumors.

FAMILIAL GLUCOCORTICOID DEFICIENCY

Infants with FGD, an autosomal recessive disorder due to insensitivity to adrenocorticotropin (ACTH), manifest hypoglycemia, feeding problems, and hyperpigmentation. As children, they are tall yet at risk for hypoglycemia precipitated by ''stress'' of various etiologies. These patients are hypocortisolemic and cortisol levels do not increase in response to exogenous ACTH; their plasma ACTH concentrations are elevated. Because mineralocorticoid production is normal, patients with FGD are eunatremic and eukalemic. Approximately 25% of patients with FGD have a loss-of-function mutation in *MC2R* (OMIM 607397, chromosome 18p11.2), the gene encoding the ACTH G-protein coupled receptor (FGD type 1—OMIM 202200). Loss-of-function mutations in *MRAP* (OMIM 609196, chromosome 21q22.1), a 6 exon gene that encodes MC2R Accessory Protein, a 172 aa polypeptide, have also been found (FGD type 2—OMIM 607398). *MRAP* aids in the processing and trafficking of the ACTH receptor from the endoplasmic reticulum where it is generated to the plasma membranes of glucocorticoid-producing cells in the zona fasciculata of the adrenal cortex where it is inserted (145). Identified abnormalities in *MRAP* include splice site deletions and nucleotide substitutions in intron 3 and missense and nonsense mutations. Yet a third chromosome site (8q11.2–q13.2) has been detected in other subjects with FGD and designated FGD type 3 (OMIM 609197); the mutated gene at this location has not been identified as yet, but presumably encodes a product that is involved with the function of the ACTH receptor. The tall stature of these patients may be due to lack of the growth inhibiting effects of cortisol (vide supra) and perhaps to the absence of adrenal androgens and their potential maturational effect on the cartilage growth plate. Paradoxically, however, skeletal maturation is often advanced in these subjects, while gonadarche occurs at an appropriate chronologic age (102). Thus, as adults these subjects may not be excessively tall. ACTH resistance is also encountered in the ''Achalasia–Addisonianism–Alacrima'' syndrome (AAAS—OMIM 231550), a disorder due to loss-of-function nonsense (Arg312ter, Arg478ter), missense (Gln15Lys, Ser263Pro), and splice site (introns 4, 11, and 14) mutations in *AAAS* (OMIM 605378, chromosome 12q13). Within its 16 exons, *AAAS* encodes a 547 aa protein (termed aladin) that is normally expressed in central and peripheral nervous systems as a member of the nuclear pore complex of proteins that is important in assisting transport between the cytoplasm and nucleus (146). In children with peripheral resistance to glucocorticoids due to defects in the glucocorticoid receptor (GCCR—OMIM 138040, chromosome 5q31), excessive synthesis of adrenal androgens leads to development of isosexual precocity in boys and heterosexual precocity in girls and transiently tall stature in childhood (147).

OVERGROWTH SYNDROMES

Overgrowth syndromes have been primarily classified by their clinical characteristics and eponymically named for the investigator(s) who described them. As the reader will quickly discern, there is substantial clinical similarity among many of the syndromes. With increasing understanding of the fundamental pathogenesis of the various overgrowth syndromes and the identification of their genetic bases, a classification based on underlying etiology rather than clinical characteristics becomes potentially feasible (Table 1). Thus, a mutation identified in one gene may be associated with one or more syndromes which may not appear to be clinically similar (clinical heterogeneity). Similarly, a clinical syndrome may often have more than one genetic basis (genetic or locus heterogeneity) as there is an apparent limit to the number of physical manifestations of disordered physiology; in addition, the clinical similarity of a specific syndrome in which several gene defects have been identified points to a genetically regulated pathway of development.

Sotos Syndrome/Cerebral Gigantism

Cerebral gigantism (OMIM 117550) or Sotos syndrome is characterized by in utero and postnatal overgrowth, acromegaloid facial features, and developmental delay (148). The abnormality is associated with haploinsufficiency of Nuclear receptor SET

domain-containing protein 1 (NSD1) (OMIM 606681, chromosome 5q35) in the majority of patients (vide infra) (149). Birth length exceeds +2 SD in 85% of neonates with clinically diagnosed Sotos syndrome (150). Mean natal length is greater in infants with mutations in *NSD1* (+1.9 SDs) than it is in clinically diagnosed subjects without an identified mutation (+1.0 SDs) (151). Birth weights do not usually exceed +2 SDs (152). Rapid linear growth is most apparent within the first year of life. During childhood occipito-frontal head circumference (OFC), height and weight are increased relative to norms and OFC remains enlarged in adults while height and weight move toward the means or stay in the upper ranges of normal (153). The stature of adults with cerebral gigantism exceeds target height by 11 cm in males and by 6 cm in females [mean (SD) adult height in males: 184.3 ± 6.0 cm, SDS 1.51 (± 0.08); females: 172.9 ± 5.7 cm, SDS 1.8 (± 1.17)] (99). Bone age is advanced in 75% of subjects with Sotos syndrome, accounting for the fact that as adults, they are not exceptionally tall. Key facial characteristics or ''gestalt'' include: sparse fronto-temporal hair, a forehead that is high and bossed, down-slanting palpebral fissures, and a pointed mandible that becomes more prominent over time (Fig. 6) (149,154,155). Mild to severe developmental delay is found in most affected individuals but may ameliorate with aging; mental retardation may be more severe in subjects with an *NSD1* mutation (vide infra) (151). Patients with cerebral gigantism may exhibit aggressive behavior (156). Associated findings include a highly arched palate and anomalies of the cardiovascular (patent ductus arteriosus, atrial septal defect), genitourinary, and central nervous systems (ventricular dilatation, prominence of the trigone, hypoplasia of the corpus callosum, septo-optic dysplasia, enlarged extracerebral fluid spaces), the latter associated with seizures and electroencephalographic abnormalities (154). There is an estimated 2% to 4% risk for tumor development in patients with Sotos syndrome including teratoma, hepatocellular carcinoma, Wilms tumor, ganglioglioma, and neuroblastoma (157). GH secretion and serum concentrations of IGF-I and acid labile subunit are generally normal in subjects with Sotos syndrome (158–160). Reduced plasma levels of IGF-II, IGFBP-4, and IGFBP-3 and accelerated proteolysis of IGFBP-3 compared to age matched norms have been observed in some subjects with Sotos syndrome (159).

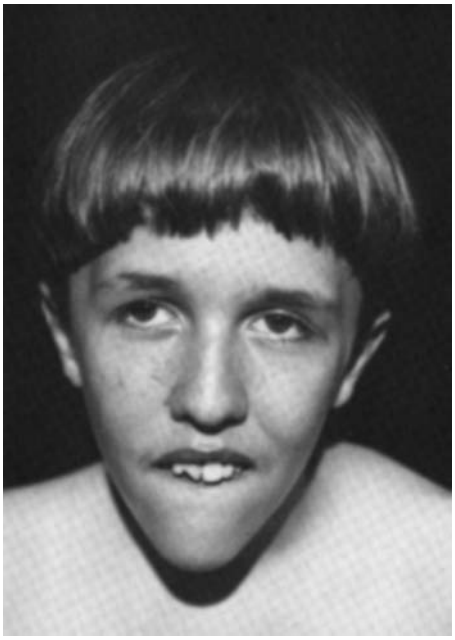

Figure 6 Facial ''gestalt'' of the child with Sotos syndrome (see text). *Source:* Reproduced with permission from Ref. 155.

In approximately 35–90% of subjects with cerebral gigantism, heterozygous loss-of-function germline mutations of *NSD1* have been found. Within its 23 exons, *NSD1* encodes a 2696 aa protein that serves as a factor that coregulates the transcriptional response that follows binding of a ligand to its respective response element; it also acts as a histone methyltransferase (149). *NSD1* enhances transcription of the gene encoding the AR, although the relationship of this activity to the clinical abnormalities characteristic of Sotos syndrome is obscure at present. The most common microdeletion of NSD1 associated with cerebral gigantism encompasses 1.9 Mb, includes the entire gene, and is more commonly found in Japanese than in white patients with this disorder (161–163). Unequal rearrangement (non-allelic homologous recombination) between highly homologous sequences of low-copy repeat regions that flank the proximal and distal breakpoints within *NSD1* appear mechanistically important in the deletion process (163–165). Most meiotic rearrangements show preference for the paternally derived chromosome 5; indeed all fathers of children with Sotos syndrome with deletion of *NSD1* harbor on their paternal chromosome 5q35 a heterozygous inversion of nucleotides flanking *NSD1*; the pathophysiological significance of this finding is uncertain (163,166). In white patients with Sotos syndrome due to microdeletions, the deletions vary in size. In addition to microdeletions, more than 100 intragenic inactivating mutations of *NSD1* have been identified; frameshift and nonsense mutations are widely distributed throughout the gene, while missense mutations cluster in the carboxyl terminal region between exons 13 to 23 (152,167). Patients with Sotos syndrome due either to point mutations or microdeletions in *NSD1* display overgrowth, developmental delay, and systemic anomalies; those with microdeletions tend to have major structural anomalies of the central nervous, cardiovascular, and genitourinary systems with severe developmental delay and less pronounced overgrowth; nevertheless, there is no clear-cut clinical phenotype–genotype correlation (154,168,169). Although the gene primarily responsible for Sotos

syndrome has been identified, the cellular pathophysiological consequences of haploinsufficiency of *NSD1* that result in cerebral gigantism are not known. In the mouse, although homozygous loss of *NSD1* is lethal, haploinsufficiency of *NSD1* does not affect viability, growth, or fertility (170).

The mutation in *NSD1* most commonly arises de novo in children of phenotypically normal parents, although some familial (autosomal dominant) cases have been reported. In no instance has germline mosaicism been observed. The recurrence risk for a second child with cerebral gigantism to be born to genotypically and/or phenotypically normal parents is usually low. However, a patient with Sotos syndrome due to an intragenic mutation (but not to a microdeletion of 5q35) has a 50% risk for having an affected offspring (152,154). That many patients with Sotos syndrome do not have a mutation in *NSD1* indicates that this syndrome is genetically heterogeneous.

The diagnosis of Sotos syndrome is based on clinical findings and confirmed by identification of mutations in *NSD1* that result in haploinsufficiency of its product. Mutations in *NSD1* have also been identified in some patients with Weaver and Beckwith–Wiedemann syndromes (vide infra). Patients with Sotos and Weaver syndromes share some characteristics, but may be differentiated by their individually distinctive facial features. Management of the patient with Sotos syndrome focuses on treatment of behavioral and developmental disabilities, monitoring of growth and body proportions, and surveillance for associated anomalies or neoplastic changes. Because the incidence of tumors in patients with Sotos syndrome is relatively low, no specific recommendations for tumor screening have been developed as yet (154).

Weaver Syndrome

Weaver (OMIM 277590) and Sotos syndromes may be allelic disorders because inactivating mutations of *NSD1* have been identified in patients with Weaver syndrome (167,171). Prenatal and postnatal overgrowth is characteristic of the Weaver syndrome. The average birth length of the male neonate with Weaver syndrome is 56 cm and that of girls is 53 cm; men achieve a mean adult height of 194.2 cm and women 176.3 cm (1). Affected children have a typically ''squared'' face with macrocrania, broad forehead, hypertelorism, large ears, elongated filtrums and retro- or micrognathia (as opposed to prognathism in subjects with Sotos syndrome). In addition, congenital hypotonia, developmental delay, hoarse, low-pitched cry, loose skin folds, inverted nipples, sparse hair, dysplastic nails, palmar hyperhidrosis, and inguinal herniae are commonly found in affected individuals (172). Macrocephaly with dilated cerebral ventricles, camptodactyly, clinodactyly, broad thumbs, and contractures of the hands and feet are common. Cervical spine anomalies are often present. Bone age is advanced with accelerated carpal bone development relative to that of the distal epiphyses; there is some epiphyseal mottling. Talipes equinovarus and talipes calcaneovalgus may be unilateral or bilateral. The iliac wings are low and broad, femoral metaphyses widened, and fourth metatarsals shortened. The disorder is primarily sporadic; however, parent-to-child (autosomal dominant) transmission has been recorded in a few instances (173). No gross chromosomal abnormalities have been identified, but mutations in *NSD1* have been found in approximately 33% of patients with Weaver syndrome (149).

Beckwith–Wiedemann Syndrome

The cardinal clinical findings in the neonate with BWS (OMIM 130650) are: macrosomia associated with in utero and postnatal somatic overgrowth with hemihyperplasia, macroglossia, and abdominal wall defects (exomphalos, omphalocele, umbilical hernia, diastis recti) (Fig. 7A) (Table 2) (154,174–176). In addition, there are anterior linear earlobe creases and posterior helical pits, visceromegaly, adrenocortical cytomegaly, renal hypertrophy with medullary dysplasia, and an increased incidence of embryonal tumors. The wide phenotypic spectrum of BWS that also includes subjects with isolated hemihyperplasia

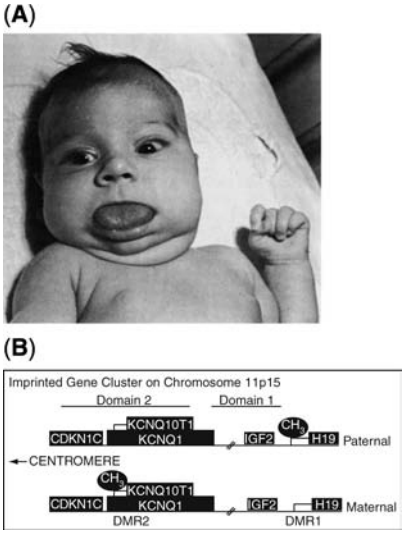

Figure 7 (**A**) Characteristic appearance of the newborn with the Beckwith–Wiedemann syndrome and macroglossia. *Source:* Reproduced with permission from Ref. 174. (**B**) The cluster of imprinted genes associated with the Beckwith–Wiedemann syndrome on chromosome 11p15.5. In imprinted domain 1, IGF2 is paternally expressed and H19 is maternally expressed. Loss of maternal methylation of DMR1 permits biallelic expression of IGF2. In imprinted domain 2, CDKN1C is maternally expressed. Inactivating mutations of CDKN1C or methylation of DMR2 prevents normal expression of this tumor suppressor gene. *Abbreviations*: IGF, insulin-like growth factor; DMR, differentially methylated region. *Source:* Reproduced with permission from Ref. 154.

Table 2 Characteristic Clinical Features in Neonates and Children with the Beckwith–Wiedemann Syndrome

Feature	Frequency (%)
Macroglossia	93
Pre and/or postnatal growth > 90th percentile	88
Abdominal wall defects	80
Ear creases or pits	76
Facial naevus flammeus	62
Renal abnormalities	59
Hypoglycemia/hyperinsulinemia	50
Hemihyperplasia	24
Cardiac malformation	7
Intestinal malrotation	5
Neoplasia	4
Developmental delay	4

Source: Adapted from Ref. 174.

is due to its genetic hetereogeneity (vide infra) (177). BWS occurs sporadically with an incidence of approximately 1 : 13,700 live births; males and females are equally affected. BWS may be suspected prenatally by accelerated increase in height of the uterine fundus and documented by prenatal fetal ultrasonography because fetal intrauterine growth is rapid in the second and third trimesters of gestation; in addition, the fetal abdomen is distended and polyhydramnios is often present (the consequence of reduced fetal swallowing due to macroglossia, omphalocele, and/or increased urine production) (178). Placental weight is often twice normal, and the umbilical cord may be unusually long (179). Occasionally, physical signs of BWS are absent at birth and appear later in life or the disorder may go unrecognized until a more severely affected family member is born (180). Within the first week after birth, hypoglycemia due to hyperinsulinemia occurs frequently because pancreatic islets are hypertrophic and beta cells hyperplastic. Excessive insulin secretion may also be related to deficiency of pancreatic islet somatostatin-producing δ cells (181,182). During childhood, heights exceed the 90th percentile in most BWS children, but adult stature is usually normal (177). Hemihyperplasia occurs in 13% to 24% of all patients with BWS, but body asymmetry is present in 49% of children who later develop a neoplasm. Approximately 7.5% to 10% of patients with BWS develop tumors. The increased risk for tumor development in BWS subjects is present primarily before eight years of age. Among the most common neoplasms in patients with BWS are Wilms tumor, hepatoblastoma, neuroblastoma, adrenocortical carcinoma, pheochromocytoma, thyroid carcinoma, leukemia, lymphoma, and rhabdomyosarcoma (183,184).

In 85% of patients, BWS occurs sporadically; it may also be transmitted as an autosomal dominant trait with variable penetrance primarily through the mother (177). Pathophysiologically, the BWS phenotype results from abnormalities of one of several genes on chromosome 11p15.5 that regulate intrauterine and postnatal somatic and organ growth. In many BWS subjects, the basic abnormality that leads to increased somatic growth appears to be augmented production of IGF-II due to overexpression of its encoding gene—*IGF2* (OMIM 147470, chromosome 11p15.5) and/or aberrations in other genes involved in the regulation of the cell cycle and cell division. *IGF2* is an imprinted gene (vide infra) that is normally expressed only by the *IGF2* allele on paternal chromosome 11 (185). No intrinsic gain-of-function mutations in *IGF2* itself have been identified in patients with BWS. Rather, overexpression of *IGF2* occurs when the gene is duplicated or when it is expressed by alleles on both of the patient's 11th chromosomes (186). Biallelic expression of *IGF2* may be due to loss of heterozygosity (e.g., disomy or isodisomy for paternal chromosome 11) or to loss of its imprinting pattern (vide infra) (187). If a fetus should acquire both 11th chromosomes from the father [uniparental (unipaternal) heterodisomy] or the donated maternal 11th chromosome should be lost and one paternal 11th chromosome duplicated [uniparental (unipaternal) isodisomy], then both paternal *IGF2* genes will be expressed leading to over-production of IGF-II. Because uniparental disomy usually occurs after fertilization due to somatic recombination, BWS patients with this anomaly have somatic mosaicsm that may account for their body asymmetry (154,188). Unipaternal disomy of chromosome 11p15.5 is present in 20% of BWS patients. In addition, a mutation in an imprinting center (IC) gene on maternal chromosome 11p15.5 (*H19*—vide infra) permits expression of maternal IGF2 as well as paternal IGF2 leading to increased IGF-II production and fetal and postnatal somatic overgrowth (Fig. 7B).

Epigenetics is the "study of heritable changes in gene function that occur without a change in the DNA sequence" (189). Epimutations are chemical modifications of DNA or histone proteins associated with DNA that change the three-dimensional structure of a gene but do not alter its nucleotide sequence. Epigenetic changes lead to tertiary structural alterations in gene DNA that make it more or less accessible to (transcription) factors that regulate gene expression. Chemical reactions that can change the tertiary structure of DNA include methylation or demethylation of nucleotides themselves, acetylation or deacetylation of histone proteins, and RNA silencing. Epigenetic changes are heritable and often parent specific. An imprinted gene is one that is normally expressed only by the allele donated by a specific parent; depending on the specific gene it may be expressed only by the mother's or the father's allele while expression by the opposite allele is repressed (189,190). The complex process by which parent-specific expression occurs is termed "imprinting." Gender-specific imprinting patterns are acquired, erased, maintained, or reestablished during development of germ cells, after fertilization, and even during early embryogenesis prior to and after implantation. Imprinted genes are grouped in chromosomal regions

termed ''imprinted domains'' that are governed in cis by an ''imprinting center'' (IC). Imprinting of a gene and its expression or repression are determined by its pattern of DNA methylation and chromatin acetylation (189). Mutations in an IC gene may lead to an error in the imprinting and consequently the expression of an associated gene.

There are two imprinted domains on chromosome 11p15 (Fig. 7B) (154,177). Imprinted domain 1 is sited near its telomere and harbors two imprinted genes—*IGF2* (paternally expressed, maternally imprinted) and *H19*—a maternally expressed, paternally imprinted gene that encodes a non-translated mRNA that may serve as a tumor suppressor. Transcription of *IGF2* is controlled by four promoters; promoter 1 is not usually imprinted while maternal promoters 2, 3, and 4 are silenced by DNA methylation (187,191). Although methylation often leads to suppression of gene expression, methylation of the differentially methylated region (DMR) 2 of exon 9 of *IGF2* increases its expression. Normally, paternal *H19* is methylated and silenced permitting paternal expression of adjacent *IGF2*. Expression of maternal *IGF2* is enabled when an ''insulator protein'' that normally binds within DMR1 to unmethylated DNA of maternal *H19* and inhibits transcription of *IGF2* is unable to do so (177). Thus, when maternal *H19* is methylated its expression is inhibited, while expression of *IGF2* is no longer repressed resulting in its biallelic expression. H19-dependent biallelic expression of *IGF2* is present in 2% to 7% of patients with BWS—primarily in sporadic cases. Imprinted domain 2 is centromeric to imprinted domain 1; it houses a number of imprinted genes but those specifically related to BWS are: *CDKN1C* (OMIM 600856), *KCNQ1* (OMIM 607542), and *KCNQ1OT1* (OMIM 604115—also termed LIT1). CDKN1C is a maternally express (paternally imprinted) gene that encodes the protein $p57^{Kip2}$, an inhibitor of CDK that depresses cell replication and is thus a tumor suppressor. Approximately 5–10% of patients with BWS harbor loss-of-function mutations in *CDKN1C*, mutations that are found most often in familial cases of BWS. *KCNQ1* is a maternally expressed (paternally imprinted) gene that encodes a voltage-gated potassium channel subunit. *KCNQ1OT1* is a paternally expressed (maternally imprinted) gene whose product is a non-coding RNA; its promoter is located within intron 10 of *KCNQ1*, the position of the IC in DMR2 of imprinted domain 2, a site critical for the maternal expression of *CDKN1C*. Maternal methylation of DMR2 is lost in 50% to 60% of subjects with sporadic BWS; this leads to loss of imprinting of *CDKN1C* and suppression of it expression resulting in loss of one component of cell-cycle regulation.

While the phenotype of the BWS patient can vary widely and phenotype–genotype correlation is not necessarily robust, there is a link between uniparental disomy for chromosome 11p15.5 and hemihyperplasia, perhaps due to the somatic mosaicism of these patients (vide supra). Subjects with mutations in *CDKN1C* have a higher incidence of abdominal wall defects than do patients with other genetic errors. However, the epigenotype (DNA methylation pattern) does relate to phenotype—neoplasia occurs with greater frequency in patients with altered DNA methylation of *H19*, while alterations in the DNA methylation pattern of *KCNQ1OT* are associated with increased frequency of abdominal wall defects and macrosomia (192). Inasmuch as mutations in *NSD1* have been identified not only in patients with Sotos syndrome (vide supra) but also in several subjects with BWS, it has been suggested that *NSD1* may be involved with imprinting at 11p15.5 (193). In transgenic mice, overexpression of *IGF2* leads to the BWS phenotype including in utero overgrowth, macroglossia, and visceromegaly (21). ''Knock-out'' of Cdkn1c results in anterior abdominal wall defects, adrenal enlargement, and renal medullary dysplasia. Thus, the BWS phenotype appears to be the result of both overexpression of *IGF2* and underexpression of *CDKN1C*.

Interestingly, multiple births, particularly monozygotic twinning, is quite common in BWS families. Most such monozygotic twins are female, and to date the offspring have always been discordant for BWS (189). It has been hypothesized that discordance is due to an error in maintenance of imprinting at DMR2 within imprinted domain 2, an epigenetic error that may itself predispose to twinning as well as to phenotypic discordance. In those pregnancies conceived with assisted reproductive technologies, there is a four-fold increase in incidence of the BWS occurring in one of monozygotic twins (189,194). The overall risk of conceiving a child with BWS by in vitro fertilization is approximately 1 : 3750.

BWS appears to be the mirror image of the Russell–Silver syndrome (OMIM 180860—intrauterine and postnatal growth retardation, body asymmetry, characteristic face with prominent forehead and small mandible, and sexual precocity). This disorder is associated with an imprinting error that leads to subnormal methylation of the DMR within exon 9 of *IGF2* and decreased production of IGF-II (195). Disordered imprinting of the *IGF2* promoters also has been reported in hepatocellular carcinoma and Wilms tumor (196,197).

The diagnosis of BWS may be suspected in utero by the rapidity of fetal growth and abnormal-abdominal wall formation. Postnatally, it is established by the clinical findings of excessive in utero and postnatal growth, omphalocele, visceromegaly, and hyperinsulinemic hypoglycemia. Cytogenetic identification of unipaternal disomy or detection of mutations (*CDKN1C*) or abnormal methylation patterns of other imprinted genes on chromosome 11p15.5 substantiate the diagnosis of BWS (177). BWS must be differentiated from other overgrowth syndrome—usually by clinical assessment. Urgent surgical management of the omphalocele and medical treatment of

hypoglycemia are mandatory. Long-term treatment of hypoglycemia in the BWS subject has been attained with the use of somatostatin analogues (181). Reduction of macroglossia may be considered if enlargement interferes with respiration, feeding, or speech. Inasmuch as patients with BWS are at high risk for tumor development, periodic screening for these lesions is imperative (177). A baseline magnetic resonance imaging study of the abdomen is recommended. As there is a greatly increased risk for hepatoblastoma and Wilms tumor, and tumor development is often heralded by nephromegaly, abdominal ultrasonography every three months for the first seven years of life has been recommended. Serum concentrations of alpha-fetoprotein should also be measured frequently.

Perlman Syndrome

The Perlman syndrome (OMIM 267000) consists of in utero overgrowth, developmental delay, renal hamartomas, nephroblastomatosis, and propensity for development of Wilms tumor, islet cell hyperplasia with neonatal hyperinsulinemic hypoglycemia, and facial characteristics such as upswept anterior scalp hair, fullness, depressed nasal bridge, anteverted upper lip, macroglossia, mild micrognathia, and gaping mouth (198). Although some of its clinical features resemble those of BWS, it is distinguished by it facial characteristics and symmetrical body proportions. Usually sporadic, Perlman syndrome has been observed rarely in siblings. Its pathogenesis is unknown, although in some patients cytogenetic anomalies of chromosome 11 have been identified.

Simpson–Golabi–Behmel Syndrome

The Simpson–Golabi–Behmel syndrome, type 1 (SGBS1—OMIM 312870) is an overgrowth syndrome associated with mutations in the extracellular proteoglycan—glypican-3 (*GPC3*–OMIM 300037, chromosome Xq26). Its clinical manifestations vary widely from mild to severe and include: fetal and postnatal overgrowth with tall adult stature, stocky habitus, microcephaly, distinctively coarse face (prognathism, upturned nasal tip, widened nasal bridge, macroglossia with tethering, cleft lip), and broad, short fingers, and hands. Associated anomalies include cleft palate, grooved tongue and lower lip, pectus excavatum, congenital heart anomalies (pulmonic stenosis, ventricular septal defect), renal cysts, and gnarled cup-shaped ears. A substantial number of infants die of cardiac arrhythmias, and the risk for development of embryonal (Wilms, hepatoblastoma) tumors is increased. The disorder is transmitted as an X-linked dominant trait that affects both genders. The Simpson–Golabi–Behmel and Beckwith–Wiedemann syndromes share clinical characteristics including: macrosomia, macroglossia, visceromegaly, cleft palate, earlobe creases, herniae, neonatal hypoglycemia, and risk for embryonal tumors. In addition, they may be related pathogenetically (vide infra). In one family, *SGBS* has been mapped to chromosome Xp22 and termed *SGBS2* (OMIM 300209).

Loss-of-function macro- and micro-deletions of GPC3 have been associated with SGBS type 1 in approximately 40% of patients reflecting the genetic heterogeneity of this syndrome. In addition, intragenic mutations resulting in frameshift, nonsense, and splice site mutations have been found in patients with SGBS type 1. *GPC-3* is selectively expressed in embryonic mesodermal tissues where it interacts with IGF-II, implying a pathophysiological relationship between SGBS and BWS (vide supra). *GPC3* is an 8 exon gene that encodes GPC-3, an extracellular 580 aa proteoglycan that is thought to control the growth of mesodermal embryonic tissue perhaps acting in concert with IGF-II with which it forms a complex, although it does not affect IGF signal transduction (199,200). In a mouse model generated by "knock-out" of *GPC*3, clinical features of SGBS including somatic overgrowth were reproduced. In mice with the deletions of both *GPC3* and *H19*, the latter permitting biallelic expression of *IGF2* by relaxation of imprinting, the phenotype was exaggerated. Further studies of double mutant mice indicated that *GPC-3* did not sequester IGF-II or IGF-I but suggested that the effects of *GPC-3* and IGF on cell proliferation and somatic growth converged at a common downstream site (199). GPCs are anchored to cell membranes through glycosylphosphatidylinositol and function in part by interacting with and enhancing the Wnt5a/JNK signaling pathway, a signal transduction system that regulates cell proliferation (200). Because inactivating mutations of *GPC3* lead to excessive growth, it may be inferred that the normal function of *GPC-3* is to restrain cell proliferation. Identification of the gene located at chromosome Xp22 that has been associated with SGBS type 2 is anticipated to reveal a product that interacts above or below the site of *GPC-3* action.

The diagnosis of SGBS depends on the clinical findings and their differentiation from other overgrowth syndromes. In addition, there are radiologic changes that include flaring of the iliac wings, increased number of vertebrae, hemivertebrae, and aberrations of the limbs. The diagnosis may be confirmed by identification of an inactivating mutation in *GPC3*, but since the disorder is genetically heterogeneous the absence of a mutation does not necessarily negate the diagnosis. Because neonates with SGBS are at risk for death due to cardiac arrhythmia, cardiac evaluation and monitoring is necessary. Periodic assessment for tumor development is also essential.

PTEN Hamartoma-Tumor Syndrome (Bannayan–Riley–Ruvalcaba and Cowden Syndromes)

The PTEN hamartoma-tumor syndrome has variable phenotypic patterns of expression (154). The Bannayan–Riley–Ruvalcaba syndrome (BRRS—OMIM 153480) is also termed the syndrome of "Macrocephaly,

Multiple Lipomas, and Hemangiomata.'' It is an autosomal dominantly transmitted, overgrowth/hamartoma syndrome with variable penetrance that has been associated with germline loss-of-function mutations of *PTEN* (*PTEN—OMIM* 601728, chromosome 10q23.31). This 9 exon gene encodes a 403 aa polypeptide with a protein tyrosine phosphatase domain in its fifth exon (vide infra) (201). Clinical features include increased intrauterine growth, segmental overgrowth, macrocephaly, prominent corneal nerves, pseudopapilledema, strabismus, pigmented macules on an enlarged penile shaft (''speckled penis''), lipomas, hemangiomas, and angiolipomas of skin, skeletal muscle, viscera, and brain, hamartomatous polyps of the ileum and colon, lipid storage myopathy, mild to severe developmental delay, and propensity to develop autoimmune thyroid disease. Birth length and weight exceed their respective 97th percentiles with birth weights frequently above 4000 g (202). Macrocephaly persists into adulthood, while height and weight normalize by three to eight years of age (99). Hypotonia and gross motor and cognitive speech delays are recognized in 70% of BRRS patients (203). Hamartomatous intestinal polyps lead to chronic anemia, diarrhea, protein losing enteropathy, and/or intusseception of the small bowel (157). The possibility of increased risk of malignancy has been debated as the condition clinically and genetically overlaps with Cowden disease (vide infra). An intracranial germinoma secreting human chorionic gonadotropin and resulting in isosexual precocious puberty in a lad with BRRS has been observed (204). Intracranial meningiomas may also occur. A second phenotypic expression of the *PTEN* hamartoma-tumor syndrome is Cowden disease (Lhermitte–Ducos syndrome—OMIM 158350)—an autosomal dominant disorder characterized by normal somatic growth, macrocephaly, multiple hamartomas, oral papillomatosis, palmoplantar hyperkeratosis, benign tumors of the hair follicle (trichilemmomas), and thyroid, breast, and endometrial neoplasms (205).

Germline mutations of *PTEN* have been identified in up to 81% of subjects with Cowden disease and approximately 60% of those with BRRS (201,206). Indeed, the overlapping complex of abnormalities found in the two disorders suggests that they might well be amalgamated into one nomenclature—PTEN MATCHS for mutations of ''PTEN associated with microcephaly, autosomal dominant (transmission), thyroid disease, cancer, hamartomas, and skin abnormalities.'' BRRS and Cowden disease are heterozygotic allelic syndromes; in occasional patients with distinctive features of one or the other syndrome, the identical mutation of *PTEN* has been detected (e.g., Arg233ter)—evidence of the important role played by other components of an individual's genetic milieu in the clinical expression of disease (205). Both disorders have occurred in the same family and some BRRS patients may develop characteristics of Cowden disease as they age (207,208). *PTEN* encodes a phosphatase with tumor-suppressive activity that dephosphorylates both lipid and protein substrates. *PTEN* normally dephosphorylates (active) phosphatidylinositol (4–6) trisphosphate to (inactive) phosphatidylinositol (5,6) bisphosphate. Thereby, it negatively regulates the phosphoinositol-3-kinase/PKB/Akt signaling pathway stimulated by cell growth factors (e.g., IGF-I) and thus indirectly controls the cell cycle and apoptosis (209). *PTEN* also negatively affects cellular interaction with extracellular matrix. Heterozygotic inactivating mutations of *PTEN* result in the accumulation of phosphatidylinositol (4–6) trisphosphate that then activates PKB/Akt leading to unbridled cell proliferation and movement (210). In patients with BRRS, interstitial deletions of chromosome segment 10q23.2–q24.1 with loss of *PTEN* have been observed as have heterozygotic inactivating germline mutations within *PTEN*. The Arg233ter mutation has been found in patients with either BRRS or the Cowden syndrome. Germline mutations in *PTEN* associated with BRRS cluster in exons 6 and 7, while those missense, nonsense, and splice-site mutations associated with Cowden disease are scattered throughout exons 2 to 8 (207). Mutations in the *PTEN* promoter region have also been described in subjects with either BRRS or Cowden disease in whom the exonic (coding) structure of *PTEN* was normal (211). Mutations of PTEN have also been identified in patients with autism and macrocephaly (OMIM 605309) but without other stigmata of BRRS. Somatic mutations in *PTEN* have been observed in prostatic neoplasms, malignant melanomas, and neural tumors.

The diagnosis of BRRS is suggested by the clinical findings and confirmed by the identification of a loss-of-function mutation in *PTEN*. However, 40% of subjects with BRRS do not have a detectable mutation in this gene—consistent with the genetic heterogeneity of this syndrome. After the diagnosis of BRRS, careful monitoring of the growth and development of the child is mandatory with periodic assessment for the development of functional tumors or other symptomatic lesions.

Proteus Syndrome

The Proteus syndrome (OMIM 176920) is characterized by postnatal segmental, disproportionate limb overgrowth at times with hemihypertrophy, enlargement of the hands, feet, and digits, macrocephaly with disproportionate growth of various cranial bones that is relentless and persistent, enlarged vertebrae, hypertrophy of the skin with epidermal nevi, plantar cerebriform subcutaneous connective tissue nevi, vascular malformations, splenomegaly, intraabdominal lipomas, and neoplasia of the gonads and other tissues (212). Cerebriform connective tissue nevi are composed of deep grooves and gyrations reminiscent of the surface of the brain that are present on the soles of the feet and palms of the hands and thorax; their presence is pathognomonic of Proteus syndrome. Malformations of the brain with developmental delay

and seizures occur. Radiologic findings include: asymmetric limb growth, macrodactyly, hyperostosis, calvarial thickening, and vertebral anomalies with scoliosis (213). This syndrome takes it name from the Greek god who could change his shape at will—Proteus, "the polymorphus," and may be mild or severe. The relentless disproportionate growth of cranial and other bones is persistent and rapid and can lead to substantial disfiguration. Affected subjects are at increased risk for deep vein thrombosis, pulmonary embolism, and sudden death. The mechanism of this complication has not been clarified as yet. The syndrome usually occurs sporadically, but is rarely familial. It occurs more commonly in males than females.

It is hypothesized that the Proteus syndrome is the result of somatic tissue mosaicism for a dominant gene (as yet unidentified) that would ordinarily be lethal when fully expressed in all cells (212,214). Approximately 20% of patients with clinically diagnosed Proteus syndrome have been reported to have a heterozygous loss-of-function mutation of *PTEN*, including 507delC and Arg335ter, the latter a mutation also identified in patients with Cowden disease and BRRS, and Arg130Gln—recorded as well in patients with Cowden disease. That many patients with the Proteus syndrome do not have a mutation in *PTEN* signifies the genetic heterogeneity of this syndrome. However, Cohen (212) feels quite strongly that those "Proteus-like" patients in whom *PTEN* mutations have been found do not have classical Proteus syndrome.

The diagnosis of Proteus syndrome is dependent on accurate identification of its protean manifestations (Table 3). It must be differentiated from neurofibromatosis type I and BRRS. Management is clearly difficult and dependent on the specific clinical problem(s) of the affected subject. Awareness of the risk of deep vein thrombosis and propensity for tumor development mandate periodic assessment for these complications.

Table 3 Diagnostic Criteria for the Proteus Syndrome

General criteria: mosaic distribution of lesions, sporadic occurrence, progressive course
Specific criteria
Cerebriform connective tissue nevus (pathognomonic)
Two findings required
Epidermal nevus
Asymmetric disproportionate growth of limbs (arms, legs, hands, feet, digits), hyperostoses of skull or external auditory meatus
Megalospondylodysplasia
Enlargement of spleen and/or thymus
Ovarian cystadenoma, parotid monomorphic adenoma
Three findings required
Dysregulated adipose tissue—lipomas, regional lipohypoplasia
Vascular malformations—capillary, venous, lymphatic
Lung cysts
Facial characteristics: dolichocephaly, long face, down-slanting palpebral fissures, ptosis, low nasal bridge, anteverted nostrils, open mouth at rest

Source: Adapted from Ref. 212.

Nevo Syndrome

The Nevo syndrome (OMIM 601450) is an autosomal recessive disorder characterized by intrauterine and postpartum overgrowth, prominent forehead, kyphosis, spindly fingers, wrist drop, volar edema, joint laxity, and hypotonia primarily in subjects of Middle Eastern origin (215). Other physical findings may include dolichocephaly, highly arched palate, large low set ears, cryptorchidism, and single palmar crease. The excessive growth of patients with Nevo syndrome may be related to hyperinsulinemia because these subjects are often insulin resistant; serum levels of GH and IGF-I are low in patients with this syndrome. Clinically, patients with the Nevo syndrome bear some resemblance to those with kyphoscoliotic Ehlers–Danlos syndrome type VIA and have similar mutations in PLOD1 (Procollagen-lysine, 2-oxoglutarate 5-dioxygenase—OMIM 153454, chromosome 1p36.3–p36.2) (216). PLOD1 is a homodimeric enzyme that catalyzes the hydroxylation of lysine in collagen subunits, a reaction essential both for further attachment of carbohydrates (galactose) and for stabilization of intermolecular collagen pyridinoline crosslinks. A number of inactivating deletions and missense and nonsense mutations in PLOD1 have been found in patients with Ehlers–Danlos syndrome type VIA including Arg319ter, Gly678Arg, and Tyr511ter. The Arg319ter mutation in PLOD1 has also been demonstrated in subjects with the Nevo syndrome; deletion of exon 17 has also been found in Nevo subjects. Thus, the Nevo and Ehlers–Danlos type VIA syndromes are likely allelic. The diagnosis of the Nevo syndrome is suspected clinically and confirmed when possible by identification of a loss-of-function mutation in PLOD1. Management is symptomatic and directed to the adverse manifestations of the disorder.

Marfan Syndrome

Marfan syndrome (MFS—OMIM 154700) is a highly penetrant but clinically diverse, autosomal dominant disorder of connective tissue due to loss-of-function mutations in FBN1 (OMIM 134797, chromosome 15q21) (217). These mutations impair synthesis of fibrillin-1, an essential glycoprotein component of the microfibrillar portion of elastic fibers. The disorder occurs with a prevalence of 2 to 3 per 10,000 live births. Affected individuals are exceptionally tall and slim with elongated limbs, fingers, and toes (arachnodactyly). Cardiovascular, ocular, central nervous system, and skeletal pathology predominate. Physical findings vary substantially between families as well as among affected members of the same family group, potentially reflecting quantitative variations in biosynthesis and deposition of fibrillin-1 (218).

Overgrowth in MFS begins in utero. Mean birth lengths approximate the normal 90th percentiles in both male and female neonates. Rapid growth velocity persists throughout childhood and adolescence

resulting in a mean adult height of 193 cm in MFS males and 175 cm in MFS females (219). Seventy-five percent of adult stature is achieved by nine years of age in boys and by 6.5 years in girls. Near adult height is achieved by 15.8 years in boys and by 14.8 years in girls. Adolescents with MFS achieve their amplified peak height velocities 2.2 to 2.4 years earlier than does the general population, while girls experience menarche a bit early than do their normal counterparts.

FBNl is composed of 65 exons with 8616 nucleotide base pairs that span 200 kb (220). Its product, profibrillin-1, is a 350 kDa, cysteine-rich glycoprotein that undergoes posttranslational processing of amino and carboxyl terminals into fibrillin, a component of the collagenous matrix of microfibrils (221). Microfibils are present in three homologous forms (fibrillin-1,–2,-3), comprised of a variety of (mostly) calcium binding epidermal growth factor-like motifs, calcium binding being integral to fibrillin structural integrity (222). Tropoelastin deposition on the fibrillin matrix supports formation of elastic fibers that anchor dermis to epidermis and suspend the lens of the eye to the ciliary body (221). In elastic arteries such as the aorta, fibrillin-1 is present in the arterial wall while fibrillin-2 predominates in the media (223). Dysfunctional elastin activity may result from disruption of microfibril assembly by mutant fibrillin or from increased susceptibility of abnormal fibrillin to proteolysis (223,224). Approximately 300 mutations of *FBNl* have been described, most unique to a single child or family. They include deletions, insertions, frameshift, missense (Arg1137Pro, Cys2221-Ser), and nonsense (Trp2756ter) mutations. Fibrillin-2 is encoded by *FBN2* (OMIM 121050, chromosome 5q23–q31); mutations in *FBN2* are associated with contractural arachnodactyly, a disorder with some features of MFS but without its ocular or cardiovascular complications. *FBN3* (OMIM 608529) maps to chromosome 19p13.3–p13.2 (223).

The clinical diagnosis of MFS is based upon criteria established as the Ghent Nosology (Table 4) (225) and requires the presence of two major manifestations in separate organ systems with the involvement of a third organ system, or a major abnormality of one organ system with minor involvement of a second organ system in an individual with a positive family history for MFS (223). The diagnosis may be further confirmed by identification of a mutation in *FBN1*. Clinical management is directed by the manifestations of the disease exhibited by the individual subject, particularly those that affect the cardiovascular system. The principal cause of premature death in patients with MFS is progressive dilation of the aortic root and ascending aorta resulting in aortic regurgitation, leading to rents in aortic endothelium and then to aortic dissection (226). Increased aortic diameter and a family history of aortic dissection are significant risk factors for this complication (227). Mitral valve disease, however, may be the earliest cardiovascular manifestation of MFS. In children and adolescents with MFS, aortic diameter is assessed by transthoracic echocardiography

Table 4 Clinical Criteria for Marfan Syndrome

Major	Minor
Cardiovascular	
Aortic root dilatation	Mitral valve prolapse
Dissection of ascending aorta	Calcifications of mitral valve (<40 y)
	Dilatation of pulmonary artery
	Dilatation/dissection of descending aorta
Ocular (2 required)	
Ectopia lentis	Flat cornea
	Myopia
	Elongated globe
Central nervous system	
Lumbosacral dural ectasia	
Skeletal (4 required)	
Pectus excavatum requiring surgery	Moderate pectus excavatum
Pectus carinatum	High narrowly arched palate
Pes planus	Typical face
Wrist and thumb sign	Joint hypermobility
Scoliosis >20° or	
Arm span-height ratio >l.05	
Protrusio acetabulae	
Diminished elbow extension (<170°)	
Pulmonary	Spontaneous pneumothorax
	Apical bulla
Skin	Unexplained striae
	Recurrent or incisional hernia

Note: The diagnosis of MFS is established by the presence of two major manifestations in different systems with involvement of a third organ or the presence of a major abnormality in one organ system, a minor abnormality in a second system, and a positive family history for this disorder.
Source: Adapted from Ref. 225.

yearly or more frequently if aortic diameter is increasing (228). Affected individuals must avoid contact sports and vigorous isometric exercise. β-Adrenergic blockade may slow the rate of aortic dilatation and protect against dissection (229). Prophylactic replacement of the aortic root when its diameter exceeds 55 mm has contributed to prolonged survival. Careful monitoring of spinal curvature is recommended in order to detect scoliosis at an early stage, because bracing may be required for progressive curvature and surgery for curves exceeding 40°. Surgical repair of pectus excavatum is sometimes indicated if respiratory compromise is significant. Periodic monitoring of visual acuity is important to avoid amblyopia. An upwardly dislocated lens may require extraction and implantation of an artificial lens (230).

In pubertal girls with MFS, growth reductive therapy with ethinyl estradiol at a dose of 0.3 mg daily for several years has been relatively ineffective in decreasing adult stature (231). With a very large dose of testosterone esters (500 mg IM every two weeks), a mean reduction in adult height of 5.5 cm (95% confidence interval 0.96–10.09 cm) as assessed by the Bayley–Pinneau method of adult height prediction has been reported; however, utilizing the Tanner–Whitehouse 2 method of adult height prediction in the same population of MFS males, a non-significant

loss of 2.2 cm was recorded (231). As with constitutionally tall children (vide supra), prediction of adult heights in MFS subjects proved difficult. In the experience of the present authors, administration of testosterone in order to foreshorten males with MFS is not recommended because of the possibility for the development of severe acne.

Homocystinuria

Homocystinuria (OMIM 236200, chromosome 21q22.3), an inborn error of methionine metabolism due to deficiency of cystathionine β-synthase (encoded by CBS), occurs with a prevalence of approximately 1 : 200,000 live births. Patients present with overgrowth and a Marfanoid-like phenotype characterized by dolichostenomelia, arachnodactyly, and tall stature, often with eunuchoid proportions; joint mobility may be limited (232). Ectopia lentis may occur in any direction but is commonly downward in contrast to the upwardly dislocated lens of MFS. Other ocular findings include iridodonesis, myopia, glaucoma, retinal detachment or degeneration, and cataracts (233). Children may also manifest blotchy erythematous skin (livedo reticularis), thining of the hair and skin, highly arched palate, crowded protruding teeth, inguinal hernia, heptatic steatosis, and myopathy. Mental retardation, usually severe, is found in as many as 50% of patients with intelligent quotients ranging from 30 to 75 units. Hypercoagulability leading to life-threatening thromboembolism, especially involving the brain, may occur in infancy. Postoperative hypercoagulopathy is of particular concern. By 30 years of age the cumulative risk of thromboembolic events exceeds 50%, and mortality by early adulthood reaches 4% in pyridoxine responders and 23% in pyridoxine non-responders (vide infra). The hypercoaguable state of homocystinuria is expressed when the patient also has a factor V Leiden mutation. Osteoporosis is often seen in affected children and adolescents, and scoliosis and kyphosis are common. Other skeletal findings include abnormalities of the chest wall and genu valgum. The mechanism of excessive growth in patients with homocystinuria is unknown. A direct growth promoting effect of homocysteine has been suggested in some studies but unconfirmed in others (234). In vitro, homocysteine has been shown to increase CDK, a promoter of mitosis (235). Clinical manifestations of homocystinuria may be related in part to errors in the tertiary formation of collagen, glycoproteins, and other molecules that require disulfide cross-links. Inactivating mutations in CBS may affect the activating or catalytic domains of the product. The Gly307Ser is a pyridoxine non-responsive mutation, the Ile278Thr and Lys384Glu mutations, among others, are vitamin B6 responsive.

Diagnostically, urinary excretion of homocysteine and methionine is increased as are serum concentrations of methionine and homocysteine, while serum levels of cysteine and cystathionine are reduced or undetectable. (Elevated urinary levels of cystathionine are also present in subjects with vitamin B-12 deficiency of various causes.) Cystathionine β-synthase activity can be directly assayed in liver, lymphocytes, and fibroblasts (236). Therapeutically, limited intake of dietary methionine is primary management. Forty percent of homocystinuric patients respond with clinical improvement to high doses of pyridoxine (vitamin B6), an essential cofactor for cystathionine β-synthase.

A Marfanoid habitus is observed also in patients the Shprintzen–Goldberg syndrome (OMIM 182212) associated with craniosynostosis, exophthalmos, maxillary and mandibular hypoplasia, aortic aneurysms, and multiple abdominal herniae; a missense mutation in *FBN1* (Cyst1223Tyr) has been detected in one patient with this syndrome. The Marfanoid habitus is also found in patients with (a) multiple endocrine neoplasia type IIb (OMIM 162300) in which a gain-of-function mutation (Met918Thr) in *RET* (OMIM 164761, chromosome 10q11.2), a gene encoding a tyrosine kinase receptor, is associated with mucosa neuromas, medullary carcinoma of the thyroid, and pheochromocytoma; (b) the Lujan–Fryns syndrome (OMIM 309520) of X-linked mild to moderate mental retardation, autistic features, schizophrenia-like symptoms, and hyperactivity; (c) the association of microcephaly and glomerulonephritis (OMIM 248760); (d) the combination of situs inversus, dextrocardia, and polyspenia (OMIM 609008); (e) extreme hypermobility due to joint laxity (OMIM 154750).

Neurofibromatosis

Neurofibromatosis (*NF1*—OMIM 162200, chromosome 17q11.2) is a neurogenetic disorder occurring with a prevalence of 1:3000 live births (121). Prominent cutaneous manifestations include café-au-lait macules and skinfold freckling involving the axillae and inguinal regions. Café-au-lait spots are epidermal groupings of neural crest derived pigmented melanocytes whose numbers and size increase during infancy with new spots appearing during the prepubertal years (237). Neurofibromas, benign peripheral nerve sheath tumors, may be visible and/or palpable just below the skin surface. While fewer than 20% of children less than 10 years of age have cutaneous neurofibromas, the number of lesions increases after puberty (238,239). Large plexiform neurofibromas, often underlying extensive café-au-lait macules or areas of hypertrichosis, can invade and compress vital structures or transform into aggressively malignant peripheral nerve sheath tumors. Fifteen to twenty percent of children with *NF1* manifest low-grade astrocytomas of the optic pathway usually in the first six years of life (240). These tumors lead to visual loss, proptosis, afferent papillary defects, strabismus, or deficits in color vision. Melanocytic hamartomas

known as Lisch nodules are minimally elevated lesions best seen by slit lamp examination of the eye and are pathognomonic for *NF1* (241).

Heights of approximately 2% to 4% of *NF1* children exceed +2 SD (121). In addition, central precocious puberty with acceleration of linear growth occurs in another 3% of children with NFl (242). Overgrowth associated with hypersomatotropism and hyperprolactinemia has been described in NF1 children with optic pathway gliomas; normalization of growth velocity has followed medical or surgical therapy. Excessive GH production in some tall children with NF1 may be the consequence of loss of somatostatinergic inhibition of GH secretion by tumor encroachment upon somatostatin-synthesizing hypothalamic neurons rather than due to primary oversecretion of GH. In some NF1 subjects, tall stature is realized without excessive GH secretion. Approximately 13% of children with NFl are small in stature with a tendency for growth to fall further behind during adolescence; most have normal GH secretion. Deficiency of GH is present primarily in short or slowly growing NF1 children who have undergone surgery or cranial irradiation for optic pathway tumors. Benign macrocephaly occurs in 25% to 50% of patients with NF1 (243). Localized areas of bony overgrowth involving the skull and face, fingers, hands, or arms have also been described (239). Other skeletal anomalies include scoliosis, dysplasia of the wing of the sphenoid, congenital thinning and bowing of long bones, and pseudofractures–pseudoarthrosis associated with hypophosphatemic osteomalacia. Attention deficit disorder and specific learning disabilities are common neuropsychiatric findings.

A microdeletion of *NF1* (chromosome 17q11.2) is the most commonly observed mutation leading to decreased neurofibromin production and clinical *NF1*. Neurofibromin, the 2818 aa product of *NFl*, inhibits cell growth as a RAS-specific guanosine triphosphatase-activating protein. RAS is a G-protein that is activated by cellular growth factors and their membrane tyrosine kinase receptors; in turn, it activates several intracellular signal transduction systems that stimulate mitogenesis. Neurofibromin activates GTPase intrinsic to RAS that in turn inactivates RAS; when neurofibromin activity is impaired, RAS activity persists and so does its mitogenic signal. Mutations in the GAP-related domain of *NF1* that reduce neurfibromin activity and thus RAS GTP-ase activity result in increased RAS-induced cell proliferation and ultimately tumor growth (238). In vitro, neurofibromin positively regulates intracellular concentrations of cyclic AMP and perhaps the expression of genes this compounds regulates (244). Augmentation of cyclic AMP also activates PKA, contributing to stimulation of cell proliferation. These pathways may account for the tall stature of some *NF1* subjects who grow without GH excess. Diagnosis of *NF1* requires fulfillment of specific criteria (Table 5). Management is determined by the clinical manifestations of the disorder.

Table 5 Diagnostic Criteria for Neurofibromatosis Type 1

Six or more café-au-lait macules >5 mm in diameter in prepubertal and >15 mm in diameter in post pubertal subjects
Axillary or inguinal freckling
Two or more neurofibromas or one plexiform neurofibroma
Optic glioma
Lisch nodules (>2)
Sphenoid dysplasia or pseudoarthrosis
Neurofibromatosis type 1 in a first degree relative

Note: Two criteria are required to establish the diagnosis of neurofibromatosis type 1.
Source: Adapted from Ref. 121.

Fragile X Syndrome

The fragile X syndrome (OMIM 309550) is characterized by developmental delay, macrocephaly, long face, large ears, prominent mandible, macroorchidism that usually develops during adolescence in males but may also be detected in utero, and jocular speech; affected subjects may be short, normal, or tall in stature (245). Fragile X (*FMR1* or FRAXA) syndrome is the most common cause of familial monogenic mental retardation and occurs in 1 : 4000 to 1 : 6000 males and in 1 : 7000 to 1 : 10,000 females of European descent (246). The condition appears to be more common in Tunisian Jews and Black Americans (247). Affected boys have delayed language skills and behavior problems. Mental retardation is moderate-to-severe in males and mild-to-moderate in females. Autistic-like behavior and hyperactivity are common. *FMRl* (chromosome Xq27.3) encodes a 70 to 80 kDa cytoplasmic protein that contains three RNA-binding motifs (248). Excessive elongation of a trinucleotide CGG (also referred to as CCG) repeat segment of 6 to 50 units that is normally present in the 5′ untranslated region of *FMR1* leads to unstable DNA and silencing of gene expression (249). Asymptomatic male carriers with repeat CGG lengths of 50 to 200 units have a premutation. When the number of CGG repeats exceeds 200, a full mutation is present and 100% of males and 50% to 70% of females with one defective allele will express the fragile X phenotype (250). Normal males with the fragile X genotype have been described who transmit the affected X chromosome to their normal daughters who in turn have female offspring with the fragile X phenotype, an example of the impact of epigenetic changes within *FMR1* on disease expression (251–253). With the expansion of CGG residues, methylation of an upstream CpG island occurs and transcription of *FMR1* is blocked. The fragile X syndrome may also arise in patients with deletions and point mutations (e.g., Ile304Asn within the RNA binding domain) of *FMR1* without CGG amplification (254). The product(s) of *FMR1* is a component of the RNA-induced silencing complex that modulates RNA interference and translation repression. Loss of the protein product(s) of *FMR1* leads to decrease in cytosolic binding of mRNA, decreased association of RNA with polyribosomes,

and disruption of shuttling of mRNA between the nucleus and cytoplasm, and consequently to errors in translation of several hundred target genes (253,255). The diagnosis of the fragile X syndrome is suspected on clinical grounds and confirmed by cytogenetic analysis and quantification of the number of CGG repeats in *FMR1*. Management is symptomatic.

Other

The chromosome 22q13.3 deletion [del(22)(qter)] syndrome (OMIM 606232) due to simple deletions, chromosomal translocations, and ring chromosome formation is associated with postnatal acceleration of growth, large head circumference, minor facial anomalies (high forehead, ptosis, epicanthal folds, dysplastic ears, and pointed chin), large fleshy hands, clinodactyly of the fifth fingers, hypoplasia of the nails, hypotonia, behavioral problems, severe expressive language, and mild developmental delay. This constellation of findings has been attributed in part to haploinsufficiency of *SHANK3* (SH3 and Multiple Ankyrin Repeat Domains 3, OMIM 606203), a gene that is expressed in neural tissues and encodes a proline-rich synapse-associated protein (256). Overgrowth has also been identified in patients with mosaicism for 20p11.2 trisomy, a region on which the gene encoding somatostatin receptor 4 is located; trisomy of 15q16.1-qter where IGF-I is encoded; and a 6q16;13q14 translocation leading to deletion of 6q16 (193). In addition, overgrowth has been described in patients with a number of uncommon syndromes (Table 1) (1).

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8

Adrenal Cortex: Hypo- and Hyperfunction

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INTRODUCTION

The adrenal gland is made of two parts, the cortex and the medulla, which have different embryonic origins. By four to five weeks of fetal life, cells from the mesoderm aggregate to form a primitive cortex between the posterior part of the dorsal mesentery and the gonadal ridge (1). Shortly thereafter, this primitive cortex becomes surrounded by a narrow band of cells termed permanent cortex. By seven to eight weeks of fetal life, the primitive cortex is invaded by chromaffin cells that develop rapidly and eventually replace most of the primitive cortical cells, forming the medulla (1). At that time, the adrenal gland is in close relation with the cranial part of the primitive kidney and not far from the genital ridge.

The adrenal medulla, which originates from ectodermal cells, has an entirely different function from the mesodermal adrenal cortex. In this chapter only the latter is discussed.

In the adult, the adrenal cortex is made of three distinctive zones. The outer zona glomerulosa secretes aldosterone; the middle zona fasciculate, and the inner zona reticularis together are involved in the secretion of the cortisol and adrenal androgens (1). We first discuss the physiological function of the adrenal cortex. Then we consider the disorders related to the hyposecretion and hypersecretion of adrenal cortical hormones.

PHYSIOLOGY

Biosynthesis of Adrenocortical Steroids

The Enzymes

Cholesterol is the precursor of all steroids of both gonadal and adrenocortical origin (2). The biosynthetic pathway of adrenal steroids is shown in Figure 1. The conversion of cholesterol to the various hormones requires the sequential action of a series of six enzymes, as listed in Table 1. All but 3β-hydroxysteroid dehydrogenase (3β-HSD) are members of a family of enzymes termed cytochromes *P*450. They are heme-containing proteins that act as mixed-function oxidases (3). Three of these enzymes have a very similar structure: cholesterol side-chain cleavage enzyme encoded by the gene CYP11A, 11β-hydroxylase encoded by CYP11B1, and aldosterone synthetase encoded by CYP11B2. The last two genes are contiguous on the long arm of chromosome 8q22. The gene CYP11A is located on chromosome 15q23–24; this enzyme requires the activity of another gene product, the steroidogenic acute regulatory protein (StAR).

The cholesterol side-chain cleavage enzyme has the ability to add a hydroxyl group on carbons 20 and 22 of the cholesterol molecule as well as to remove a side chain between carbons 20 and 22 (4). The 3β-HSD converts pregnenolone to progesterone as well as 17α-hydroxypregnenolone to 17α-hydroxyprogesterone; this is accomplished by reduction of the 3β-hydroxyl group into a 3-ketone and isomerization of the 5,6 double bond to a 3,4 double bond. Its gene is located in chromosome 1p13.1 The 17α-hydroxylase enzyme (chromosome 10 q24–25) has the ability of both 17α-hydroxylation and 17, 20-lyase. The latter activity transforms steroids with 21 carbons into steroids with 19 carbons. 21-Hydroxylase (CYP21) and 11β-hydroxy-lase (CYP11B1) are specific for the function of adding a hydroxyl group in carbons 21 and 11, respectively. Aldosterone synthetase (CYP11B2) adds an 11β-hydroxyl group and an 18-hydroxyl group and is also capable of 18-oxidation (5). Whereas the CYP11A, 3β-HSD, and CYP21 genes are expressed in all the zones of the adrenal cortex, the CYP17 and CYP11B1 genes are expressed only in the zona fasciculata–reticularis and the CYP11B2 gene is expressed only in the zona glomerulosa. This accounts for the specificity of steroid production by the various zones of the cortex.

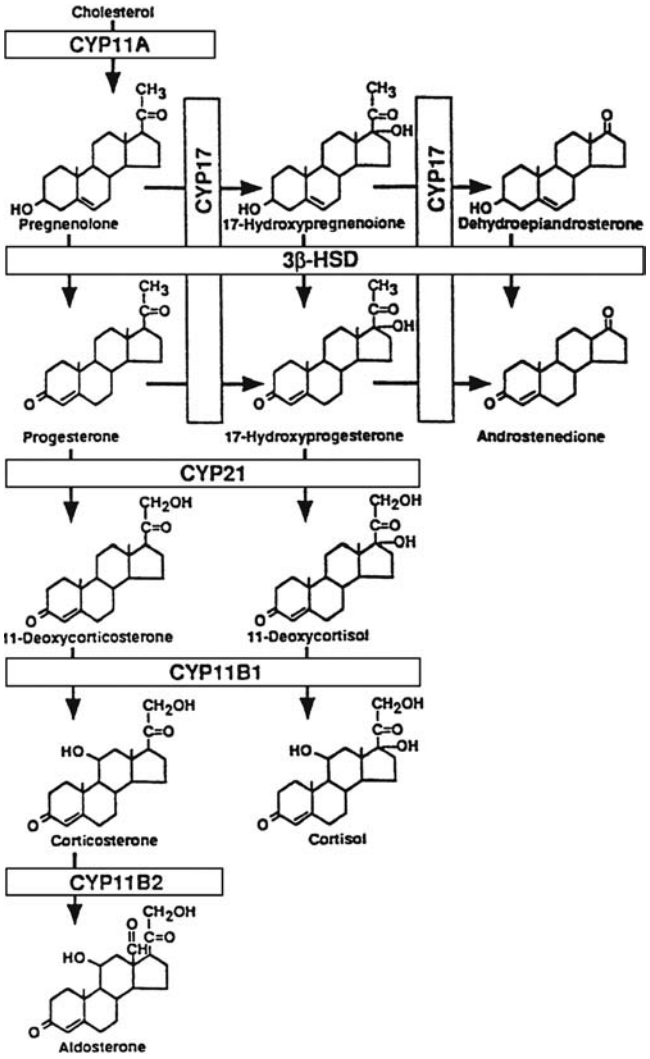

Figure 1 Biosynthesis of adrenocortical steroids. The pathway from cholesterol to cortisol, aldosterone, and adrenal androgens requires the action of five cytochrome P450 (CYP11A, CYP17, CYP21, CYP11B1, and CYP11B2), one dehydrogenase (3β-hydroxysteroid dehydrogenase and a regulatory protein, steroidogenic acute regulatory protein). *Source*: From Ref. 2.

Subcellular Location of the Various Enzymes

As shown in Figure 2, cholesterol is stored in the adrenal cell as cholesterol esters. Under the influence of an esterase, cholesterol becomes available and is transported to the mitochondria, where it is converted to pregnenolone (2). This steroid then moves into the endoplasmic reticulum, where 3β-HSD, 21-hydroxylase, and 17-hydroxylase enzymes are located.

The resulting steroids include 11-deoxycorticosterone (DOC) and 11-deoxycortisol as well as two C-19 carbon steroids, androstenedione, and dehydroepiandrosterone (DHEA). At that point, DOC and 11-deoxycortisol return to the mitochondria, where they are converted into corticosterone, and cortisol, respectively. This is basically the end of the biosynthetic process in the cells of the zona fasciculata. In the cells of the zona glomerulosa, there is no 17-hydroxylase activity and therefore, no formation of cortisol or androgens. However, the mitochondria of these cells include CYP11B2 enzyme, which transforms DOC into corticosterone, 18-hydroxycorticosterone, and aldosterone.

Finally, it must be noted that the activity of all cytochrome P450 enzymes requires a gain of electrons. These electrons are transferred from NADPH. In the mitochondria this transfer is made via two intermediaries, adrenodoxin, and adrenodoxin reductase, whereas in the microsomes the transfer requires only the presence of an adrenodoxin (2).

Table 1 Nomenclature for the Various Steroid Biosynthetic Enzymes and the Respective Genes

Enzyme activity	Gene	Chromosomal locus
Cholesterol side-chain cleavage enzyme (20-hydroxylase, 22-hydroxylase, 20,22-lyase)	CYP11A	15q23–24
3β-Hydroxysteroid dehydrogenase (3β-HSD)	3β-HSD	1p13.1
17α-Hydroxylase and 17,20-lyase	CYP17	10q24–25
21-Hydroxylase	CYP21	6p21
11β-Hydroxylase (some 18-hydroxylation)	CYP11B1	8q22
Aldosterone synthetase (11β-hydroxylation, 18-hydroxylation, and 18-oxidation)	CYP11B	8q22

The Placental–Fetal Adrenal Unit

Early in fetal life, the adrenal cortex is capable of secreting steroids. It lacks 3β-HSD, however, and the major hormones secreted include pregnenolone, 17α-hydroxypregnenolone, DHEA, and 16α-hydroxy-DHEA. All these steroids circulate mainly as sulfate conjugates, which are then transferred to the placenta (Fig. 3) (1). This organ is rich in sulfatase, which makes the native steroids available to the greatly active placental 3β-HSD. The resulting progesterone and 17α-hydroxyprogesterone are returned in part to the fetus and are used by the fetal adrenal to make aldosterone and cortisol and by the fetal gonad to make testosterone.

The placental 3β-HSD also transforms DHEA and its 16α-hydroxylated derivative into androstenedione and its 16α-hydroxylated derivative. In the next step, placental aromatase transforms androstenedione into estrone and estradiol, whereas 16α-hydroxyandrostenedione is metabolized into estriol. Most of these estrogens are excreted by the mother. The large amounts of estriol excreted by the mother are related to the large amounts of 16α-OH-DHEA secreted by the adrenal cortex of the fetus.

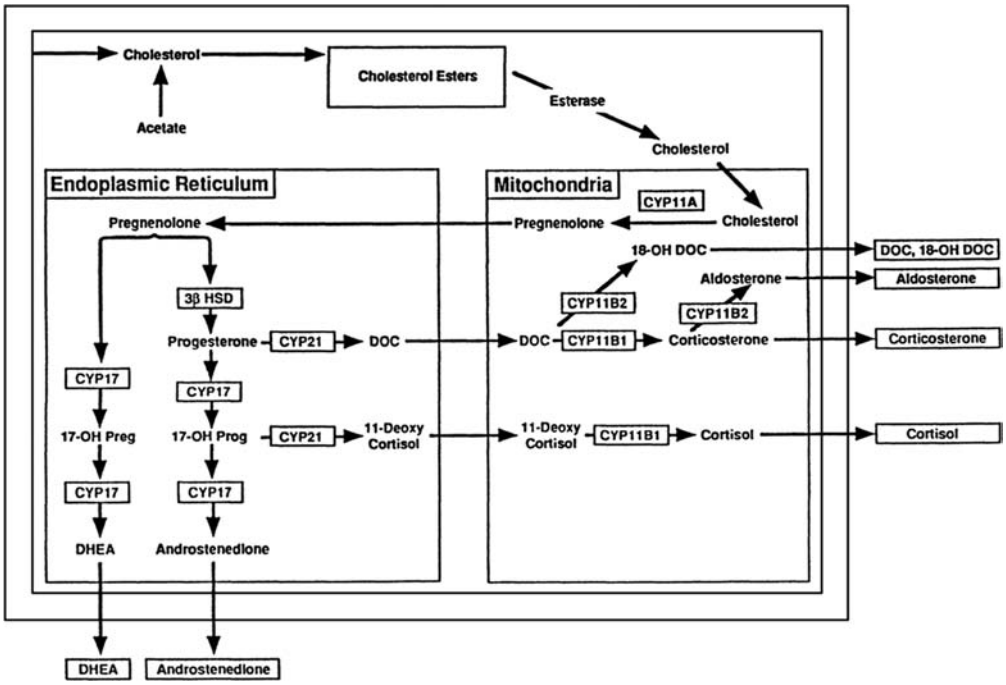

Figure 2 Subcellular location of the various steps of steroidogenesis in an adrenal cell. Cholesterol from blood or from intracellular synthesis is stored as cholesterol esters. An esterase makes cholesterol available when needed. CYP11A is located in the mitochondria. The pregnenolone formed moves to the endoplastic reticulum where it is submitted to the effects of 3β-hydroxysteroid dehydrogenase, CYP17, and CYP21. The resulting 11-deoxycorticosterone and 11-deoxycortisol return to the mitochondria where the CYP11B1 and CYP11B2 cytochromes are located. The main steroids secreted by the adrenal cortex are shown outside of the adrenal cell. *Source*: From Ref. 2.

Control of Adrenal Steroid Secretion

Regulation of Cortisol Secretion

The ability of the adrenal gland to synthesize cortisol is dependent upon the secretion by the hypothalamus of corticotropin-releasing hormone (CRH). By the use of the short loop of the portal vessel system from the hypothalamus to the anterior pituitary, CRH reaches the corticotrophs and triggers the secretion of adrenocorticotropic hormone (ACTH).

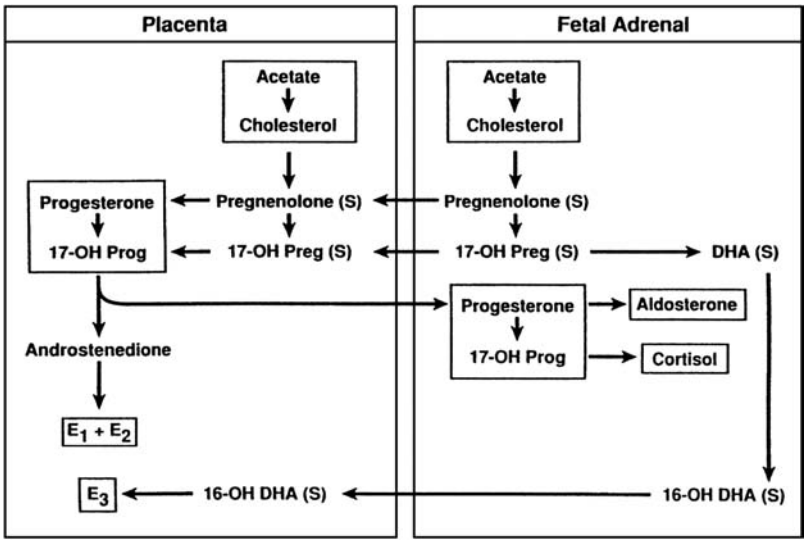

Figure 3 The placenta–fetal adrenal unit. The fetal adrenal can readily synthesize pregnenolone, 17-hydroxypregnenolone, and 16-hydroxy-dehydroepiandrosterone. However, it has little or no 3β-hydroxysteroid dehydrogenase. The placenta, which is rich in this enzyme, transforms the fetal steroids into progesterone, 17-hydroxyprogesterone, and estriol. In the next step, progesterone and 17-hydroxyprogesterone are returned to the fetal adrenal, which can then synthesize aldosterone and cortisol. *Source*: From Ref. 1.

Corticotropin-Releasing Hormone and Adrenocorticotropic Hormone

CRH is a 41 amino acid straight-chain peptide (6). It is secreted mainly by the median eminence. However, it has also been detected in the cortical part of the brain. CRH binds with high affinity to receptors located on the membrane of the corticotrophs of the anterior pituitary. This in turn activates the formation of cyclic AMP, which then activates a series of protein kinases, resulting in increased transcription of the pro-opiomelanocortin gene; appropriate processing of this mRNA results in ACTH formation. ACTH has a half-life in blood of a few minutes. Although the native hormone has 39 amino acid residues, the first 1 to 24 amino-acid sequence has as much activity as the ACTH itself (7). Like other peptide hormones, ACTH binds to specific membrane receptors of the adrenocortical cells to increase the formation of cyclic AMP and activation of various protein kinases.

The ACTH stimulation of cortisol secretion includes acute and chronic phase (2). In the acute phase, which takes only a few minutes, cholesterol is made available for steroidogenesis by activating the effect of an esterase on stored cholesterol esters. The more chronic phase is related to a stimulation of transcription of the various cytochrome P450 genes. A so-called steroidogenetic factor 1 (SF-1) appears to be responsible for this stimulation (8). Of great interest is the recent finding that SF-1 is also a transcription factor. Along with DAX-1, it plays a major role in the formation of steroidogenic tissues, specifically the adrenal glands and the gonads (9).

Mechanisms Regulating Cortisol Secretion

Four physiological mechanisms play an important role in the secretion of cortisol: pulsatile secretion and diurnal variation, stress, and negative feedback.

Pulsatile Secretion and Diurnal Variation in Cortisol. The collection of blood samples at frequent intervals has shown that cortisol is secreted in a pulsatile manner (10). Previously (11) it was observed that the plasma concentration of cortisol showed a specific diurnal variation, the highest peak taking place between 4 and 6 AM. The concentrations then tend to decrease for the rest of the day, being at their lowest in the evening and during the night (Fig. 4).

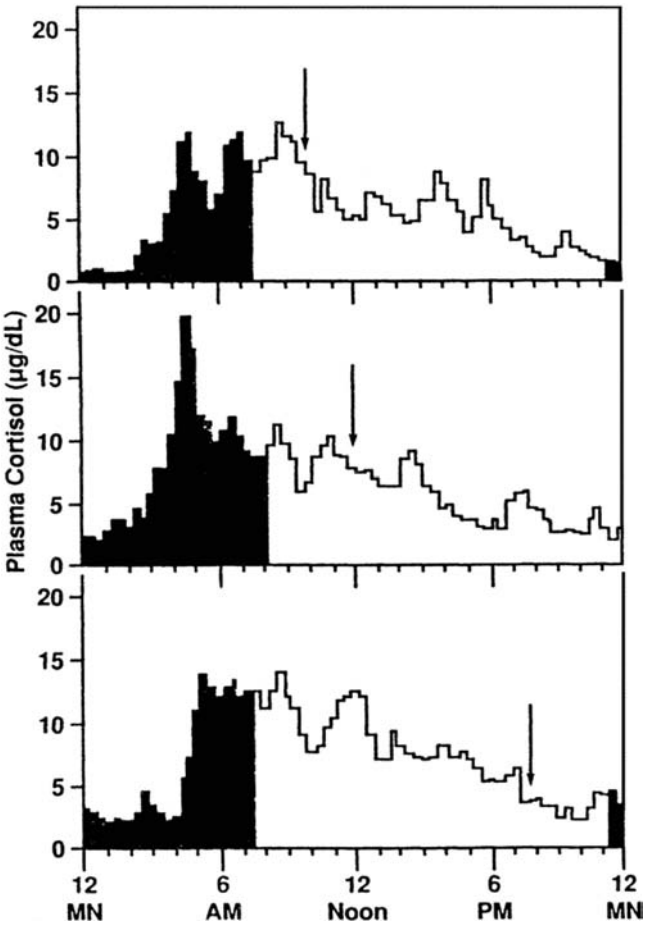

Figure 4 Diurnal variation of plasma cortisol in three normal adult subjects. Black areas represent periods of sleep. *Source*: From Ref. 12.

Stress. Surgical stress, such as trauma and tissue destruction; medical stress, such as acute illness, fever, and hypoglycemia; and emotional stress related to psychological upset result in most cases in an important increase in cortisol secretion. How these various types of stress influence steroid output is not clear. However, studies of the immune system have shown that leukocytes secrete a series of peptide hormones, the interleukins (13). Their formation is markedly increased during stress, and two of them, interleukin-1 and interleukin-6, have been shown to stimulate CRH secretion and therefore to increase cortisol secretion (14).

Negative Feedback. Another important physiological mechanism controlling cortisol secretion is negative feedback. Under normal conditions, there is equilibrium between the rate of secretion of ACTH and that of cortisol. When the plasma concentration of cortisol increases markedly, it has a negative effect on the secretion of CRH and ACTH. By this mechanism, cortisol levels in blood regulate the rate of output of CRH and ACTH.

Cortisol Secretion Rate

In normal children of various ages and in adult subjects, the rate of cortisol secretion increases with body size (15). When the values are corrected for body surface area, the rates are similar at various ages; the average $\pm$ standard deviation (SD) was 12 ± 2 (Fig. 5) with a range of 8 to 16 $mg/m^2/24\,hr$. However, the circadian variation of the clearance rate of cortisol

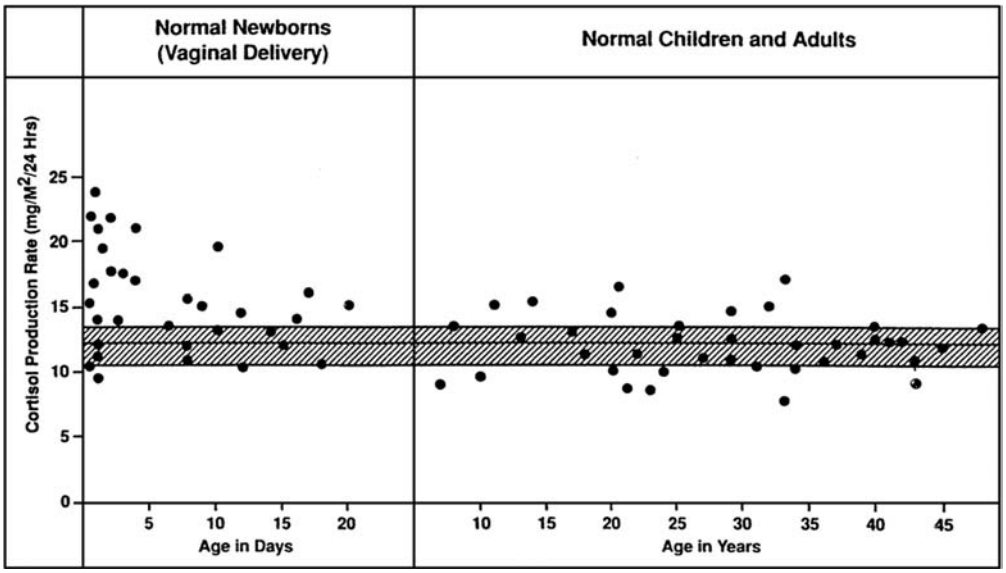

Figure 5 Cortisol secretion rate in newborn infants, children, and adults. The values have been corrected for body surface area. The rates expressed in mg/m^2/24 hr, were fairly constant throughout life except in the first few days when they were somewhat higher. *Source*: From Ref. 1.

resulted in an overestimation. An appropriate correction gave a lower normal range of 6 to 14 mg/m^2/24 hr. Using stable isotope dilution/mass spectrometry Esteban et al. (16) reported a cortisol secretion rate for 12 normal subjects of 5.7 ± 1.5 mg/m^2/24 hr. Kerrigan et al. (17), using deconvolution analysis, found in similar value in 18 normal male children.

Regulation of Aldosterone Secretion

Aldosterone is secreted by the cells of the zona glomerulosa of the adrenal cortex. It is mainly under the control of angiotensin II. As shown in Figure 6, the liver produces a large protein, angiotensinogen, which is cleaved by a proteolytic enzyme, renin, which is secreted by the juxtaglomerular cells of the kidney. A converting enzyme then transforms angiotensin I into angiotensin II.

Potassium concentration in plasma and ACTH also play a role in the control of aldosterone secretion. The effect of ACTH is an acute stimulation related to the rapid increase in availability of cholesterol from the cholesterol ester reserve (18). As shown in Figure 7, an intravenous (IV) injection of ACTH very quickly raises plasma aldosterone levels, but after 60 minutes the levels tend to decrease.

Regulation of Adrenal Androgen Secretion

Adrenal androgens are secreted in large amounts during fetal life. Their production decreases rapidly after birth and is not resumed until puberty. The factor that triggers the pubertal secretion of adrenal androgens is not ACTH because its levels are not different before and after puberty. However, large amounts of ACTH given chronically markedly increase the secretion of adrenal androgens. It has been postulated that a pituitary peptide other than ACTH but not yet characterized is responsible for this stimulation, and it has been named adrenal androgen-stimulating hormone (19).

General Metabolism

As shown in Figure 8, adrenal steroids are transported by blood to reach their target tissues. The liver is one of the target tissues as well as an important site for the catabolism of the steroids. The main glucocorticoid is cortisol. Between 80% and 90% is bound to a specific glycosylated α-globulin known as corticosteroid-binding globulin (CBG), also called transcortin (20). Transcortin has a very high affinity for cortisol, but it can also bind progesterone, prednisolone, and, with less affinity, aldosterone (21). Another 7% of the total circulating cortisol is loosely bound to albumin, and 2% to 3% is not bound to protein. This unbound cortisol is the fraction of the total available to target cells. In these cells, cortisol binds to a specific glucocorticoid receptor. The receptor–steroid complex binds to the glucocorticoid receptor elements located in the promoter area of responsive genes. By activating the transcription of such genes, glucocorticoids express their biological activity.

The main mechanism of catabolism is a reduction of the steroid molecule and eventually conjugation with glucuronic or sulfuric acid to make products that are water soluble and readily excreted by the kidney as urinary metabolites.

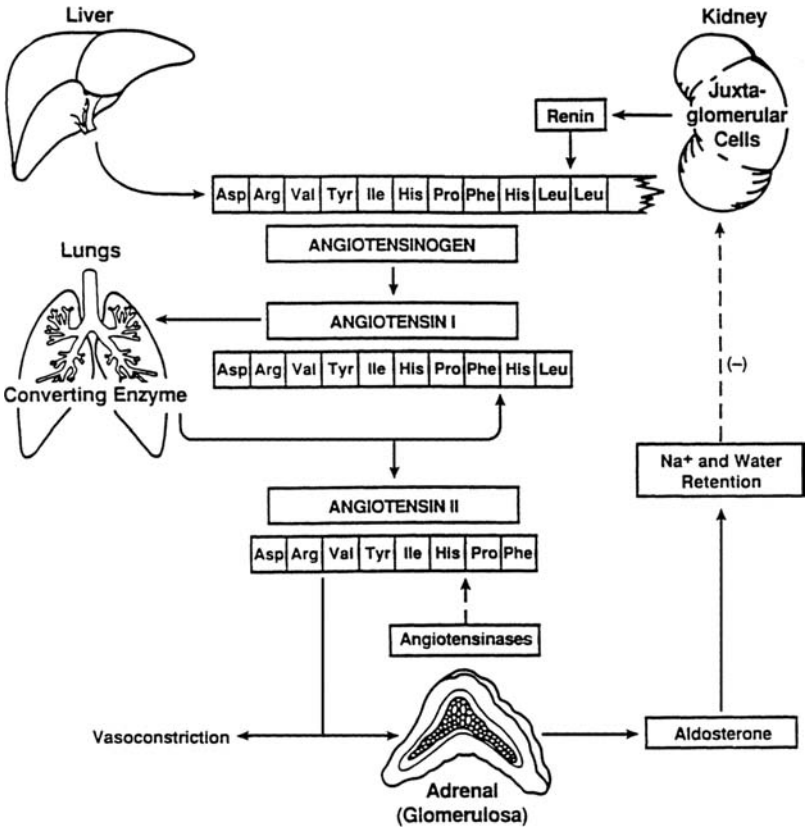

Figure 6 Control of aldosterone secretion. Angiotensinogen of hepatic origin is cleaved by renin from the kidney. The resulting angiotensin I is further cleaved into angiotensin II, an 8 amino-acid peptide that has properties of vasoconstriction on vessels and of activating secretion of aldosterone by the cells of the glomerulosa. *Source*: From Ref. 1.

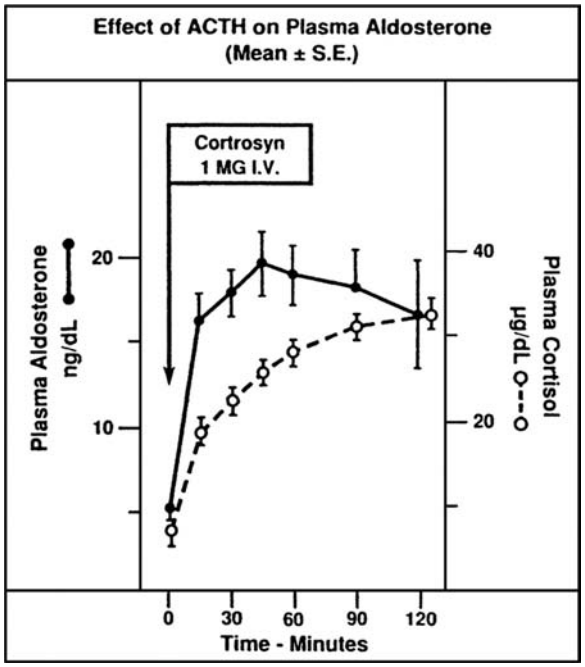

Figure 7 Effects of 1,24-adrenocorticotropic hormone on the plasma concentration of cortisol and aldosterone in 16 normal subjects. *Source*: From Ref. 15.

Although aldosterone can bind to CBG, most of the binding sites of this protein are occupied by cortisol. For this reason, aldosterone is physiologically mainly bound to albumin. Its half-life in blood is 20 to 30 minutes, compared with 60 to 80 minutes for cortisol. In target cells, aldosterone binds to its own specific receptor, the mineralocorticoid receptor. The mode of action of this steroid–receptor complex is similar to that described for cortisol.

Tests of Adrenocortical Function

Many types of tests have been proposed for the determination of adrenocortical function. We outline here only the tests considered important and practical for diagnostic purposes.

Tests Related to Glucocorticoid Function

8 AM Plasma Cortisol Concentrations

It is necessary to obtain plasma levels of cortisol at a specific time of the day (8 AM) because of the diurnal variation discussed earlier.

However, even this precaution may not be sufficient because of the episodic secretion of the steroid. In general, a single plasma value is of limited clinical significance. There are conditions for which one must determine the plasma concentrations of cortisone and corticosterone. Normally, their concentrations are

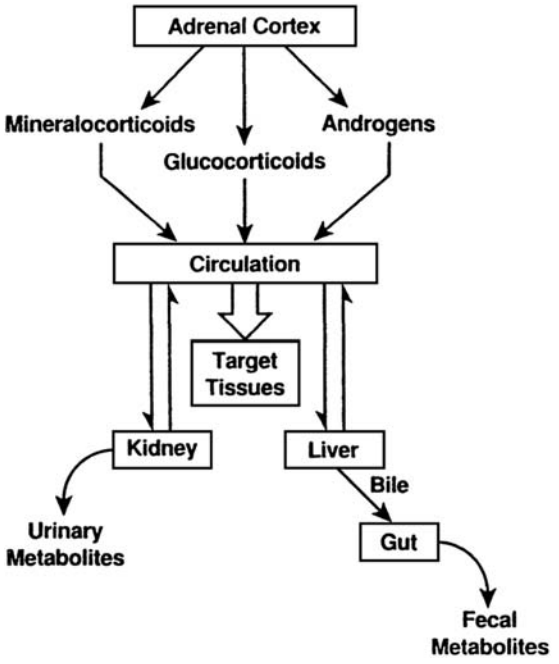

Figure 8 General metabolism of adrenal steroids. In the blood circulation, the steroids are mainly bound to specific proteins. The small unbound fraction is available to target tissues where the steroids express their biological effects. At the same time, steroids are metabolized and conjugated by the liver. The conjugates are returned to the circulation and excreted in bile by the kidney as urinary metabolites. *Source*: From Ref. 1.

approximately one-tenth that of cortisol at 8 AM. Changes in the ratios of these steroids to cortisol in a single blood sample may therefore be indicative of a specific abnormality of adrenal secretion.

Urinary 17-Hydroxycorticosteroids

About 30% of secreted cortisol is excreted as urinary 17-hydroxycorticosteroids (17-OHCS). For this reason, determination of 24 hours urine excretion gives a good indication of the 24 hours cortisol secretion. Indeed, this test is of interest in ruling out Cushing's disease. In normal subjects, the mean $\pm$ SD urinary 17-OHCS is $2.5 \pm 1.0\,mg/m^2/24\,hr$ (15).

Urinary Free Cortisol

Approximately 0.25% to 0.5% of secreted cortisol is excreted as cortisol itself. Because urinary free cortisol reflects the amount of unbound cortisol available to target cells (i.e., nonprotein-bound cortisol), this test is considered of greater biological importance than that of urinary 17-OHCS. In our opinion, however, both tests should be obtained in a single 24 hours urine specimen when screening for Cushing syndrome.

Adrenocorticotropic Hormone Test

At present, the standard technique involves the IV bolus administration of 0.25 mg 1,24-ACTH (Cortrosyn). Blood samples are obtained at 0, 60, and 120 minutes after ACTH injection. As seen in Figure 7, plasma cortisol increases to about 30 μg/dl (18).

A normal baseline and a normal increment by 120 minutes rule out primary adrenal insufficiency. This test is also useful when determining the mineralocorticoid function of the adrenals. Recent studies have shown that administration of a very low amount of ACTH (1 μg) may be more sensitive than the standard 250 μg test in the detection of dysfunction of the hypothalamopituitary–adrenal axis. This would be particularly true in mild adrenal insufficiency, as can be seen in the case of pituitary disease or with the use of inhaled steroids as reported by Mayenknecht et al. (22) and Nye et al. (23).

Metyrapone (Metopirone) Test

Given acutely, Metopirone has the property of blocking 11b-hydroxylation. This results in decreased secretion of cortisol and corticosterone with a decrease in negative feedback on the hypothalamic–pituitary axis. As a consequence, there is an increase in ACTH secretion with an increased secretion of 11-deoxysteroid, specifically 11-deoxycortisol (compound S), and 11-DOC. The present standardized test consists of the administration of a single oral dose of Metopirone ($300\,mg/m^2$/per dose) at midnight and measurement the next day at 8 am of plasma 11-deoxy-cortisol. If adrenocortical function is normal and if the pituitary is capable of increasing its ACTH secretion, the plasma concentration of 11-deoxycortisol rises to values of 7 to 22 μg/dl (24). A marked increase in ACTH concentrations is also observed in normal subjects.

Dexamethasone Suppression Test

Dexamethasone is a potent glucocorticoid that, given in small amounts suppresses ACTH secretion and secondarily decreases cortisol secretion. The suppressing effects of the test are measured by the determination of either plasma ACTH and cortisol or urinary excretion of free cortisol and of total 17-OHCS. In the low dose or single Dexamethasone suppression test, $1.25\,mg\ Dexamethasone/m^2/24\,hr$ is administered for two days. In the high-dose or triple-Dexamethasone suppression test, $3.75\,mg\ dexamethasone/m^2/24\,hr$ is administered for an additional two days.

The Corticotropin-Releasing Hormone Test

Following the IV administration of CRH (100 μg/dose), there is a moderate but significant response in both cortisol and ACTH, the maximum response being about 60 minutes after injection. In Cushing's disease, there is a greatly exaggerated response of both plasma cortisol and ACTH, whereas in ectopic ACTH syndrome there is no change, as shown in Figure 9 (25).

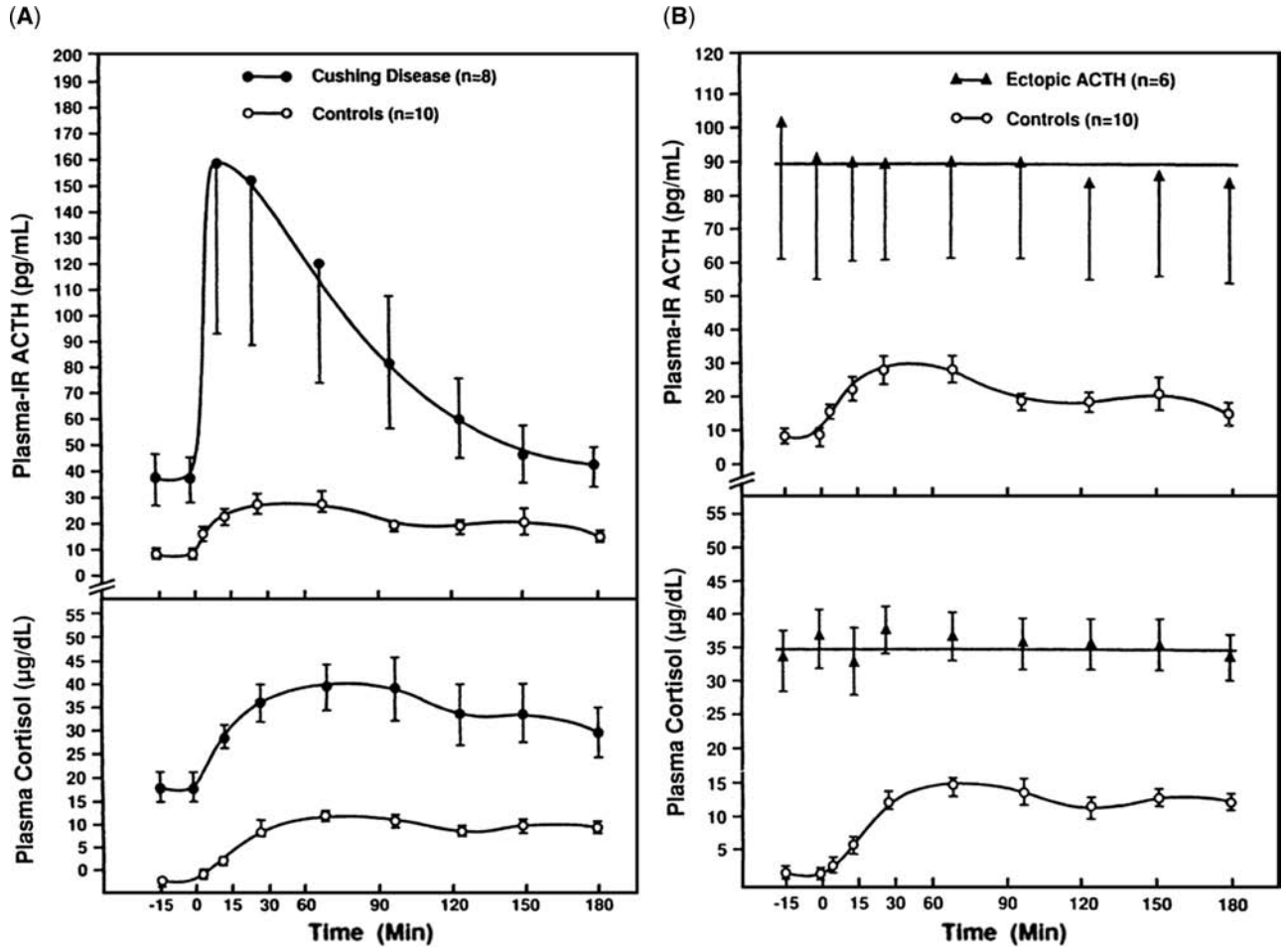

Figure 9 The corticotropin-releasing hormone (CRH) stimulation test. The figure shows the response of plasma cortisol and adrenocorticotropic hormone (ACTH) to IV administration of 100 mg CRH (**A**) in adult controls and in patients with Cushing's disease and (**B**) in patients with Cushing's due to ectopic ACTH. *Source*: From Ref. 25.

Tests Related to Mineralocorticoid Secretion

Plasma Aldosterone and 11-Deoxycorticosterone

Concentrations of plasma aldosterone are quite variable, changing rapidly in relation to body posture, the standing values being greater than those while supine (18). Plasma concentrations of aldosterone are also influenced by chronic changes in sodium intake, the concentration being 15 to 30 ng/dl on a low-sodium diet (<17 mEq/24 hr) and 2 to 12 ng/dl on a normal-sodium diet (150–200 mEq/24 hr in adults).

The plasma concentrations of DOC, like those of aldosterone, tend to be higher in early infancy (from 8–5 ng/dl) than later in childhood (5–10 ng/dl), as reported by Lashansky et al. (26).

As shown in Figure 7, ACTH acutely increases aldosterone concentration. There is also a three-to five-fold increase in DOC.

Urinary Excretion of Aldosterone and Deoxycorticosterone

A small fraction of secreted aldosterone is excreted as a 21-oxoglucuronide conjugate. Following hydrolysis at pH 1.0, aldosterone is freed and is measured by radioimmunoassay. Under normal sodium intake, the values are 3 to 10 µg/24 hr and, on a low-sodium diet, they are 20 to 50 µg/24 hr.

HYPOADRENOCORTICISM

Adrenal insufficiency can be caused by an abnormality of the adrenal glands (primary hypoadrenocorticism). In such cases, the decreased cortisol secretion results in an homeostatic increase in CRH/ACTH secretion. Primary hypoadrenocorticism may be congenital and related to a specific genetic abnormality (Table 2).

Table 2 Classification of Congenital Forms of Primary Hypoadrenocorticism

Congenital adrenal hyperplasia
DHCR-7 (Smith–Lemli–Opitz syndrome)
CYP11A/steroidogenic acute regulatory protein
3β-HSD-2
CYP17
CYP-21
CYP11B1
POR (oxydoreductase)
Congenital adrenal aplasia/hypoplasia
Congenital adrenal aplasia
Adrenal hypoplasia due to mutations of transcriptions factors. DAX-1 (x-linked adrenal hypoplasia)
Mutations of steroidogenetic factor 1 transcription factor
IMAGe association [IUGR, metaphyseal dysplasia, adrenal deficiency (AD), gonadal abnormalities]
Autoimmune AD
Isolated autoimmune AD
Autoimmune polyglandular syndrome (APS) type 1 (AD and hypoparathyroidism/candidiasis)
APS type 2 (AD and hypothyroidism/diabetes mellitus)
APS type 4 (AD and other disorders)
Metabolic lipid disorders
Adrenoleukodystrophy/Adrenomyeloneuropathy (ABCD gene)
Zellweger syndrome (PEX-1 gene)
Wolman syndrome (Acid lipase deficiency)

It can also be acquired and related to hemorrhage, infections, or tumor (Table 3).

Adrenal insufficiency can also be secondary to an hypothalamic/pituitary abnormality (secondary hypoadrenocorticism).

Finally, the adrenal insufficiency may be due to a hormone resistance. It includes conditions of CRH unresponsiveness, ACTH unresponsiveness, cortisol, and aldosterone resistance. These cases of hormone resistance are caused by hormone receptor mutations.

PRIMARY HYPOADRENOCORTICISM—CONGENITAL FORMS

As shown in Table 2, there are multiple causes of congenital primary adrenal insufficiency. They can be classified in four major groups: (A) congenital adrenal hyperplasia (CAH), (B) congenital adrenal hypoplasia, (C) autoimmune adrenal deficiency (AD), and (D) metabolic disorders.

Table 3 Classification of Acquired Forms of Primary Hypoadrenocorticism

Massive bilateral adrenal hemorrhage
Adrenal hemorrhage of the newborn
Adrenal hemorrhage of acute infection (Waterhouse–Fridericksen syndrome)
Thrombocytopenia and heparine therapy
Infectious disorders
Tuberculosis
Fungal infection (histoplasmosis coccidiomycosis)
Viral (HIV)
Noninfectious disorders and adrenal tumors
Amyloidosis
Histiocytosis X
Sarcoidosis
Tumors (some related to p53 mutation)

Congenital Adrenal Hyperplasia

There are six major forms of CAH, each caused by a mutation of one of the six enzymes required for the biosynthesis of cortisol. In each of these forms, the decrease in cortisol secretion results in a decrease in negative feedback at the level of the hypothalamus-pituitary (1,2). The resulting increase in CRH/ACTH output attempts to return the cortisol secretion to normal if the mutation permits some degree of enzymatic activity. At the same time, the increased ACTH secretion results in markedly elevated production of the cortisol precursors before the mutant block.

The Smith–Lemli–Opitz syndrome is now considered as a form of CAH. It is due to mutation of the DHCR7 gene (locus: 11q12–13) whose product is the enzyme required for the formation of cholesterol from 7-dehydrocholesterol. Because cholesterol is so important for multiple cell functions, most mutations of DHCR7 are lethal. The less nefarious mutations result in severe phenotypes which include craniofacial abnormalities, mental deficiency, failure to thrive, genitourinary, and adrenal dysfunction.

Lipoid Adrenal Hyperplasia is caused by mutations of the StAR gene and on occasion the CYP11A gene.

The 3β-HSD deficiency results in salt loss. It also involves testicular steroidogenesis resulting in ambiguous genitalia in male fetuses.

A completely inactivating mutation of CYP17 is expected to result in lack of cortisol/aldosterone secretion, as well as an absence of androgen/estrogen secretion. Hence, 46,XY fetuses will lack masculinization of their external genitalia.

Depending upon the severity of the mutation of CYP21, there are three sub-forms of 21-hydroxylase deficiency: the salt-losing form, the simple virilizing form, and the attenuated (or nonclassical) form.

Mutations of CYP11B1 gene result in the hypertensive form of CAH. Like the 21-hydroxylase, the 11-hydroxylase produces masculinization of the external genitalia of the female fetus.

All forms of CAH are autosomal recessive traits and they represent 70% to 75% of all cases of primary hypoadrenocorticism. The 21-hydroxylase deficiency accounts for about 90% of all CAH and the 11-hydroxylase deficiency for about 5%. These disorders are discussed in a separate chapter of this book (Vol. 2; Chap. 9).

Congenital Adrenal Aplasia/Hypoplasia

Congenital Adrenal Aplasia

This is believed to be caused by a developmental disorder of the adrenal anlage during fetal life. Symptoms occur shortly after birth and are characterized

by acute shock with tachycardia, hyperpyrexia, cyanosis, and rapid respiration. Untreated, it evolves to total vascular collapse and death. The symptoms of congenital adrenal aplasia are similar to those present in other conditions, such as septicemia and intracranial hemorrhage. The rapid evolution of adrenal aplasia to death is the reason it is often diagnosed at autopsy.

X-Linked Adrenal Hypoplasia Congenita

Among families presenting multiple cases of adrenal insufficiency, several of them suggested the possibility of an X-linked trait. In 1980, Guggenheim et al. (27) reported an association of glycerol kinase deficiency (GKD) with Duchenne muscular dystrophy (DMD). Later, these disorders were reported to be associated with an Xp21 interstitial deletion (28).

Pathophysiology

The deletions of the X chromosome associated with adrenal hypoplasia congenital (AHC), GKD, and DMD have permitted mapping the locus of these three disorders, as well as the locus of chronic granulomatous disease, retinitis pigmentosa, and ornithine transcarboxylase deficiency, the last gene being closest to the centromere, and AHC being closest to the end of Xp (29). However, it is not clear how the deletion of the AHC locus results in hypoplastic adrenals. The adrenal cortex in patients studied at autopsy shows a small number of large adrenocortical cells, hence the name cytomegalic adrenal hypoplasia given by pathologists.

Clinical Manifestations and Mapping of Xp21

AHC appears early in infancy with signs of acute adrenal insufficiency. However, the signs may occur later in childhood and be somewhat milder. Growth failure with short stature is often observed. In AHC caused by a deletion involving as well other genetic loci, such as GKD and DMD, signs of GKD, and myopathy are also present.

In a series of patients presenting with the association of AHC, GKD, and DMD, mental retardation was reported in most of the cases, suggesting the existence of a contiguous locus involved in mental function (30). However, it must be noted that subjects with adrenal insufficiency may have other reasons for mental retardation such as hypoglycemic episodes and abnormal electrolyte imbalance. It is believed that the gene locus of adrenal hypoplasia congenita is the DAX-1 gene (31,32). DAX-1 plays a major role in the differentiation of fetal cells into both adrenal glands and gonads; it is also necessary for the formation of the hypothalamus, in cooperation with the SF-1 transcription factor. This may explain why abnormalities of the DAX-1 gene result in hypogonadotropic hypogonadism (HH) in addition to AHC (33).

Finally, it is of interest to note that a duplication of the DAX-1 locus results in testicular abnormalities in 46,XY subjects (34). This is related to the complex relationships of DAX-1 with the SRY and SF-1 proteins during male sex differentiation.

Laboratory

Hormonal and electrolyte studies are required in cases of adrenocortical insufficiency. Low basal cortisol and aldosterone levels responding poorly to ACTH administration, elevated ACTH concentrations, along with hyponatremic, hyperkalemic acidosis are characteristic.

GKD association is demonstrated by elevated serum and urine glycerol levels. Because glycerol is measured in the triglyceride assay, there is pseudohypertriglyceridemia. Muscle biopsy showing elevated serum creatine kinase confirms DMD. Results of a luteinizing hormone-releasing hormone (LHRH) test helps in determining HH (35).

Genetics

The clinical abnormalities of AHC and associated disorders in patients with Xp21del demonstrate a relation of cause to effects between the chromosomal deletion and the specific symptoms.

All cases of AHC, GKD, and DMD associated with an X deletion occur in male subjects, as expected of X-linked recessive traits. Unaffected mothers of patients have been shown to be heterozygous for the deletion Xp21del (28). However, one case in a female patient has been reported (30).

Treatment

Steroid replacement for both glucocorticoids and mineralocorticoids is necessary. If GKD is present, appropriate therapy must be given. Unfortunately, DMD is not specifically treatable at this time.

Mutations of Steroidogenic Factor 1 Transcription Factor

Adrenal Embryology

SF-1 is a member of the family of nuclear hormone receptors which contain ''zinc fingers'' and play a role of transcription factor. Originally SF-1 was found to be a regulator of the various cytochromes *P*450 required for steroid synthesis (36). Later, it was reported that SF-1 also controlled the expression of the 3β-HSD gene, the StAR, the ACTH receptor, and the Müllerian Inhibiting Factor.

Most transcription factors have multiple ubiquitous roles and this is true for SF-1. Its transcripts were found in very early embryogenesis not only in adrenals and gonads but also in ventro-medial hypothalamus and pituitary gland (37).

Clinical Manifestations

It is probable that many of the mutant SF-1 are lethal resulting in early abortion. However, an heterozygous

mutation at codon 35 (Gly35glu substitution) in a 46,XY infant resulted in a female phenotype with normal Müllerian structures (uterus; fallopian tubes) and streak gonads typical of complete gonadal dysgenesis or Swyer syndrome. In addition, the infant presented with adrenal hypoplasia (38).

Treatment

Typically, early glucocorticoid and mineralocorticoid replacement will be needed for survival. In addition, the 46,XY patients may present intersex condition. It is expected that some of the 46,XX patients will present with gonadal dysgenesis (streak gonads), in addition to adrenal hypoplasia. However, there is a report of a 46,XX girl with an heterozygous Arg255leu mutation who presented with adrenal hypoplasia but normal ovaries (39).

Intrauterine Growth Retardation, Metaphyseal Dysplasia, Adrenal Hypoplasia and Genital Abnormalities Association

An association of intrauterine growth retardation, metaphyseal dysplasia, adrenal hypoplasia and genital abnormalities (IMAGe) has been reported (40,41). Male infants present micropenis, cryptorchidism, and occasionally hypospadias. The association may be familial with autosomal recessive inheritance (42).

As other disorders which include hypoadrenocorticism, adrenal hormone replacement will be necessary.

Autoimmune Adrenocortical Deficiency

Among the various forms of primary hypoadrenocorticism, autoimmune AD is second in frequency of causes, representing about 20% of the patients, CAH subjects being 70%. Its prevalence is about 1/25,000 births.

Autoimmune AD results from the effects of adrenocortical antibodies which produce a lymphocytic infiltration of the cortex. The inflammatory infiltration is followed by scarring and destruction of the cortical cells. Essentially, the secretion of adrenocortical steroids becomes insufficient despite CHR/ACTH hypersecretion.

Although the immune disorder is congenital, the formation of antibodies and the progressive destruction of the adrenals appear later in life—sometimes at 20 to 30 years of age. Witebsky, Rose and colleagues (43,44) have defined the criteria needed for the diagnosis of autoimmune disorders.

Autoimmune AD can be isolated or associated (Table 4) to other autoimmune disorders (autoimmune polyglandular syndrome or APS). Depending on the associated glandular disorders (45), it is classic to distinguish APS type 1 (candidiasis, hypoparathyroidism, and AD) and APS type 2 (AD with thyroid autoimmune disease or type 1 diabetes).

Isolated Autoimmune Adrenal Deficiency

This AD is associated with adrenocortical cells antibodies and 21-hydroxylase (CYP-21) antibodies. It is often found with HLA type DR3. Females tend to be equally affected than males. Although the immune disorder is congenital, signs of AD occur about 10-times more often in young adults than in infants or children.

Autoimmune Polyglandular Syndrome Type 1

The group of disorders of APS type 1 includes chronic candidiasis, chronic hypoparathyroidism, AD and usually in this order, candidiasis being first. In some patients, there can be additional components (Table 4): hypergonadotropic hypogonadism, vitiligo, alopecia, pernicious anemia, chronic hepatitis, and on rare occasion, Sjögren syndrome, autoimmune thyroid disease, Crohn's disease, atrophic gastritis, diabetes mellitus (DM) type 1. Because of the multiple added components, the APS type 1 is a very serious condition. In addition, most patients are children rather than adults. This form of autoimmune AD is four to five times less frequent than isolated AD and APS

Table 4 Autoimmune Adrenocortical Deficiency: Isolated AD, Autoimmune Polyglandular Syndrome

	Isolated AD	APS type 1	APS type 2
Components	AD	Candidiasis Hypoparathyroidism AD	AD Auto-thyroid and/or diabetes mellitus type 1
Other components			
Hypogonadotropic	0		
Hypogonadism	0	+0.6	+0.1
Vitiligo	0	+0.2	+0.1
Alopecia	0	+0.4	+0.05
Pernicious anemia	0	+0.2	0
Hepatitis	0	+0.2	+0.05
Gene	Related to HLA-DR3	Autoimmune regulator gene	Related to HLA-DR3
Familial	Sporadic	Autosomal recessive	Dominant

Abbreviations: AD, adrenocortical deficiency; APS, autoimmune polyglandular syndrome.

type 2. However, its prevalence varies a great deal among world populations, with great frequency in Finland, reflecting a ''founder effect'' (46).

It has been demonstrated that APS type 1 is caused by a mutation of a single gene, the autoimmune regulator gene (AIRE) of chromosome 21p22.3 (47,48). The AIRE protein includes a leucine rich region and two zinc-finger motif, typical of nuclear transcription factors. Multiple mutations have been reported (49,50). Heterozygous carriers are usually normal, suggesting an autosomal recessive trait.

Autoimmune Polyglandular Syndrome Type 2

In addition to AD, the APS type 2 includes autoimmune thyroid disease and/or DM type 1. Thyroid disease or DM type 1 or both are needed for this clinical diagnosis. Additional components are rare (Table 4). The association of AD and thyroid disorder is frequent and is named Schmidt's syndrome. DM type 1 is less frequent. Antibodies to adrenal cortex and to 21-hydroxylase are detected regularly (45).

Patients are older adolescents and young adults, females being more susceptible than males. Prevalence is about the same as isolated AD, and three to four times more frequent than type 1.

In type 2, the AIRE gene is normal. There is a strong association with HLA-DR3 but the specific cause has not been determined.

Other Autoimmune Polyglandular Syndromes

In addition to the well defined APS types 1 and 2, an APS type 3 has been proposed which would include any of the typical disorders previously described, except for AD. Whether this would represent a specific disorder with a specific etiology is not clear.

A type 4 polyglandular syndrome has also been suggested (45). It would be somewhat similar to isolated AD but would also include some of the complements of types 1 and 2.

Metabolic Lipid Disorders

These abnormalities of lipid metabolism result in progressive neurodegenerative disorders and, in most cases, abnormal hormone secretion of the steroid producing glands (adrenals and gonads).

Adrenoleukodystrophy and Adrenomyeloneuropathy

Pathophysiology

Also known as Siemerling–Creutzfeldt's or Schilder's disease, the basic biochemical abnormality is an elevation in plasma and various tissues of the concentration of the very-long-chain fatty acids (VLCFAs), C24, C25, and C26 (51). They probably accumulate because of a defect in their normal breakdown in cellular organelles known as peroxisomes. On pathological examination, the adrenal cortex at first shows cytoplasmic striations containing cholesterol esterified with VLCFA. Later the adrenal cells appear to be filled with the abnormal cholesterol esters, and in the final stage the cells tend to atrophy and die (52).

Clinical Manifestations

There are two major clinical forms: the childhood inflammatory cerebral adrenoleukodystrophy (ALD) and the noninflammatory adrenomyeloneuropathic (51).

The cerebral ALD is a progressive disease of the brain that manifests in its early stage by mild symptoms, such as unusual behavior, a mild decrease in visual acuity, and a loss of muscle strength in some limbs. Over a period of a few years, the symptoms progress to dementia, blindness, quadriparesis, and end in death. At various times during this degenerative process, symptoms of adrenal insufficiency may occur, involving both cortisol and aldosterone function.

The adrenomyeloneuropathy (AMN) is a distal axones disorder resulting in dorsal column abnormalities. The patients also present hypoadrenocorticism.

Although ABCD-1 gene mutations are responsible for both ALD and AMN, the expression is greatly variable.

Genetics

The locus of ALD and AMN maps to the long arm of the X chromosome (Xq28) (53,54). Because of the location of the gene on the X chromosome, the disorder is expressed only in male subjects. Recently the gene was identified (55). It encodes a 745 amino acid protein that includes six membrane-spanning segments and an adenosine triphosphate (ATP)-binding domain. The gene has been shown to be a member of the family of ATP-binding cassette (ABC) transporters. The role of these transporters is to facilitate the motion of various molecules through cell membranes. The specific gene in ALD is ABCD-1; it is working as a dimere which transports peroxisomes through cell membranes. A large number of mutations have been reported (51,56).

Variable Expression of Adrenoleukodystrophy and Adrenomyeloneuropathy

Generally, the symptoms of ALD appear at around six to seven years of age. In such patients the neurological deterioration is somewhat rapid, leading to a bedridden state in two to three years. There is also a group of boys in whom the illness appears between 12 and 20 years of age: in these subjects, the neurological deterioration is usually much slower.

AMN involves peripheral nerves rather than the brain. In most cases the neurological signs occur at or after 21 years of age (57). However, some patients with AMN may have early symptoms of mild adrenal insufficiency and skin hyperpigmentation. These men also present with primary gonadal failure (58). It must also be noted that some cases present with either only neurological manifestations or only addisonian symptoms.

All the forms mentioned earlier are considered allelic and are all X-linked recessive traits. Except in the rare cases of de novo mutation, the mothers of ALD patients are obligate heterozygotes. These women are free of symptoms, but some may present with neurological abnormalities late in life, usually between 40 and 50 years of age.

Treatment

The therapy of the AD and gonadal deficiency is a straightforward hormonal replacement. However, there is no specific treatment of the neurological disorder: only palliative therapy is helpful. However, a recent therapeutic trial with Lorenzo's oil has been conducted on boys identified by a positive plasma VLCFA assay.

It was concluded that the hexacosanoic acid reduction produced by administration of Lorenzo's oil was associated with a reduced risk of developing magnetic resonance imaging (MRI) abnormalities (59).

When the central nervous system abnormalities have appeared, attempts at supplementation with glycerol trioleate and glycerol triercate improve the condition only temporarily. Bone marrow transplantation has also been attempted but without clear success.

Zellweger Syndrome

Whereas ALD/AMN is an X-linked trait, Zellweger syndrome is autosomal recessive (60). In addition, the biochemical defect is different: in ALD/AMN there is a normal number of peroxisomes which cannot be translocated but in Zellweger syndrome their number is markedly decreased due to an abnormal biogenesis (61). The greater the deficit, the worse the clinical picture will be due to an abnormal biogenesis. The clinical spectrum includes the Zellweger syndrome itself which has the most severe phenotype (cerebro-hepato-renal form) and the milder forms of Infantile Refsum's Disease.

The genetic basis is a mutation of an acyl-CoA oxidase gene which is required for the formation of peroxisomes. The gene has been named PEX-1; it encodes a 143 kDa ATPase. The nature and locus of the mutations are responsible for the various allelic forms (62).

Wolman Disease (Acid Lipase Deficiency)

Wolman disease is caused by a deficiency of lysosomal acid lipase (63). This enzyme is an esterase that hydrolyzes cholesterol esters and triglycerides. In such patients the normal esterified lipids accumulate in various cells. It is also called generalized xanthomatosis with calcified adrenals (64). Symptoms occur in the first month of life as failure to thrive, anemia, hepatomegaly, and splenomegaly. There is also vomiting and diarrhea as well as jaundice. Shortly thereafter, one can notice the calcified, enlarged adrenal gland, and decreased cortisol secretion. The locus for lysosomal acid lipase deficiency has been mapped to chromosome 10 (65). This is an autosomal recessive trait. There is no treatment for this extremely rare disorder, which ends in death rapidly.

PRIMARY HYPOADRENOCORTICISM—ACQUIRED FORMS

The adrenal receives its blood supply from a network of arteriols coming from the capsule of the gland. Local trauma or acute infection can produce a massive subcapsular hemorrhage resulting in loss of adrenal function. Infectious disorders, noninfectious disorders, as well as tumors can destroy the adrenal cortex. If the processes are bilateral, it will result in signs of acquired hypoadrenocorticism (Table 3).

Massive Bilateral Adrenal Hemorrhage

Adrenal Hemorrhage of the Newborn

Adrenal hemorrhage occurs more often after a prolonged labor and traumatic delivery, usually of a large male infant. The normal adrenal has a rich network of small vessels between the capsule and the cortex. This is the site of bleeding in adrenal hemorrhage.

Children with massive bilateral adrenal hemorrhage appear in acute shock caused by adrenal insufficiency and incipient blood loss. On the other hand, if the hemorrhage is unilateral, there are usually no adrenal symptoms. The typical laboratory finding is hypoglycemia with hyponatremic, hyperkalemic acidosis. On physical examination a mass can be felt in the flanks. Sonography reveals a mass that tends to displace the kidney downward (66). Residual calcification may be visible on X ray of the abdomen three to six weeks after the bleeding occurred and as the hemorrhage resolves. With time, the calcifications themselves disappear. The differential diagnosis includes renal vein thrombosis. An ACTH test is useful in differentiating this condition from bilateral adrenal hemorrhage.

Experience shows that bilateral hemorrhage are extremely rare. Ultrasound screening of abdomen in neonatal period has shown that adrenal hemorrhage are unilateral in most of the time (67,68). Furthermore, many cases were asymptomatic and regressed spontaneously. It has also been observed that many neonatal suprarenal masses were localized neuroblastoma (69).

Adrenal Hemorrhage of Acute Infection

An adrenal crisis may occur during an acute infection, such as fulminating meningococcemia, pneumococcal, streptococcal, Haemophilus, and diphtheria infections. The acute adrenal insufficiency occurring with meningococcemia has also been called Waterhouse–Friderichsen syndrome. The subcapsular hemorrhage is thought to be related to the effects of arterioantitoxin.

Such an acute adrenal crisis occurring at the time of a fulminating infection has an extremely poor prognosis. It is clear that rapid and energetic treatment of the infection, as well as therapy with adrenal steroids in stress dose (IV Solucortef) is necessary.

Recent reports have demonstrated that *Staphylococcus aureus sepsis* can be accompanied by the Waterhouse–Frederichsen syndrome (70) and purpura fulminans (71).

Thrombocytopenia and Heparin Therapy

Clearly any bleeding disorder or heparin treatment can result in adrenal subcapsular hemorrhage. In such cases, an MRI or computed tomography (CT) will show a markedly enlarged adrenal. Only in bilateral hemorrhage would we expect adrenal insufficiency.

Infectious Disorders

In the days of Thomas Addison's report of 1855, tuberculosis was the most common pathogenesis of primary AD (72). Other infections have been reported, such as fungal infections (histoplasmosis and coccidiomycosis). Recently, it was also reported associated with human immunodeficiency virus infection (73). At present, the most important mechanism of destruction of the adrenals is an autoimmune disorder (45). Addison's disease is much less frequent in children than in adult subjects (Table 5).

Addison's Disease—Clinical Features

The clinical features are directly related to the decreased production of adrenal steroids. All or some of the symptoms of adrenal insufficiency outlined in Table 5 may appear. Initially, there is usually general fatigue, muscle pain, weight loss, gastrointestinal symptoms, and hypotension related to salt loss. An acute adrenal crisis may occur at the time of a minor infection or febrile illness. The skin hyperpigmentation is distributed mainly in pressure areas (axillae and groin), as well as in the buccal and vaginal mucosa, the creases of the hand, and the nipples. The hyperpigmentation is related to the increased pituitary secretion of b-lipotropin, which occurs concomitantly with the increased ACTH secretion.

Depending on the cause of Addison's disease, some other specific symptoms can be expected. In cases involving infectious agent, there are also signs of this infection. If the cause is an autoimmune disorder, positive adrenal antibodies will be detected.

Table 5 Signs and Symptoms of Adrenal Insufficiency

Glucocorticoid deficiency
Fasting hypoglycemia
Increased insulin sensitivity
Decreased gastric acidity
Gastrointestinal symptoms (nausea, vomiting)
Fatigue
Mineralocorticoid deficiency
Muscle weakness
Weight loss
Fatigue
Nausea, vomiting, anorexia
Salt craving
Hypotension
Hyperkalemia, hyponatremia, acidosis
Adrenal androgen deficiency
Decreased pubic and axillary hair
Decreased libido
Increased b-lipotropin levels
Hyperpigmentation

Addison's Disease—Diagnosis

The diagnosis of Addison's disease is made based on the demonstration of a low cortisol concentration in plasma concomitantly with elevated ACTH levels. Early in the development of the disorder, the cortisol levels may be normal because of adrenal hyperstimulation by high levels of ACTH. The short ACTH test described earlier shows a low or normal cortisol baseline but no increase on ACTH stimulation.

Addison's Disease—Treatment

The typical treatment is replacement of the missing hormones, specifically glucocorticoids, and mineralocorticoids. In children, the glucocorticoids are replaced by oral cortisol or prednisolone (Table 6), whereas, mineralocorticoids are replaced with Florinef (fludrocortisone). In adolescent girls and adult women, the lack of adrenal androgens may need to be compensated for by administration of a mild androgenic preparation to improve the libido and the growth of pubic hair.

Noninfectious Disorders and Adrenal Tumors

Sarcoidosis, Histiocytosis, Amyloidosis, and Scleroderma can affect the adrenal cortex and suppress its hormonal function (74). However, these abnormalities must be bilateral in order to result in hypoadrenocorticism.

Table 6 Maintenance and Stress Dosage of Glucocorticoid and Mineralocorticoid in Treatment of Adrenal Insufficiency

Therapy	Mean	Range	Stress
Glucocorticoid replacement ($mg/m^2/24\,hr$)			
Oral cortisol (one-third dose every 8 hr)	18	(12–24)	35–55
Oral prednisolone (one-half dose every 12 hr)	2.5	(2–4)	5–7.5
Oral prednisone (one-half dose every 12 hr)	3.0	(2–4)	6–9
Mineralocorticoid replacement (mg/day)			
Oral fludrocortisone Acetate (Florinef)	0.1	(0.05–0.125)	0.1

Adrenal tumors can also destroy the adrenal cortex (75). In the United States, adrenocortical tumors are extremely rare. Furthermore, most of these tumors are functional, resulting in increased cortisol, or androgen secretion, or a combination of both. To cause hypoadrenocorticism, the tumor would have to be nonfunctional and bilateral.

SECONDARY HYPOADRENOCORTICISM DUE TO DEFICIENT CRH AND/OR ACTH SECRETION

The hypothalamus secretes the CRH which reaches directly the anterior pituitary via the portal vessel system.

Hypopituitarism is characterized by a deficient secretion of one, some, or all pituitary hormones (76). In infancy and childhood, the main deficiencies are those of growth hormone, thyroid-stimulating hormone, and ACTH (77). A deficiency of gonadotropins and prolactin is detected only at puberty. In this chapter we will consider ACTH deficiency (Table 7).

Hypothalamic Disorders

Various congenital malformations of the brain, particularly midline defects, can result in a deficiency of secretion of hypothalamic hormones, including CRH. The most frequently recognized malformation is the septo-optic dysplasia as originally described by de Morsier. This condition includes agenesis of the septum pellucidum, hypoplasia or aplasia of optic nerves and chiasma resulting in various degrees of visual impairment, and abnormality of the hypothalamus causing secondary hypopituitarism. The midline defects may be mild, with partial hypopituitarism and no eye disorder. It may also be extensive and associated with cleft palate and cleft lip. An absence of gonadotropin secretion during fetal life may result in micropenis in male infants.

Various brain tumors or their surgical/radiation treatment can result in hypothalamic lesions including destruction of CRH producing cells.

Table 7 Classification of Secondary Hypoadrenocorticism

Hypothalamus
Congenital malformations (some forms of septo-optic dysplasia)
Tumors and tumor treatment (surgery, radiation)
Histiocytosis X, sarcoidosis, hemochromatosis
Pituitary
Congenital aplasia/hypoplasia
Tumor (craniopharyngioma)
Tumor treatment (surgery, radiation)
Hypophysitis
Cessation of glucocorticoid therapy
Removal of a unilateral adrenal tumor
Infants born to mothers treated with glucocorticoid
Respiratory distress syndrome of the newborn
Anencephaly

Disorders that result in the destruction of normal tissues (hemochromatosis, sarcoidosis, and histiocytosis) can cause hypothalamic and/or pituitary dysfunction. A similar situation can occur following various types of infections, such as meningitis or encephalitis.

Prolonged therapy with high doses of glucocorticoids result in suppression of CRH/ACTH secretion; cessation of therapy produces a secondary hypoadrenocorticism which will be discussed below.

Pituitary Disorders

Pathophysiology

Congenital malformation of the pituitary gland can also occur and may be the cause of empty sella turcica. Head trauma either at delivery or later in life may result in hemorrhage in the area of the hypothalamus or pituitary gland. This in turn may cause hypopituitarism with ACTH deficiency.

Tumors arising in the sella (craniopharyngioma) or the hypothalamus likewise result in hypopituitarism. Radiation of a brain tumor may have the same effect. In some patients, no specific cause for the hypopituitarism can be detected.

Such cases can involve several hormones of the anterior pituitary (idiopathic panhypopituitarism), or on rare occasions, only ACTH secretion (idiopathic isolated ACTH deficiency).

Clinical Manifestations

The congenital malformations described earlier can be manifested by hypoglycemia if ACTH and cortisol secretions are deficient. The hypoglycemia may be more marked if there is concomitant growth hormone secretion deficiency. Persistent hypoglycemia in a newborn requires the collection of a blood sample for the determination of true glucose, cortisol, growth hormone, and insulin concentrations. In hypopituitarism the concentration of all these hormones is very low. In contrast, in nesidioblastosis, the concentration of insulin, growth hormone, and cortisol are usually elevated concomitantly with low glucose levels.

Aldosterone secretion is controlled by the renin-angiotensin system, so that there are usually no electrolyte or water abnormalities in hypopituitarism. When a destructive process has taken place, however, there can be involvement of the neuronal cells that secrete antidiuretic hormone (ADH), resulting in a disturbance of electrolytes and water balance characteristic of diabetes insipidus. In addition, following neurosurgery, inappropriate ADH secretion can occur that results in electrolyte abnormalities.

By definition, patients with idiopathic isolated ACTH deficiency have normal thyroid function as well as normal growth and normal sexual maturation at puberty (78). Because females rely on adrenal androgens for the developement of pubic hair at puberty, women with idiopathic isolated ACTH deficiency present scant pubic hair.

Laboratory Diagnosis

It is expected that a decreased cortisol secretion would result in hypoglycemia. Indeed, hypoglycemia is marked in patients who have a combined deficiency of ACTH and growth hormone but is usually mild in the absence of growth hormone deficiency. A low cortisol concentration in an 8 AM blood sample may be observed, but values are often low-normal to normal.

A single oral dose of Metopirone (metyrapone) given at midnight and an 8 AM plasma sample obtained the next morning show a decreased response of plasma 11-deoxycortisol (compound S) in subjects with ACTH deficiency.

However, many subjects can respond to the marked stress resulting from the administration if IV Metopirone or IV pyrogen (79,80). When reviewing a large group of patients with hypopituitarism, Brasel et al. (81) found that about 50% of the patients had normal adrenocortical function; most of the others had normal basal cortisol but a poor response to the regular Metopirone test. Among the latter subjects, only those who had an organic lesion could not respond to the IV Metopirone test. The 1 ug ACTH test is particularly helpful in detecting the mild adrenal insufficiency characteristic of panhypopituitarism. Finally, in hypopituitarism, it is important to check the function of all pituitary hormones.

When the diagnosis of hypopituitarism is established, MRI of the head may show some of the characteristics of septo-optic dysplasia or other brain malformation or injury. In head trauma, the MRI shows evidence of hemorrhage. Brain tumors can also be visualized by MRI studies.

Various degrees of impairment of mental development can be present, particularly in hypopituitarism secondary to meningitis, or encephalitis. Because hypopituitarism can involve various combinations of tropic hormones, the clinical manifestations in childhood may include symptoms of growth hormone deficiency and hypothyroidism.

Treatment

Treatment consists of appropriate replacement of the deficient hormones. If basal cortisol levels are abnormally low, maintenance as well as stress therapy is required. When basal levels are normal, then stress therapy only is needed. In addition, patients who have thyroid-stimulating hormone and or growth hormone deficiency need appropriate replacement. Finally, in ADH deficiency, treatment with intranasal or oral desmopressin (DDAVP) is necessary.

Cessation of Glucocorticoid Therapy

It is well established that the administration of glucocorticoids suppresses the secretion of CRH by the hypothalamus and of ACTH by the pituitary gland, resulting in secondary adrenal cortex atrophy (82). However, when the dosage is less than the replacement level, for whatever period of time, or if the dosage is greater than replacement but for a duration of less than four weeks, no adrenocortical atrophy is expected. By contrast, if the dosage is greater than replacement and the duration of treatment is greater than four weeks, suppression is expected. Experience has shown that recovery occurs within six weeks in about half of patients and within six months of all subjects (83).

Glucocorticoid therapy is often administered orally. However, it can also be given topically. Many skin conditions are treated with various types of glucocorticoid creams. Some of these preparations include very potent steroids such as Betamethasone. If applied in large amounts for long periods on large skin areas, as in children with persistent eczema, it can result in full suppression of the hypothalamic-pituitary-adrenal axis and sometimes is signs of Cushing syndrome.

Similarly, ophthalmologic preparations contain high concentrations of potent synthetic glucocorticoids. Prolonged use will also result in partial or complete adrenal suppression (84).

Inhaled glucocorticoids have become widely recognized as the preferred therapeutic modality for persistent asthma but they can produce suppression of endogenous cortisol secretion (85,86). Treatment should aim at optimal disease control and normal development and growth. This can be achieved in the majority of cases with doses of inhaled budesonide ranging from 200 to 400 μg/day administered in one or two daily doses; however, the use of higher doses for the treatment of severe asthma could lead to suppression of the hypothalamic-pituitary-adrenal axis and to growth suppression. Recent studies in asthmatic children following a low dose ACTH test revealed mild adrenal suppression in a quarter of the children treated with moderate doses of inhaled steroids; a 200 μg/day dose of fluticasone propionate caused less adrenal and growth suppression than did a 400 μg/day dose of budesonide. An 800 μg of inhaled budosenide only once daily in the morning had a sparing effect on short term growth and collagen turnover, when compared with 400 μg administered twice daily (86,87).

Measurement of serum DHEA sulfate concentrations were recently shown to be a practical method to evaluate adrenocortical function and to detect its suppression during inhaled steroid treatment (88).

Clinical Features

Hypoglycemia may be observed in some patients. Growth delay is mainly related to the duration and dose of glucocorticoid therapy rather than its cessation. The greatest problem may occur at the time of a major medical or surgical stress. Prolonged use of glucocorticoids can result in dependence and addiction (82). In such cases, withdrawal of treatment may result in nonspecific symptoms.

Laboratory Tests

A 1 μg ACTH test is simple and informative.

Treatment

Clearly there is no need for treatment in subjects who have been treated for less than four weeks or for those who have received less than replacement therapy for any period of time. For other patients, treatment is required only at times of stress using a dosage equivalent to two to three times replacement and only for the period of stress. Because more than 90% of subjects recover adrenocortical function after six months of cessation of therapy, additional stress doses of steroid are not required after this period of time.

Removal of a Unilateral Adrenal Tumor

Tumors that produce excessive amounts of cortisol usually secrete steroids independently of ACTH stimulation. For this reason, such a subject usually presents with suppressed CRH/ACTH secretion and an atrophic contralateral adrenal. During the surgical removal of such tumors, the patient should receive stress dosages of glucocorticoids. After surgery, the dosage should be decreased progressively and the patient should then be considered as are those discussed earlier for cessation of glucocorticoid therapy.

Infants Born to Mothers Treated with Glucocorticoids

Cortisol administered during pregnancy can cross the placenta, but the fetal concentration is only about 10% of maternal levels. This is in part because the placenta is rich in the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) that transforms cortisol into cortisone. This same enzyme transforms prednisolone into prednisone. Experience has shown that pregnant women treated with prednisone at a dosage of two to five times replacement therapy gave birth to infants whose cortisol secretion rate was normal shortly after birth (89). Nevertheless, such infants should be evaluated for the possible development of hypoglycemia. Because of the physiology of the control of aldosterone secretion, no electrolyte abnormality is expected in infants born of mothers treated with glucocorticoids. In contrast to cortisol or prednisone, dexamethasone readily crosses from the mother to the fetus and is used for fetal therapy.

In contrast, dexamethasone can readily cross the placenta and suppress fetal adrenal function.

Respiratory Distress Syndrome

There are some differences of opinion on the optimal use of surfactant and glucocorticoids in the treatment of respiratory distress syndrome. If steroids are used, dexamethasone is the choice because it readily crosses the placenta. Therapy may be continued for a period of time in the neonatal period and may therefore result in suppressed adrenocortical function when treatment is stopped (90). In such cases, therapy might be needed at times of stress.

Anencephaly

Adrenal glands are very small in patients with anencephaly, probably secondary to the absence of pituitary tissue, leading to adrenal insufficiency.

HYPOADRENOCORTICISM SECONDARY TO END-ORGAN UNRESPONSIVENESS

Most hormones effect their actions by binding either to cell membrane receptors or intracellular receptors. CRH binds to its membrane receptor of the pituitary cells in order to permit ACTH secretion. In turn, ACTH binds to receptors of cells of the adrenals to induce cortisol secretion. Finally, cortisol binds to its nuclear receptors in order to affect its multiple functions. The gene for this receptor protein is localized on 5q31–32 (91). A similar scheme is present for aldosterone and its effects.

Theoretically, any mutation of the gene encoding CRH or its receptor, ACTH or its receptor, cortisol or its receptor will result in hypoadrenocorticism. This chapter discusses adrenocortical unresponsiveness to ACTH, cortisol resistance and aldosterone resistance (Table 8).

Congenital Adrenocortical Unresponsiveness to Adrenocorticotropic Hormone

ACTH insensitivity has been shown to be familial (92,93) and congenital (94,95). It is due to a mutation of the ACTH-receptor gene.

Pathogenesis

This disorder is caused by an inability of the adrenal cells of the zona fasciculata and reticularis to respond to ACTH. The lack of ACTH response is caused by a genetic abnormality of the ACTH receptor (96).

Clinical Manifestations

It is characterized by feeding problems in early life, failure to thrive, hypoglycemia, and hyperpigmentation of the skin (94,95). In some cases, the symptoms seem to occur later in infancy, perhaps because the frequent feedings of the neonatal period prevented major hypoglycemic episodes.

Table 8 Hypoadrenocorticism Due to End-Organ Unresponsiveness

Corticotropin-releasing hormone unresponsiveness
Adrenocorticotropic hormone unresponsiveness
Cortisol resistance
Aldosterone resistance

Laboratory

As already noted, glucose levels are quite low but there is no electrolyte abnormality. During a hypoglycemic episode, a blood sample demonstrates high growth hormone, and low insulin concentrations with low cortisol levels. ACTH measurement shows elevated values, even when cortisol values are normal.

Genetics

It has been shown that unresponsiveness to ACTH is an autosomal recessive trait.

In 1992, Mountjoy et al. (97) isolated a gene for ACTH-receptor and one for 5-alpha-MSH. Later, it was found that these two genes were part of a family of receptors for the various MSHs and named ''MC''. MC2 is specific to the adrenal for ACTH. The other MCs bind the alpha, beta, and gamma MSH is at various degrees in various tissues, as well as ACTH.

Multiple gene mutations have been reported in cases of ACTH insensitivity (96,98,99). Heterozygous subjects for these mutations do not present any abnormality of adrenal function.

It is of interest that about half of the families exhibiting ACTH insensitivity do not present any mutation of the MC2 gene, suggesting that other genetic abnormalities can result in a similar phenotype.

Treatment

Patients require only glucocorticoid replacement therapy, the mineralocorticoid function being normal.

The Adrenal Insufficiency, Achalesie, and Alacryma Syndrome

Some cases of congenital adrenocortical unresponsiveness to ACTH have been reported associated with various neuropathies. It was suggested that some relationship with AMN could occur.

Other subjects developed sodium loss, later in life suggesting a possible relationship with other syndromes, including autoimmune disorders.

However, within that diversity, a specific association of adrenal insufficiency, achalasia, and alacryma has been recognized in several patients. Hence, this disorder was named ''Tripe A syndrome'' (100,101).

The adrenal insufficiency occurs in childhood along with the alacryma. The achalesia can precede the other signs by one to two years. The hypoglycemia and hyperpigmentation can be very marked. Cortisol replacement therapy is very effective in relieving the symptoms.

The Triple A syndrome is an autosomal recessive trait. Allgrove (100) suggested that the locus was on chromosome 12q13 (102). Further investigations suggested a locus at 9q12. Various authors have theorized that the candidate gene should probably related to the type 2 keratin gene family (103).

Cortisol Resistance

This is a rare disorder that has been discovered in a small number of families (104,105). In all cases the resistance appeared partial; none of the affected subjects completely lacked glucocorticoid activity. The main laboratory characteristics of cortisol resistance are markedly increased plasma concentrations of cortisol and ACTH, as well as elevated excretion of urinary free cortisol and total 17-OHCS. In addition, a standard dexamethasone suppression test is partially negative. Such laboratory findings are typical of patients with Cushing's disease, yet subjects with cortisol resistance do not present with any of the symptoms of this disorder. The elevated levels of ACTH result in increased secretion not only of cortisol but also of DOC and corticostrone. These two steroids are responsible for the signs of mineralocorticoid excess, including hypertention, hypokalemia, and metabolic alkalosis. As previously mentioned, the genesis of the resistance syndrome is related to an abnormal glucocorticoid receptor. The gene for this receptor has been mapped to chromosome 5q31-q32 (91). In one family, a point mutation was found in the receptor gene. In all the families reported with this condition, the mode of inheritance has been found to be autosomal dominant.

Aldosterone Resistance

Aldosterone resistance is most probably a heterogeneous group of disorders that express themselves clinically as an unresponsiveness of the kidney to aldosterone. One of the first patients described in 1958 by Cheek and Perry (12) presented with a salt-losing syndrome that responded poorly to mineralocorticoid therapy but was adequately corrected by sodium chloride supplementation. Most of these patients have shown an improvement with age and often did not require further therapy after one or two years of age. In other affected subjects, however, therapy had to be continued.

Aldosterone resistance represents at least two different entities because autosomal dominant and autosomal recessive modes of inheritance have been reported (106,107). The gene encoding the mineralocorticoid receptor was cloned and mapped to chromosome 4q31 (108). This receptor and that of glucocorticoids are remarkably similar in structure (109,110). This new knowledge should help us in understanding better the various forms of this disorder.

TREATMENT OF HYPOADRENOCORTICISM

AD is often recognized because of an acute adrenal crisis. Such crisis needs immediate treatment; otherwise it can be lethal in very few days. Later, maintenance for glucocorticoids and mineralocorticoid therapy must be instituted. Finally, stress conditions must be treated with two to three times the maintenance dose.

Acute Adrenal Insufficiency

In the acute adrenal crisis, a deficiency of cortisol and aldosterone as well as dehydration must be treated. Fluid and electrolyte replacement to expand blood volume and increase blood pressure must be instituted immediately, particularly in the neonate or small child, who otherwise may decompensate rapidly. During the first hour of therapy, the patient should receive 20 ml/kg, 0.9% sodium chloride in 5% glucose solution. The IV solution should then be continued to deliver 60 ml/kg over the following 24 hours.

With this therapy, serum electrolytes improve, with plasma concentrations of sodium and chloride returning to normal but serum potassium often remaining elevated. At some time during the period of fluid replacement therapy, steroid replacement must be instituted. Cortisol sodium succinate (Solucortef) is given as a IV bolus at a dosage of 25 mg/m^2 and is followed by a similar dose added to the 24 hours IV maintenance fluid solution. Twenty to thirty-five milligrams Solucortef has a mineralocorticoid activity equivalent to 0.1 mg Florinef. After the acute crisis, maintenance therapy is instituted.

Maintenance Therapy

Glucocorticoid Replacement

Cortisol is the drug of choice because it is the major glucocorticoid secreted physiologically by the adrenal cortex. In infants and children, synthetic preparations with high potency are not recommended for replacement treatment because their proper dosage is difficult to adjust. Furthermore, cortisol has some mineralocorticoid activity, whereas the synthetic preparations have little or none. On the basis of a cortisol secretion rate of 6 to 12 mg/m^2/24 hr, a dosage similar to this secretion given over a 24 hours infusion should be a maintenance dose. The oral dosage is approximately twice the physiological secretion rate, because some oral cortisol is destroyed by the gastric acidity. Because of the short half-life of cortisol, the total 24 hours dose must be given in thirds of every eight hours (Table 6).

Experience has shown that children of school age and adolescents have problems remembering to take the midday dose. Prednisone, which has a longer half-life than cortisol, can be used; half of the daily dose being given every 12 hours (Table 6). It is about six times more potent than cortisol. Prednisolone is the only glucocorticoid available presently in liquid form. It is about seventimes more potent than cortisol. There are two commercial preparations: Pediapred contains 1 mg prednisolone per 1 ml; Prelone contains 5 mg prednisolone per 1 ml.

Mineralocorticoid Replacement

As previously noted, the secretion rate of aldosterone in human subjects is similar from two weeks of age to adulthood. Therefore, replacement therapy remains constant regardless of the age of the patient. The only preparation available is 9 alpha-fluoro-cortisol acetate (fludrocortisone, Florinef). It is given orally as a single dose of 0.05 to 0.150 mg/24 hr. Mineralocorticoid therapy is effective only if salt is ingested simultaneously. Infant formula are very low in salt (8–10 mEq/day), and such patients may require a modest sodium chloride supplement (15–30 mEq/day), particularly when they show serum electrolyte abnormalities and elevated plasma renin levels. When the infants start eating regular table food, the additional salt becomes unnecessary.

Therapy During Stress

Subjects who receive glucocorticoid therapy for more than one month have an unresponsive hypothalamic-pituitary-adrenal axis. As a result, they require additional glucocorticoids during stress. For minor infection with low-grade fever, increased medication may not be required. In moderate stress the dosage is increased to twice maintenance, and in more severe stress to three to four times replacement (Table 3). If a patient is unable to retain oral therapy during an acute illness, the parents must administer an IM injection of Solucortef (50 mg/m^2). Following the IM injection, the parents are advised to discuss the problem with their physician or to attend the hospital emergency room. If this cannot be done within eight hours following the first injection, another IM Solucortef is required every eight hours.

Treatment at the Time of Surgery

At present we recommend using Solucortef, A dose of 50 mg/m^2 is given IV just before the start of anesthesia. This is followed by a second dose of 50 mg/m^2 administered as a constant infusion throughout the surgical procedure. A third 50 mg/m^2 dose of Solucortef is then given at a constant rate for the rest of the first 24 hours period. During the time that the patient is unable to take oral treatment, constant infusions of Solucortef are continued at 50 to 75 mg/m^2/24 hr.

During neurosurgery, patients are given large amounts of dexamethasone to avoid brain edema. Although dexamethasone is a very potent glucocorticoid, it has no mineralocorticoid effect.

In both medical and surgical stress it is important to limit the time of increased glucocorticoid dosage to the period of acute stress and to return to maintenance therapy as soon as improvement occurs. Otherwise, the patient may be overtreated and will then present with symptoms of Cushing's disease.

ISOLATED MINERALOCORTICOID DEFICIENCY CAUSED BY MUTATIONS OF CYP11B2 GENE

As previously discussed, the cytochrome P450 encoded by the CYP11B2 gene is capable of making

the conversion of DOC to aldosterone by a series of three enzymatic steps: addition of the 11β-hydroxyl group to form corticosterone, 18-hydroxylation (also called corticosterone methyloxidase type I) to form 18-hydroxycorticosterone, and 18-dehydrogenation (also called corticosterone methyloxidase type II) to form aldosterone. Although the same enzyme appears to be involved, two types of aldosterone deficiency, 18-hydroxylase, and 18-dehydroxylase have been reported in the literature.

18-Alpha-Hydroxylase Deficiency

These patients present with a general failure to thrive related to mild salt wasting. Laboratory studies show decreased aldosterone and 18-hydroxycorticosterone with a concomitantly elevated secretion of corticosterone and DOC (111,112). Mineralocorticoid replacement therapy results in the resumption of normal growth in infancy and early childhood. Later in life, treatment becomes unnecessary.

18-Dehydrogenase Deficiency

The onset of this disorder is also in infancy or early childhood. It is characterized by a failure to thrive and symptoms of mineralocorticoid deficiency, including hyponatremia, hyperkalemia, and acidosis. However, these patients rarely present in acute adrenal crisis. This is probably because some of the precursors of aldosterone that have some sodium-retaining activity are produced in increased amount. Hormonal study shows an increase in plasma renin activity as well as an increase in DOC, corticosterone, and 18-hydroxycorticosterone, along with low aldosterone (113). It is of interest that these patients present with an increased excretion of urinary 17-OHCS; this is because the urinary metabolites of 18-hydroxycorticosterone give the Porter–Silber reaction (114). A very large Iranian Jewish pedigree has been reported with this salt-wasting disorder (115,116). Recently mutations in the CYP11B2 gene were reported by Pascoe et al. (117). Treatment consists of mineralocorticoid replacement therapy. It is of interest to note that later in life many of these patients have successfully withdrawn from treatment.

STEROID SULFATASE DEFICIENCY (X-LINKED ICHTHYOSIS)

This is an X-linked trait expressed only in male subjects, the mothers who are hemizygotus, being normal.

A deficiency of steroid sulfatase results in the accumulation of sulfated products, such as 3β-hydroxysteroids (particularly DHEA-S) and cholesterol sulfate. The ichtyosis is thought to be related to the accumulation in the skin of a large amount of cholesterol sulfate (118). This syndrome is not an adrenocortical insufficiency because cortisol and aldosterone secretions are normal. The normal fetus has low steroid sulfatase activity and relies on the placenta to accomplish this function. Because the placenta is made of maternal cells and at least one of the maternal X chromosomes is normal, the steroid sulfatase activity is adequate during pregnancy.

The steroid sulfatase gene has been mapped to the X-chromosome (Xp22.3) near the pseudoautosomal region of the terminal part of the short arm.

HYPERADRENOCORTICISM

The syndromes of hyperadrenocorticism can be classified in four subgroups, depending on the predominance of a specific type of adrenocortical steroid. In each of these entities, however, levels of other steroids can also be elevated.

- Hypercortisolism, characterized by elevated cortisol secretion.
- Virilized adrenal tumors, characterized by abnormal secretion of adrenocortical androgens.
- Feminizing tumors, related to increased estrogen secretion.
- Hyperaldosteronism, caused by excessive aldosterone secretion.

HYPERCORTISOLISM

Pathophysiology

Except for the iatrogenic hypercortisolism related to glucocorticoid therapy given in pharmacological dosages, this disorder is rare in the newborn period and in childhood. Its frequency increases in adolescence. Harvey Cushing described patients with hypercortisolism related to a pituitary adenoma (119) and the condition has been termed Cushing's disease. When the hypercortisolism is related to an adrenal tumor, it is termed Cushing's syndrome.

Adrenal cortical tumors have an incidence of 0.3 per million children-year, about one-half of them resulting in Cushing's syndrome. In adults and, rarely, in children, malignant tumors not related to an endocrine gland can produce an excessive amount of ACTH, in which case the disorder is called Ectopic ACTH syndrome. On occasion, the tumor may produce CRH, which in turn results in increased ACTH and cortisol secretion. Also in adults, chronic alcoholism has been shown to result in increased CRH secretion, which in turn increases ACTH and cortisol output.

Clinical Manifestations

The symptoms of hypercortisolism are similar whatever its cause. Because of the ubiquitous effects of glucocorticoid on general metabolism, the symptoms are multiple and varied (Table 9) (120).

In muscles, the breakdown of proteins and decreased glucose uptake results in major muscle wasting.

Table 9 Effects of Hypercortisolism on Some Body Systems

Effects	Laboratory	Symptoms
On muscle cells		
Increased breakdown of proteins	Increased amino-acids and negative nitrogen balance	Muscle wasting
Decreased glucose uptake		
Decreased glycogen concentrations		
On fat cells		
Increased lipolytic enzymes	Hyperlipidemia Hypercholesterolemia	Redistribution of fat (truncal obesity)
On carbohydrate metabolism in liver		
Increased gluconeogenic	Hyperglycemia	Insulin-resistant
Enzymes and AA transferases	Glycosuria	Diabetes
On bones		
Decrease osteoblast activity	Hypokalemia compensated by increased PTH	Osteopenia
Increase osteoclast activity		Growth retardation
Decrease gut Ca absorption		
Increase renal Ca excretion		
On electrolyte and water metabolism		
K loss	Hypokalemia	Muscle weakness
Na retention	Hypernatremia (slight)	Hypertension

In fat cells, the increased lipolysis produces a lipid increase in blood. This in turn, results in redistribution of body fat with typical troncular obesity-abdomen, chest, cervical fat pad (''buffalo hump''). The increased fat in the face produces the ''moon facies'' appearance. In older children and adults, the troncular obesity contrasts with the muscle wasting of the limbs. However, in infants, the obesity is usually generalized.

The gluconeogenesis results in hyperglycemia, glycosuria, and insulin-resistant diabetes.

Hypercortisolism has multiple effects on bones and Ca metabolism. There is suppression of osteoblastic activity with increased resorption of protein bone matrix. An increased renal Ca excretion combined with decreased Ca absorption from the gut results in hypocalcemia. This hypocalcemia is compensated by a mildly increased PTH secretion. At the same time, the PTH increases the osteoclastic activity. The overall effect is a marked osteopenia.

Because hypercortisolism decreases growth hormone and IGF-1 secretion, there is, with time, a marked statural growth retardation.

Hypercortisolism affects electrolyte metabolism. There is some Na retention which is responsible for the hypertension of Cushing syndrome. The K loss is rather marked, resulting in muscle weakness.

Thinning of skin and formation of purple striae on abdomen, thighs, and buttocks is related to the effect of hypercortisolism on collagen metabolism. Easy bruisability of Cushing is related to the capillary friability.

Hypercortisolism increases gastric acid secretion which is thought to be related to the high frequency of peptic ulcers.

The elevated ACTH secretion that causes a large percentage of cases of Cushing's syndrome increases not only cortisol output but also adrenal androgen secretion. A number of adrenocortical tumors produce increased secretion of both cortisol and adrenal androgen. In these cases, there will also be signs of virilism.

Treatment with pharmacologic amounts of glucocorticoids as well as Cushing's syndrome can, in some subjects, produce emotional instability with depression alternating with euphoria.

Laboratory Diagnosis

Demonstrate the Presence of Hypercortisolism

This is reliably done by obtaining a 24 hours urine specimen and determining both total urinary 17-OHCS and free cortisol. In children and adults, the urinary excretion of 17-OHCS is similar when the values are corrected for body size. The mean $\pm$ SD for urinary 17-OHCS is $2.9 \pm 1.2\,mg/M^2/24\,hr$. The mean $\pm$ SD for urinary free cortisol is $22.5 \pm 4.0\,\mu g/M^2/24\,hr$. Therefore, values of the urinary. 17-OHCS below $5.5\,mg/m^2/24\,hr$ and urinary free cortisol of less than $30\,\mu g/m^2/24\,hr$ rule out hypercortisolism. Among obese subjects, about 30% (Fig. 10) have urinary 17-OHCS values above $5.5\,mg/m^2/24\,hr$ (15) but many of them have normal urinary free cortisol. Such subjects require a low-dose dexamethasone suppression test ($1.25\,mg$ dexamethasone$/m^2/24\,hr$ for two days). A good suppression suggests obesity and lack of it suggests hypercortisolism.

A single evening salivary cortisol has been reported to be a good outpatient screening test for Cushing syndrome. Salivary cortisol reflects the unbound, biologically active serum cortisol, and is therefore not influenced by alterations of cortisol protein binding. It represents 3% to 6% of the total serum cortisol concentration. It seems to have the same diagnostic significance as the 24 hours urinary free cortisol

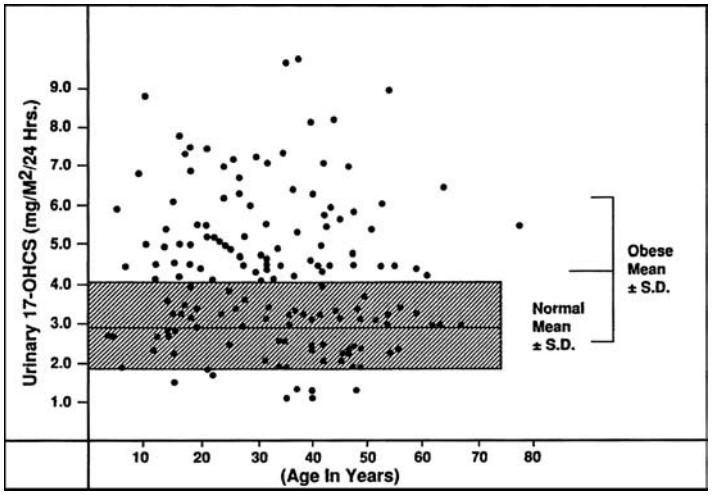

Figure 10 Urinary 17-hydroxycorticosteroids corrected for body surface area (mg/m^2/24 h) in 160 obese individuals of various age. Their values are compared to the mean$\pm$ SD of control subjects (*shaded area*). About 30% of obese subjects had excretions above the control mean $+2$ SD. *Source*: From Ref. 14.

determination and it would seem to be a reliable first line screening test (121).

Determine the Cause of the Disorder

This is done by carrying out a CRH stimulation test (25) and/or a high-dosage dexamethasone suppression test (3.75 mg/m^2/24 hr for two days). The response of plasma ACTH and cortisol to CRH administration is presented in Figure 9. A marked response in the CRH stimulation test and a good suppression in the high-dosage dexamethasone suppression test suggest an hypercortisolism that is pituitary dependent, that is, Cushing's disease. In contrast, a lack of response to the CRH stimulation and a lack of suppression (25) to the high-dosage dexamethasone suppression test strongly favor hypercortisolism being caused by an adrenal tumor or possibly a tumor producing ectopic ACTH.

Magnetic Resonance Imaging of the Head and Abdomen

In theory, MRI of the head will demonstrate the presence of a pituitary adenoma. Unfortunately, the adenoma can be very small and is visualized in about only one-third to one-half of cases. A recent study concluded that "post contrast spoiled-gradient-recalled acquisition in the steady state" MRI is a superior technique in identifying pituitary tumors as it can be performed in thin sections of 1 mm thickness, so that the spatial resolution of the acquired images is substantially improved. This technique has been proven effective in adults and more recently in children and adolescents (122).

MRI or CT of the abdomen visualizes adrenal tumors 1 cm or more in diameter. In hypersecretion of ACTH, bilateral adrenal hyperplasia is observed by MRI in some cases.

Bilateral Petrosal Sinus Sampling

This has been proposed as a means to localize a small pituitary adenoma that is not visible on the MRI scan. Catheters are placed in the left and right inferior petrosal sinuses and blood samples are obtained before and 10 to 30 minutes after administration of CRH (100 μg/m^2). Before CRH administration, high baseline concentrations of ACTH are expected from the side of the tumor. The differential in ACTH increases following CRH helps to determine the location of the microadenoma on the left or right side of the pituitary gland. Although this technique has been reported by some investigators to be successful (123), others have found it to be traumatic for the patient, resulting in a fair amount of body radiation, and not always accurate.

Treatment

Clearly, the treatment is dictated by the cause of the hypercortisolism. The treatment of Cushing's syndrome caused by an adrenal tumor is surgical. An adenoma or well-encapsulated carcinoma without metastasis offers the best opportunity for a complete cure. In contrast, the prognosis of malignant adrenal tumors that produce glucocorticoids is generally poor.

If the adrenal tumor is localized before surgery, a transthoracic approach affords excellent exposure and ease of removal. If the tumor is not well localized, a transperitoneal approach is indicated. Patients with metastasis can be treated with the drug nitrotane (o,p′-DDD), but results have been disappointing and the drug is not always well tolerated. Cisplatin has also been used with limited success.

Autonomous adrenal tumors secrete cortisol in high concentrations and suppress ACTH secretion, resulting in atrophy of the contralateral adrenal gland. Postsurgical treatment of this situation has been discussed earlier.

In Cushing's disease with bilateral adrenal hyperplasia, several forms of therapy have been used. In the past, bilateral adrenalectomy was widely employed, resulting in immediate cure of hypercortisolism but also in adrenal insufficiency that required glucocorticoid and mineralocorticoid replacement for life and the possibility of development of a pituitary tumor (124). Radiation of the sella turcica has also been used extensively in adults but not in children because it often results in destruction of growth hormone-secreting cells; however, newer stereotactic techniques of delivery of radiation have given good results (125).

Transphenoidal microsurgery is now the treatment of choice for the removal of pituitary microadenomas and has been successful in bringing about a complete cure in many patients (126,127). This form of therapy requires an experienced neurosurgical team to obtain the best chances of success (128). In a few patients, surgery has resulted in panhypopituitarism. Recurrence of the pituitary adenoma can also occur (129). Depending on the reports, a recurrence can occur in 10% to 20% of cases. In such cases, conventional fractionated radiotherapy is a therapeutic alternative. Unfortunately, it is associated with complications such as hypopituitarism, cranial nerve neuritis, and visual field defects. Gamma knife radiosurgery uses cobalt-60 to deliver a single high dose of high energy beams targeted to defined intracranial sites. This treatment will be helpful in patients who have failed to achieve hormonal control following pituitary surgery and may also be an initial therapeutic approach in patients with moderate size of microadenomas, sufficiently distant from the optic chiasm. Several studies have demonstrated how gamma knife radiosurgery induces far fewer complications than those noted with conventional radiotherapy (130,131).

Several drugs have been used in the treatment of Cushing's disease. Cyproheptadine, a serotonin antagonist, can suppress ACTH secretion but it is generally not recommended as long-term medical management of the disease and it often does not return the cortisol secretion rate to normal. Bromocriptine, a dopamine agonist, although useful for the treatment of prolactin and growth-hormone-secreting pituitary adenomas, has not been of value in treating ACTH-secreting pituitary tumors. Metopirone and aminoglutethimide, which suppress adrenal secretion, have been recommended only for the short-term, presurgical treatment of Cushing's disease.

Treatment of ectopic ACTH syndrome consists of the removal of the ACTH-secreting tumor. In cases of iatrogenic Cushing's syndrome, stopping the excessive glucocorticoid therapy is recommended.

VIRILIZED ADRENAL TUMORS

Clinical Manifestations

These tumors result in masculinization of prepubertal children. In boys, it produces a pseudoprecocious puberty (132,133). Sexual hair (pubic, axillary, and sometimes facial hair) develops and the penis is enlarged to adult size, with frequent erections. However, the testes remain prepubertal or slightly enlarged. In girls, the masculinizing syndrome is characterized by pubic and axillary hair with an enlarged and erectile clitoris (Fig. 11).

In both boys and girls, the excessive secretion of androgens accelerates growth, with an advancement of height age and bone age along with an increase in muscle mass.

Incidence and Associations

The incidence of all types of adrenal tumors is extremely low. It has been reported as 0.2 per million children-years in Denmark and 0.3 per million children-years in the United States.

In 1997, Sandrini, Ribeiro, and DeLacerda (134) reported a frequency of adrenocortical tumor in the states of Parana' and Sao Paulo in Southern Brazil which was 10 to 15 greater than the world rate. Later studies (135) showed that 35 out of 36 patients had the same germline mutation of the gene of the tumor suppressor protein P53. The locus of P53 gene is on chromosome 17p and the point mutation was in exon 10 encoding an Arg 337Hist amino acid substitution. This mutation appears to interfere with dimerization and tetramerization necessary for the suppression effect. Studies of adrenocortical tumor cells showed a complete absence of wild type P53.

The relatives of children presenting with an adrenocortical tumor and the R337H mutation showed that 34.5% of 695 subjects in the carrier parental line had also the R337H mutation while none of 232 subjects of the other parental line had no mutation (136).

The conclusions of these recent studies are:

- The R337H mutation of P53 greatly increases the risk of adrenocortical carcinomas.
- This mutation did not increase the risk of other cancers.
- About 95% of the patients presented with virilism; in almost half of them, the virilism was associated with signs of hypercortisolism.
- The penetrance of the adrenal tumor is low at 9.9%.
- The regional frequency of the R337H mutation explained the concentration of cases of adrenocortisolism in Southern Brazil.

Adrenal tumors have been associated with several syndromes, specifically, Li–Fraumeni syndrome (137), Beckwith–Wiedemann syndrome, Multiple Endocrine Neoplasia type 1, and body hemihypertrophy.

Adrenal tumors have also been reported in adult patients with CAH. These subjects were poorly treated or had stopped treatment. Indeed, we have seen two CAH adults, untreated for many years, who required adrenalectomy because their adrenals were the size of the kidneys and produced compression.

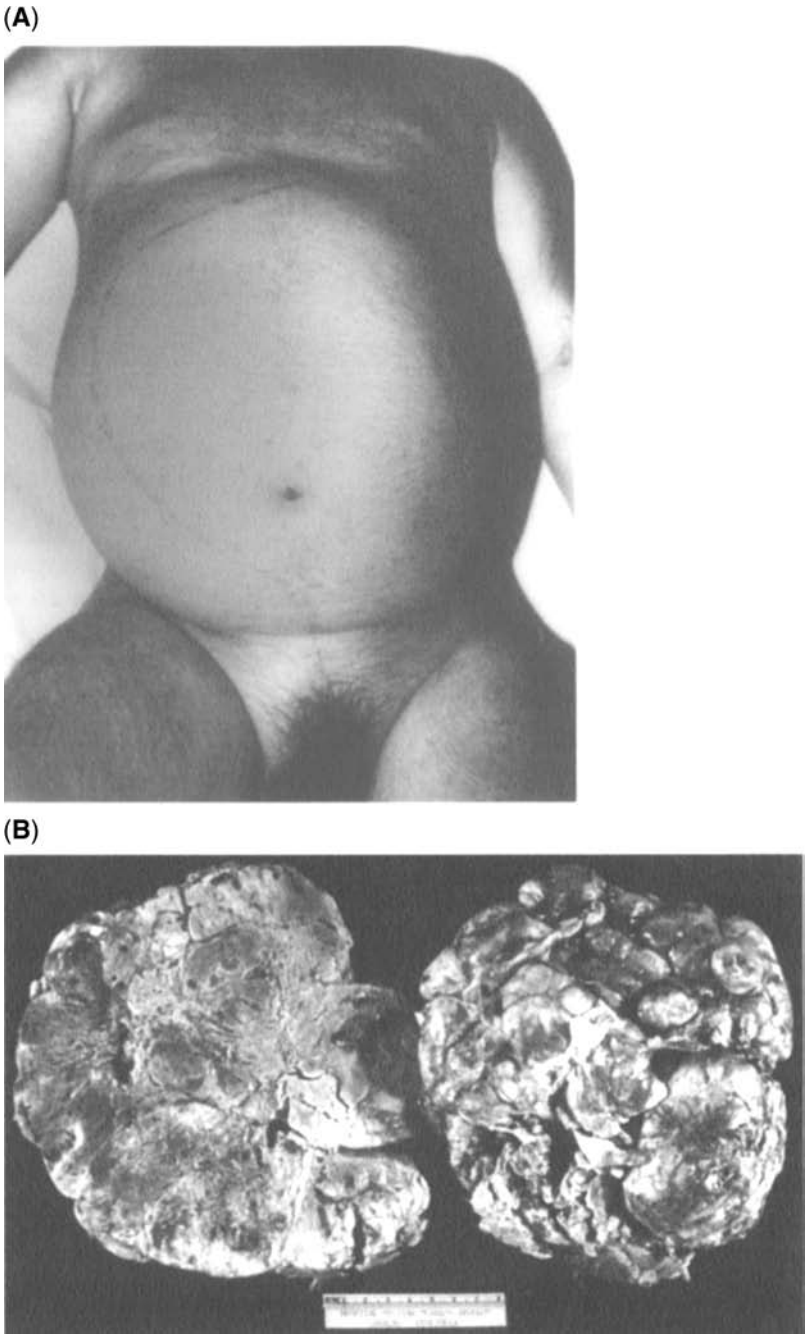

Figure 11 (**A**) Abdomen of a 4-year-old girl with virilizing adrenocorticol carcinoma. (**B**) Surgical specimen. *Source*: From Ref. 106.

Pathology

Virilizing adrenal tumors are usually carcinomas containing malignant cells. Experience shows that the degree of malignancy is low, however, and it is rare to observe metastasis at the time of surgery. In cases of longstanding, the tumor may invade the capsule of the adrenal and may metastasize to the kidneys, liver, lungs, and bones. On occasion, an adrenal adenoma may also be virilizing. However, it is often difficult to determine microscopically whether the tumor is malignant or benign. Moreover, the clinical evolution does not always parallel the pathological appearance.

Laboratory Studies

These tumors secrete large amounts of androgens, as demonstrated by the elevated urinary excretion of total 17-ketosteroids and of DHEA, along with abnormally high plasma concentrations of DHEA, DHEA-S, testosterone, and androstenedione. However, the levels can be quite variable as shown in Table 10.

Table 10 Plasma Concentration of Androgens in Patients with Adrenal Tumors

Patient no.	Unconjugated (ng/dL)			Sulfates (μg/dl)		
	Sex	Age	T	A	DHEA	DHEA-S
A—virilizing	F	1½	1,457	789	450	118
B—virilizing	F	1½	41	249	2,066	926
C—virilizing	F	1½	168	1606	1950	1484
D—feminizing	F	3½	124	130	1086	806
Normal prepubertal			10–20	0–50	0–100	10–60
Normal adult female			23–75	74–230	215–855	82–338

Abbreviations: T, Testosterone; A, Androstenedione; DHEA, Dehydroepioandrosterone.

In contrast, with CAH, the excessive androgen secretion of virilizing adrenal tumors is not suppressed by dexamethasone administration. In premature adrenarche, the increase in DHEA, DHEA-S, and androstenedione is only at or below adult values and can also be suppressed by dexamethasone.

The differential diagnosis also includes virilizing gonadal tumors. In boys, Leydig cell tumors produce mainly testosterone with minimal amounts of DHEA and DHEA-S. In girls, virilizing ovarian tumors occur only after puberty and include the various types of tumors seen in adult women (adrenal rest tumor, hilar cell tumor, and arrhenoblastoma). A CT scan or MRI without and with contrast will be necessary to localize an adrenal mass.

Treatment

The tumor should be excised carefully, without damaging its capsule. Every effort should be made to remove the tumor in bloc. Early surgery can result in full cure. If present, metastases should be removed as completely as possible. In such cases, radiotherapy or chemotherapy with o,p′-DDD, Cisplatinum, or Keotconazole can be attempted. Unfortunately, such chemotherapy is very toxic and of limited usefulness.

FEMINIZING ADRENAL TUMORS

These tumors are even rarer than virilizing adrenal tumors in infants and children. Extrapolating from experience in adults, approximately half of feminizing adrenal tumors are malignant (139).

It would appear that the feminization is related to an increase of aromatase p450 expression in the tumor cells (140).

Clinical Manifestations

In boys, the major sign is gynecomastia; the testes are prepubertal in size, but pubic hair is often present, being related to the concomitant secretion of androgens and estrogens by the tumor. In girls, there is breast development, as seen in precocious puberty; pubic hair can be present, and breakthrough vaginal bleeding may occur. In children of both sexes, there is rapid statural and osseous development, height age, and bone age being significantly advanced.

Pathology

For feminizing adrenal tumors, the diagnosis of malignancy is often difficult.

Laboratory Diagnosis

Urinary and plasma estrogen levels are usually elevated. Most of the tumors also secrete an excess of androgens, resulting in increased urinary 17-ketosteroids and plasma DHEA, DHEA-S, androstenedione, and to some extent, testosterone (Table 10). This excessive secretion of steroids is not dexamethasone suppressible. Often the laboratory results are not characteristic. CT scan and/or MRI is needed to make the definitive diagnosis of adrenal tumor.

Differential Diagnosis

Because of the lack of specificity of the clinical signs and hormone assays, the diagnosis of feminizing adrenal tumor is usually difficult.

Gynecomastia is a physiological finding in pubertal boys, and it is not easy to differentiate the breast enlargement that accompanies precocious puberty in a young boy from that of a feminizing adrenal tumor. A modest increase in testicular size in precocious puberty may not be differentiated from infantile testes in feminizing tumor. An LHRH test may be useful, showing a pubertal increase in LH in precocious puberty.

In prepubertal girls, a feminizing adrenal tumor must be differentiated from premature thelarche and idiopathic sexual precocity. Estrogens are markedly increased in an adrenal tumor but not in premature thelarche. A positive LHRH test should be helpful in determining sexual precocity.

Treatment

The tumor should be removed surgically promptly after the diagnosis has been established. If the tumor did not secrete excessive amounts of cortisol or 11-deoxycortisol, the prognosis is usually good.

HYPERALDOSTERONISM

The various syndromes of hyperaldosteronism are outlined in Table 11. The classification is based on

Table 11 Syndrome of Hyperaldosteronism

With elevated plasma renin activity:
Primary hyper-reninemia
Secondary hyperaldosteronism
Bartter/Gitelman syndrome
With low plasma renin activity
Primary hyperaldosteronism (Conn syndrome)
Dexamethasone suppressible hyperaldosteronism
Apparent mineralocorticoid excess
(Familial 11β-dehydrogenous deficiency)

whether there is an increased plasma renin activity, or if it is suppressed.

Primary Hyperreninemia

The most common cause is renal ischemia, whether unilateral or bilateral. Such ischemia results in excessive secretion of renin by the juxtaglomerular apparatus. Tumors of the juxtaglomerular apparatus have also been reported as a rare cause of renin excess (141).

Secondary Hyperaldosteronism

An increase in plasma renin activity with increased aldosterone secretion is a physiological mechanism for the maintenance of serum electrolyte concentrations and fluid volume. It occurs with sodium loss, potassium retention, or decreased intravascular volume. Sodium loss occurs during diarrhea or excessive sweating. It also happens with administration of diuretics in renal tubular acidosis or salt-losing nephritis. The edema of the nephrotic syndrome or the ascites of cirrhosis of the liver causes a decrease in blood volume that results in compensatory increased aldosterone secretion. In all these conditions, the hyperaldosteronism is an attempt to reestablish an electrolyte-water balance, and this is termed secondary hyperaldosteronism. It may also occur in hypertension related to a unilateral renal disease with increased plasma renin activity.

The increased aldosterone secretion characteristic of the nonsalt-losing form of 21-hydroxylase deficiency can also be considered a secondary hyperaldosteronism because it occurs in response to the salt-losing tendency created by excessive secretion of 17-hydroxyprogesterone and progesterone.

Bartter Syndrome

This disorder is thought to be related to a renal tubular defect of chloride reabsorption. This results in passive loss of sodium, which in turn activates the renin-agiotensin-aldosterone system. In addition, the hyperaldosteronism results in hypokalemic alkalosis.

Clinical Manifestations

Patients usually present in infancy with failure to thrive, vomiting, weakness, and dehydration. Blood pressure is normal. There is hypochloremic metabolic alkalosis with hypokalemia and usually normal blood sodium. Plasma renin activity and aldosterone are elevated. One finds an increased urinary excretion of chloride and potassium with elevated excretion of prostaglandin. Renal biopsy shows hyperplasia of the juxtaglomerular apparatus (142).

Pathophysiology

In the recent past, a great deal of progress has been made in our understanding of the Bartter syndrome.

There are two chloride channel genes with 90% identity which probably arose by gene duplication. The ClCKA is expressed in the thin limb of the Henle Loop, whereas the ClCKB is expressed in the thick ascending limb of the Henle loop (143). To be functional, both chloride channels require the coexpression of Barttin, a small protein with two transmembrane domains. The ClCKB/Barttin system is located on the basolateral side of the cells.

On the apical side of the cells, the system of chlorine transfer requires two genes: the apical sodium, potassium, chloride channel (or NKCC2), and the ROMK gene for potassium out-flow.

The NKCC2 permits the in-flow of sodium-potassium chloride and the ROMK permits the out-flow of excess potassium. The chlorine is reaching the ClCKB/channel-Barttin to be reabsorbed while the Na is reabsorbed by the ATPase system.

It is of interest that mutations of any one of four genes involved in chlorine reabsorption can result in Bartter syndrome. Mutations of NKCC2 results in Bartter type I (144); mutation of ROMK results in type II (145), mutation of ClCKB results in type III (146), and mutation of Barttin results in type IV (147).

Treatment

There is no specific therapy, and an attempt is made to correct the electrolyte abnormalities. In some patients, the use of prostaglandin synthetase inhibitors, such as indomethacin or salicylates, can be beneficial.

Primary Hyperaldosteronism

In 1955, Conn (148) described a disorder termed primary aldosteronism that was caused by an aldosterone-producing tumor of an adrenal gland. The symptoms included arterial hypertension, hypokalemic alkalosis, muscle weakness, and polyuria. Subsequently, it was demonstrated that the plasma renin activity was markedly decreased. This syndrome is encountered mainly in adults.

Among hypertensive subjects with elevated aldosterone secretion, only 10% to 20% of them will present primary hyperaldosteronism.

Clinical Manifestations

The full clinical picture of this disorder is directly related to the hyperaldosteronism. Aldosterone increases potassium excretion, resulting in hypokalemia.

This in turn results in muscle weakness with various types of paresthesias and sometimes unusual types of periodic paralysis. It is thought that the chronic hypokalemia is also responsible for the polyuria and resulting polydipsia. Aldosterone increases the retention of sodium, but the hypernatremia is largely compensated for by increased water retention. The increase in blood volume in turn results in hypertension, both systolic and diastolic.

Laboratory Diagnosis

The typical finding is a high aldosterone secretion with low plasma renin activity. The low plasma renin activity differentiates the syndrome from the high renin levels seen in secondary hyperaldosteronism. A CT scan or MRI is necessary to demonstrate the presence of a mass in one of the adrenals. Because the adrenocortical tumor may be quite small, it may not be seen by MRI. Catheterization of the renal veins and selective adrenal vein sampling for measurement of aldosterone may demonstrate a very large secretion on one side and a lack of aldosterone on the other.

Pathology

Usually, the tumor is an adenoma, but on occasion it can be a carcinoma. There can also be bilateral nodular hyperplasia of the adrenal cortex or focal hyperplasia with glomerular cells arranged in a nodular fashion. Nodular hyperplasia is often the cause of primary hyperaldosteronism.

Treatment

The adrenal tumor should be removed. Because it does not secrete cortisol, there is usually no need for glucocorticoid therapy during surgery.

In bilateral nodular hyperplasia, bilateral adrenectomy may be considered. In such cases, however, medical treatment with spironolactone, an inhibitor of aldosterone biosynthesis, is preferable if it can control the hypertension.

Dexamethasone-Suppressible Hyperaldosteronism (Glucocorticoid-Remediable Aldtosteronism)

In 1966, Sutherland et al. (149) reported a clinical condition characterized by hypertension, increased aldosterone secretion, and low plasma renin activity that was fully relieved by administration of dexamethasone (150). Most recent progress in the molecular biology of steroid biosynthesis has been able to demonstrate that the disorder was related to a chimeric gene encoding for cytochrome P450 possessing aldosterone synthetase activity. It is inherited as an autosomal dominant trait.

Clinical Manifestations

Symptoms and laboratory abnormalities in this disorder are identical to those found in primary hyperaldosteronism caused by an adrenal adenoma (151). They include hypertension, hypokalemia, elevated plasma aldosterone concentrations, and low plasma renin activity. However, and in contrast to primary hyperaldosteronism, the hypertension, hypokalemia, increased aldosterone secretion, and low plasma renin activity can be returned to normal by the administration of dexamethasone. It has been noted that affected subjects always present with hypertension, but the degree of hypokalemia is variable.

Pathogenesis

Two genes CYP11B1 and CYP11B2, encode for a cytochrome P450 possessing 11β-hydroxylase activity. However, CYP11B1 is mainly expressed in the zona fasciculata, under ACTH control, and CYP11B2 is mainly expressed in the zona glomerulosa, under angiotensin II control. The product of the CYP11B2 gene is also called aldosterone synthetase, which is capable of activating 18-hydroxylation and 18-oxidation in addition to 11β-hydroxylation. Both genes are located on chromosome 8q22.

It was recently proposed that a hybrid gene, CYP11B1/CYP11B2, as shown in Figure 12, is the cause of the disorder (152–154). The promoter of the hybrid gene is derived from the CYP11B1 and is therefore responsive to ACTH, but the chimeric protein encoded by this gene has the function of the aldosterone synthetase, hence, the reason for the ACTH-dependent hyperaldosteronism. Because the chimeric gene is expressed in the zone fasiculata, which also expresses the CYP17 gene, there can be secretion of cortisol, which has been hydroxylated in the carbon 18. Indeed, the secretion of 18-hydroxy and 18-keto-cortisol is characteristic of dexamethasone-suppressible hyperaldosteronism. A series of recent reports has identified such a chimeric gene, which includes the 5′ sequences of the CYP11B1 gene and the 3′ sequences from the CYP11B2 gene.

Treatment

As expressed by the name of the syndrome, dexamethasone, or other glucocorticoid administration in replacement dosages turns off ACTH secretion as well as cortisol output. Although gluco-steroid-remediable aldosteronism remains rare as a cause of hypertension in children, a recent report (152) suggested that it should be considered in severe cases with a positive family history of early-onset hypertension.

Familial Deficiency of 11β-Dehydrogenase

This congenital disorder is characterized by an apparent mineralocorticoid excess (155,156). A gene encoding 11β-dehydrogenase has been cloned and mapped to chromosome 1 (157). This enzyme has the property of metabolizing cortisol into cortisone. In

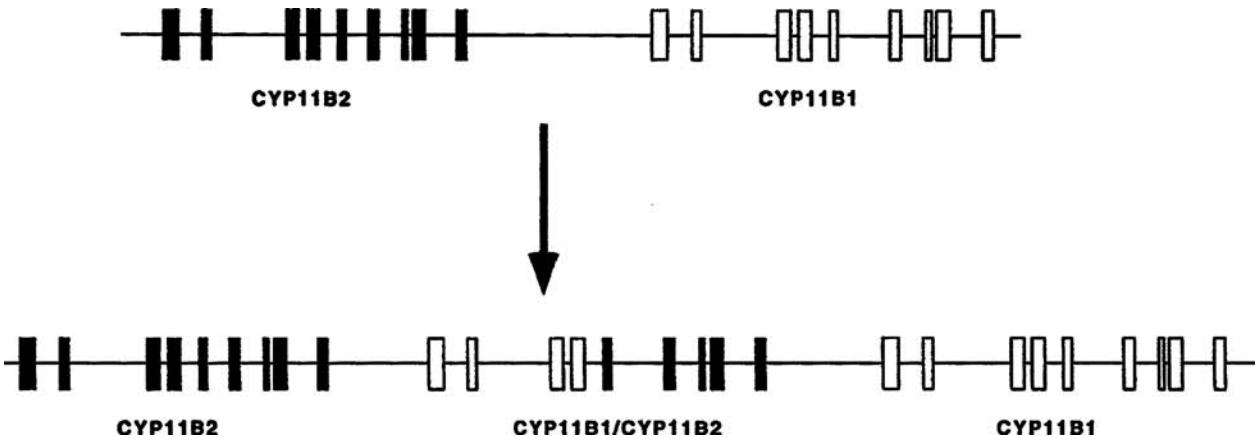

Figure 12 Gene organization of *CYP11B1* and *CYP11B2* genes (*upper part*) and organization of the hybrid *CYP11B1/CYP11B2* gene resulting from unequal crossing over (*lower part*). The resulting hybrid gene can be stimulated by ACTH as it includes the promotor of the B1 gene and can from aldosterone as it includes the 3′ end of the B2 gene. *Source*: From Ref. 2.

blood, the ratio of cortisol to cortisone is 5:1 to 10:1. In contrast, the kidney is rich in 11β-dehydrogenase, and only cortisone is found in this organ (158,159). Under physiological conditions, the receptor protein for mineralocorticoids has equal affinity for cortisol and aldosterone, but cortisone does not bind.

Pathophysiology

In this syndrome, a deficiency of 11β-HSD in the kidneys results in binding of cortisol to the mineralocorticoid receptor. In view of the large concentration of cortisol relative to aldosterone, there is sodium retention and water retention. The increased blood volume results in hypertension, and the increased mineralocorticoid activity generates hypokalemia. Under such conditions, plasma concentrations of aldosterone, and renin activity are low.

Treatment

The severe hypertension in this disorder is generally resistant to any medical therapy. Dexamethasone binds to the glucocorticoid receptor with high activity but does not interact with the mineralocorticoid receptor. The full suppression of cortisol secretion by dexamethasone does not improve the hypertension, however, suggesting that the increased mineralocorticoid activity of the syndrome is mediated by both the renal glucocorticoid receptor and the mineralocorticoid receptor (160).

It has been reported that subjects who ingest large amounts of licorice develop hypertension and hypokalemia, with low levels of plasma aldosterone and plasma renin activity. It has been shown (161) that, licorice contains glycyrrhizic acid, which has the property of inhibiting 11β-HSD activity.

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9

An Update of Congenital Adrenal Hyperplasia

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INTRODUCTION

Congenital adrenal hyperplasia (CAH) is a family of inherited disorders of adrenal steroidogenesis. Each of these disorders results from a deficiency in one of the several enzymatic steps necessary for normal steroid synthesis. Since the earliest case of CAH documented in 1865 by the Neapolitan anatomist De Crecchio (1), numerous investigators have unraveled the mechanisms of adrenal steroid synthesis and the associated enzyme defects responsible for the clinical syndromes. This report includes recent advances in the investigation and understanding of these disorders.

PATHOPHYSIOLOGY

The adrenal glands synthesize three main classes of hormones: mineralocorticoids, glucocorticoids, and sex steroids. Figure 1 shows a simplified scheme of the adrenal synthesis of these steroids from the cholesterol precursor molecule. Each enzymatic step is indicated.

The pituitary regulates adrenal steroidogenesis via adrenocorticotropic hormone (ACTH). ACTH stimulates steroid synthesis by acting on the adrenals to increase the conversion of cholesterol to pregnenolone, the principal substrate for the steroidogenic pathways. The central nervous system controls the secretion of ACTH, its diurnal variation, and its increase in stress via corticotropin-releasing factor (2,3). The hypothalamic–pituitary–adrenal feedback system is mediated through the circulating level of plasma cortisol; any condition that decreases cortisol secretion results in increased ACTH secretion. Cortisol therefore exerts a negative feedback effect on ACTH secretion.

In most forms of CAH, an enzyme defect reduces cortisol synthesis, thus impairing cortisol-mediated negative feedback control of ACTH secretion (Fig. 2). Oversecretion of ACTH ensues, which stimulates excessive synthesis of the adrenal products of those pathways unimpaired by the enzyme deficiency and causes an accumulation of precursor molecules. The enzyme deficiency of 21-hydroxylase is the most common type of CAH, accounting for 90% to 95% of all cases, and 11β-hydroxylase deficiency is the second most common, occurring in 5% to 8% of cases.

The clinical symptoms of the different forms of CAH result from both the hormones that are deficient and those that are produced in excess. In the most common case, that of 21-hydroxylase deficiency, the aldosterone and cortisol pathways are blocked and the androgen pathway, which does not involve 21-hydroxylation, is overstimulated. The characteristic virilization caused by 21-hydroxylase deficiency is due to excessive secretion of adrenal androgens.

As in 21-hydroxylase deficiency, an enzymatic deficiency of 11β-hydroxylase also results in decreased cortisol synthesis with consequent overproduction of cortisol precursors and sex steroids, again producing virilization. In contrast to 21-hydroxylase deficiency, however, excess accumulation of the aldosterone precursor deoxycorticosterone (DOC), a steroid with salt-retaining activity, causes the additional finding of hypertension in many patients with 11β-hydroxylase deficiency.

Disorders of adrenal steroidogenesis have also been described in association with deficiencies of 3β-hydroxysteroid dehydrogenase, 17α-hydroxylase/17, 20-lyase, and steroidogenic acute regulatory protein (StAR). A summary of the biochemical features of these disorders is presented in Table 1.

CLINICAL FEATURES

The most prominent clinical feature of both 21- and 11β-hydroxylase deficiency is virilization. Because

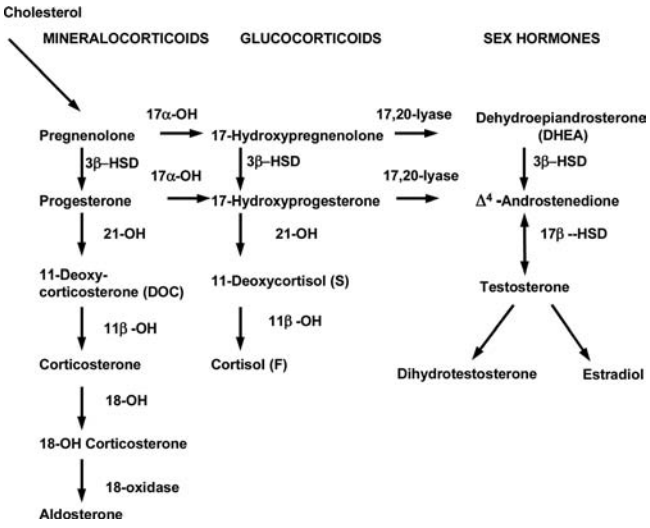

Figure 1 Simplified scheme of adrenal steroidogenesis showing abnormal secretion of hormones in CAH resulting from 21-hydroxylase deficiency. *Abbreviations*: CAH, congenital adrenal hyperplasia; OH, hydroxylase; HSD, hydroxysteroid dehydrogenase.

adrenocortical function begins by month 3 of gestation, a fetus with 21- or 11β-hydroxylase deficiency is exposed to oversecreted adrenal androgens at the critical time of sexual differentiation. In a female fetus, the excessive adrenal androgens masculinize the external genitalia and female pseudohermaphroditism results. In rare cases, the masculinization is so profound that the urethra is penile (5). The internal genitalia (i.e., uterus and fallopian tubes), which arise from the müllerian ducts, are normal because the female fetus does not possess Sertoli cells of the testes, the source of müllerian inhibiting factor. The female

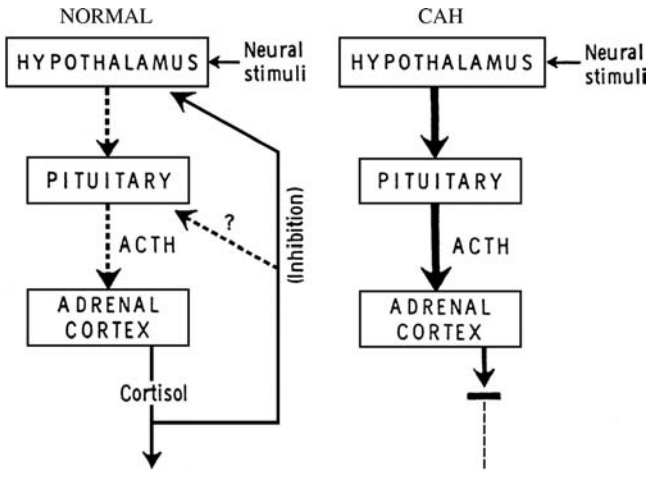

Figure 2 Regulation of cortisol secretion in normal subjects and in patients with CAH. *Abbreviation*: CAH, congenital adrenal hyperplasia. *Source*: From Ref. 4.

genital abnormalities are present only in the androgen-responsive external genitalia. Males with 21- or 11β-hydroxylase deficiency do not manifest genital abnormalities at birth but may demonstrate hyperpigmentation.

The simple virilizing form of 21-hydroxylase deficiency is characterized by excess adrenal androgen secretion, which causes prenatal virilization of the genetic female and postnatal virilization of both boys and girls. In the salt-wasting form, besides the excess adrenal androgens, there is aldosterone deficiency causing low serum sodium, high serum potassium, and vascular collapse. Both newborn boys and girls with this form of CAH are subject to early, life-threatening, salt-wasting crises within the first few weeks of life.

The various clinical and biochemical features associated with the different forms of CAH are indicated in Table 1. Continued oversecretion of adrenal androgens as a result of untreated 21- or 11β-hydroxylase deficiency results in progressive penile or clitoral enlargement; advanced bone age and tall stature in early childhood with ultimate short stature caused by premature epiphyseal closure; early appearance of facial, axillary and pubic hair; and acne. In 11β-hydroxylase deficiency, hypertension is frequently, although not necessarily, an additional finding. Girls with CAH who are not treated may not develop breasts or spontaneously menstruate. Further virilization may include hirsutism, male habitus, deepening of the voice, or male-pattern alopecia. In untreated males, the testes may remain small due to suppression of gonadotropins. They may also develop intratesticular adrenal rests, which can cause infertility, although some untreated men have been fertile (6,7).

In 3β-hydroxysteroid dehydrogenase deficiency (3β-HSD), steroid synthesis in both the adrenal cortex and in the gonads is affected, and only Δ^5 steroid precursors are formed and secreted. Circulating Δ^5 precursors undergo conversion peripherally to active Δ^4 steroids and are believed to cause virilization of the external genitalia in genetic females with 3β-HSD. Genetic males have incomplete external genital development due to deficient Δ^4 androgen production in the gonads (8,9). Thus, genital ambiguity results in both sexes. Similar to 21- and 11β-hydroxylase deficiency, the appearance of the external genitalia at birth does not predict the severity of the enzyme defect. There have been reports of a milder, nonclassical form of 3β-HSD deficiency, however, mutations in the gene for 3β-HSD have yet to be identified in these patients (10,11).

Steroid 17α-hydroxylase/17,20-lyase deficiency, which accounts for approximately 1% of all CAH cases, affects steroid synthesis in the adrenals and gonads (12). Patients have impaired cortisol synthesis, leading to elevated ACTH which increases serum levels of DOC and corticosterone, resulting in low-renin hypertension, hypokalemia, and metabolic alkalosis. Affected females are born with normal external genitalia. Affected males are born with

Table 1 Clinical and Laboratory Features of Various Disorders of Adrenal Steroidogenesis

Deficiency (syndrome)	Genital ambiguity	Postnatal virilization	Salt metabolism	Diagnostic hormones	Treatment
21-Hydroxylase				17-Hydroxyprogesterone (17OHP)	Hydrocortisone (HC), 15–20 mg/m/day orally (PO), and fludrocortisone acetate (9αFF), 0.05–0.2 mg/day PO
Classical Salt-wasting (SW)	F	Yes	Salt-wasting	Δ^4-Androstenedione (Δ^4-A) Aldosterone	
Simple virilizing	F	Yes	Normal (↑ renin)	17-OHP, Δ^4-A	HC (same); addition of 9αFF (same) if ↑ renin
Nonclassical (symptomatic and asymptomatic)	No	Yes	Normal	17-OHP, Δ^4-A	HC, 10–15 mg/m/day or dexamethasone, 0.25–0.5 mg/day h.s., or prednisone 5–10 mg/day
3β-Hydroxysteroid dehydrogenase					
Classical	M (±F)	Yes	Salt-wasting	17-OHP 17-hydroxypregnenolone (Δ^5 17-OHP) Dehydroepiandrosterone (DHEA) Δ^4 –A	HC and 9αFF as for SW 21-hydroxylase deficiency
Nonclassical	No	Yes	Normal	Δ^5 17-OHP DHEA	HC as for nonclassic 21-hydroxylase deficiency
11β-Hydroxylase classical (hypertensive congenital adrenal hyperplasia)	F	Yes	Salt retention (PRA↓)	DOC 11-deoxycortisol (S) Δ^4-A plasma renin activity	HC, 15–20 mg/m/day
Nonclassical	No	Yes	Normal	S DOC	HC, dexamethasone, or prednisone as for nonclassic 21-hydroxylase deficiency
17α-Hydroxylase/17,20-lyase	M	No	Salt retention (PRA↓)	DOC, corticosterone (B)	HC, 15–20 mg/m/day[a]
StAR (congenital lipoid hyperplasia)	M	No	Salt-wasting	None	HC, 15–20 mg/m/day 9αFF, 0.05–0.2 mg/day[a]

[a]With addition of sex steroid replacement at puberty.

Abbreviations: DOC, deoxycorticosterone; StAR, steroidogenic acute regulatory protein; PRA, plasma renin activity.

under-virilized genitalia due to deficient testosterone production. 17α-Hydroxylase/17,20-lyase deficiency is often recognized at puberty in female patients who fail to develop secondary sex characteristics.

Congenital lipoid adrenal hyperplasia is an extremely rare and severe form of CAH in which cholesterol is not converted to pregnenolone. Deficient uptake of cholesterol into the mitochondria leads to profoundly impaired synthesis of all adrenal and gonadal steroids. The term ''lipoid'' refers to the accumulation of cholesterol and cholesterol esters in the adrenocortical tissue. Recent studies have revealed that abnormalities of the StAR are responsible for this disorder (13,14). StAR is involved in the transfer of cholesterol from the outer to the inner mitochondrial membrane, the rate-limiting step in steroidogenesis. Males with congenital lipoid hyperplasia are born with female-appearing external genitalia. The lack of virilization in genetic males is twofold. Initially there is an absence of StAR-dependent steroidogenesis in the adrenals and testes, although small amounts of steroid can be made through StAR-independent steroidogenesis. However, this process then stimulates the uptake of more extracellular cholesterol, which accumulates in the testes and adrenal glands, thereby damaging the cells and destroying any ability of steroidogenesis. In contrast, genetic females have normal female genitalia at birth, and because the ovaries produce no steroids until puberty, they undergo spontaneous feminization at puberty from StAR-independent steroidogenesis. They subsequently accumulate cholesterol in the ovaries, resulting in cellular damage and the eventual loss of any further steroidogenesis. All patients present with salt-wasting, and if not detected and treated, lipoid CAH is usually fatal in infancy (15).

CLINICAL FORMS OF ADRENAL HYPERPLASIA CAUSED BY 21-HYDROXYLASE DEFICIENCY

Two major phenotypes are recognized in 21-hydroxylase deficiency: classical and nonclassical (late onset; Fig. 3). Within the latter class of patients are those who demonstrate the biochemical defect but lack any overt stigmata of hyperandrogenism. Table 2 delineates the differences between classical and nonclassical 21-hydroxylase deficiency.

Classical

Classical CAH is a well-known genetic disorder transmitted by an autosomal recessive gene. The biochemical and clinical abnormalities of this form of CAH are clearly present in patients both prenatally and postnatally. Deficiency of the enzyme 21-hydroxylase impairs cortisol synthesis, thus stimulating increased secretion of corticotropin-releasing hormone and ACTH, and excess production of precursors such

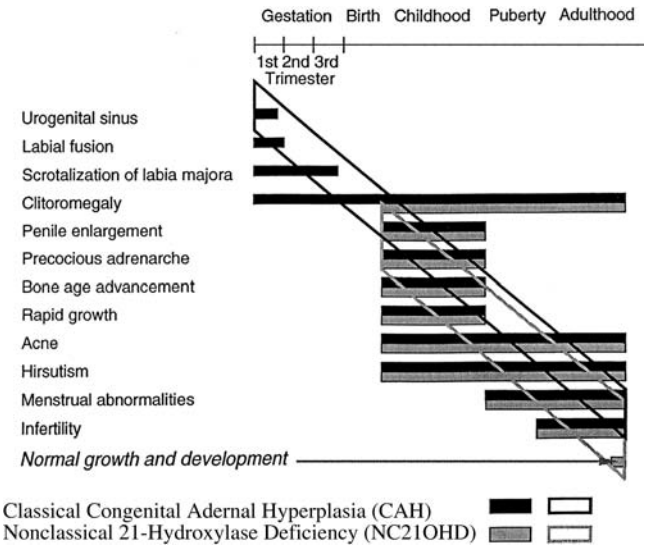

Figure 3 Clinical spectrum of steroid 21-hydroxylase deficiency. There is a wide spectrum of clinical presentation in 21-hydroxylase deficiency, ranging from prenatal virilization with labial fusion to precocious adrenarche, to pubertal or postpubertal virilization. During their lifetimes, patients may change from symptomatic to asymptomatic. *Source:* From Ref. 16.

as progesterone and 17-hydroxyprogesterone. These precursors are then channeled into the androgen pathway, which does not require 21-hydroxylase (17–24). As expected, the urinary excretion of the metabolites of these steroids is also increased (25,26). Abnormalities in cortisol secretion are also associated with alterations in the secretion of other pituitary hormones such as GH and TSH (27,28).

In genetic females with congenital 21-hydroxylase deficiency, the developing fetus is exposed to excessive adrenal androgens, equivalent to the male fetal levels, secreted by the hyperplastic adrenal cortex. External genitalia in the genetic female range from mildly ambiguous to completely virilized. The internal genitalia (uterus and fallopian tubes) are not affected by the excess androgens. Boys with 21-hydroxylase deficiency do not manifest genital abnormalities at birth. Postnatally, in untreated boys and girls, continued excessive androgen production results in rapid somatic growth, advanced epiphyseal maturation, progressive penile or clitoral enlargement, early appearance of facial, axillary, and pubic hair, and acne. Without treatment, early epiphyseal closure and short stature result (29).

In three-quarters of cases with classical 21-hydroxylase deficiency, salt-wasting occurs, characterized by hyponatremia, hyperkalemia, inappropriate natriuresis, and low serum and urinary aldosterone with concomitantly high plasma renin activity (PRA). The increase in the proportion of salt-wasting cases in recent years may be attributed in part to enhanced ascertainment because of advancements in diagnostic capabilities, as well as increased survival because of the availability of exogenous mineralocorticoid supplements. Salt-wasting results from inadequate secretion of salt-retaining steroids, particularly aldosterone. In addition, hormonal precursors of 21-hydroxylase may act as mineralocorticoid antagonists in the marginally competent sodium-conserving mechanism of the immature newborn renal tubule (30–33). It has been observed that an aldosterone biosynthetic defect apparent in infancy may be ameliorated with age (33,34) and spontaneous partial recovery from

Table 2 Comparison of Classical and Nonclassical 21-Hydroxylase Deficiency

Feature	Classical	Nonclassical
Disease frequency	1:14,000	1:100 all Caucasians 1:27 Ashkenazi Jews
Prenatal virilization	Females	No
Postnatal virilization	Males and females	Variable
Salt-wasting	60–75% cases	No
17-Hydroxyprogesterone levels after adrenocorticotropic hormone challenge	Extreme elevation ($>$ 20,000 ng/dl)	Moderate elevation (2,000–15,000 ng/dl)
Genotype of CYP21	Severe allele/severe allele Occasionally severe/mild	Mild allele/mild allele Mild allele/severe allele
Associated HLA haplotype	B47;DR7	B14;DR1
Common mutations:		
Simple virilizing	I172N Intron 2, A→G	V281L P30L P453S
Salt-wasting	Deletion Lg. Conversion Intron 2, A→G Exon 3, -8 bp Codons 234–238 Q318 → Stop codon R356W	

salt-wasting in adulthood has been described in patients with severe salt-wasting in infancy. This variation in the ability to produce mineralocorticoids may be attributable to another adrenal enzyme with 21-hydroxylase activity (35). Therefore, it is desirable to follow the sodium and mineralocorticoid requirements carefully by measuring PRA or direct renin concentration in patients who have been labeled neonatally as salt-wasters.

Although it has been claimed that salt-wasting correlates with severe virilism (36), it is important to recognize that the extent of virilism may be the same in simple-virilizing and salt-wasting CAH. Thus, even a mildly virilized newborn with 21-hydroxylase deficiency should be observed carefully for signs of a potentially life-threatening crisis within the first few weeks of life.

Nonclassical 21-Hydroxylase Deficiency

An attenuated, late-onset form of adrenal hyperplasia was first suspected by gynecologists in clinical practice who used glucocorticoids for the treatment of women with physical signs of hyperandrogenism, including infertility (37,38). The first documentation of suppression of 21-hydroxylase precursors in the urine of such individuals after glucocorticoid therapy was by Baulieu and co-workers in 1957 (39). The precise diagnosis of a mild 21-hydroxylase defect was made possible when a radioimmunoassay for 17-hydroxyprogesterone (17-OHP), the direct precursor of the enzyme in the adrenal zona fasciculata, was developed (40). The autosomal recessive mode of genetic transmission of the nonclassical form of 21-hydroxylase deficiency (NC21-OHD) became apparent through family studies of classical 21-OHD (41–43). The establishment of linkage to HLA (44,45) confirmed the existence of this disorder as an allele of classical 21-OHD (41,46). The HLA associations for nonclassical 21-OHD (44,47,48) are distinct from those found in classical 21-OHD and differ according to ethnicity (45,49,50).

The clinical symptomatology of NC21-OHD is variable and may present at any age. NC21-OHD can result in premature development of pubic hair in children; to our knowledge, the youngest such patient was noted to have pubic hair at six months of age (42). In a review of 23 cases presenting for evaluation of premature pubarche, seven children demonstrated a 17-OHP response to ACTH stimulation consistent with the diagnosis of nonclassical 21-hydroxylase deficiency, a prevalence of 30% in this pre-selected group of pediatric patients at high risk (51). Other investigators found 7 of 46 children (15%) with premature pubarche demonstrating an ACTH-stimulated 17-OHP response greater than that of obligate heterozygote carriers of the 21-hydroxylase deficiency gene (52). Elevated adrenal androgens promote accelerated linear growth velocity accompanied by early fusion of epiphyseal growth plates in children with this disorder, frequently resulting in a shorter than expected height based on mid-parental height (53).

Severe cystic acne refractory to oral antibiotics and retinoic acid has been attributed to NC21-OHD. In one study of 31 young female patients with acne and/or hirsutism tested with low-dose ACTH stimulation after overnight dexamethasone suppression, no cases of 21-hydroxylase deficiency were found (54). In another study comparing the responses of 11 female patients with acne and eight (female) control subjects to a 24-hour infusion of ACTH, elevated urinary excretion of pregnanetriol in six patients was suggestive of a partial 21-hydroxylase deficiency (55).

Additionally, male pattern alopecia has been noted as the presenting symptom with or without hirsutism in young women with this disorder (56–58). Menarche may be normal or delayed, and secondary amenorrhea is a frequent occurrence. The syndrome of polycystic ovarian disease includes a subgroup of women with NC21-OHD. The pathophysiology of this phenomenon probably relates to adrenal sex steroid excess disrupting the usual cyclicity of gonadotropin release and/or the direct effects of adrenal androgens upon the ovary, leading ultimately to the formation of ovarian cysts, which then may autonomously produce androgens.

Retrospective analysis of the etiologies of hirsutism and oligomenorrhea revealed that 16 of 108 (14%) young women presenting to our group for endocrinologic evaluation of these complaints had nonclassical 21-hydroxylase deficiency (59). In other published series, the prevalence of nonclassical 21-hydroxylase deficiency in hirsute, oligomenorrheic women ranged from 1.2% to 30% (60–64). The disparity in frequency of nonclassical 21-hydroxylase deficiency reported by different authors may be attributed to differences in the ethnic groups studied because the disease frequency is ethnic-specific. The frequency of nonclassical 21-hydroxylase deficiency is 1:27 in Ashkenazi Jews, 1:53 in Hispanics, 1:63 in Slavics, 1:333 in Italians, and 1:100 in the general Caucasian population (49).

In boys with NC-21OHD, early signs of puberty such as facial hair, acne, axillary hair or odor, and growth acceleration may be present. A highly reliable constellation of physical signs of adrenal (as opposed to testicular) androgen excess in boys is the presence of pubic hair, enlarged phallus, and relatively small testes. In men, signs of androgen excess are difficult to appreciate and may theoretically be manifest only by short stature and/or adrenal sex steroid-induced suppression of the hypothalamic–pituitary–gonadal axis, resulting in diminished fertility.

Oligospermia and subfertility have been reported in men with NC-21OHD (65,66). Reversal of infertility with glucocorticoid treatment in three men has been reported (66–68).

The presence of 21-hydroxylase deficiency is sometimes uncovered during the evaluation of incidental adrenal masses (69). An increased incidence

of adrenal incidentalomas has in fact been found in male and female patients homozygous for CAH (82%) and also in heterozygote subjects (45%), probably arising from hyperplastic tissue areas and not requiring surgical intervention (70).

A subset of NC-21OHD individuals are overtly asymptomatic when detected (usually as part of a family study), but it is thought, based on longitudinal follow-up of such patients, that symptoms of hyperandrogenism may wax and wane with time. The gene defect in these so-called cryptic 21-hydroxylase-deficient subjects is the same as that found in symptomatic nonclassical patients.

PUBERTAL MATURATION IN CLASSICAL CONGENITAL ADRENAL HYPERPLASIA

Onset of Puberty

In most patients treated satisfactorily from early life, the onset of puberty in both girls and boys with classical CAH occurs at the expected chronological age (71–74). The pattern of gonadotropin response to luteinizing hormone-releasing hormone (LHRH) is appropriate for age in prepubertal and pubertal girls with well-controlled CAH (75,76). Physiologic secretion of gonadotropins, however, may not be entirely normal (77,78).

True precocious puberty may occur in some well-treated children with CAH, perhaps correlated with bone age. Another setting in which central puberty sometimes occurs in CAH is after initiation of glucocorticoid therapy, producing a sudden decrease in sex steroid levels and leading to hypothalamic activation. LHRH analogs may be employed as an adjunct to therapy with hydrocortisone in such children (79). Long-term data on final height in CAH patients suggest that LHRH analogs (80,81) along with growth hormone treatment (82,83) are not only effective in arresting the pubertal process but also improve final height.

In most untreated or poorly treated adolescent girls and in some adolescent boys, spontaneous true pubertal development does not occur until proper treatment is instituted (Table 3) (72,73,84–86). Studies suggest that excess adrenal androgens (aromatized to estrogens) inhibit the pubertal pattern of gonadotropin secretion by the hypothalamic–pituitary axis (72). The inhibition probably occurs via a negative feedback effect; whether it is primarily at the hypothalamus or pituitary is not known. This inhibition is reversible by suppression of the adrenal hormone production by glucocorticoid treatment.

Following onset of puberty, in the majority of successfully treated patients, the milestones of further development of secondary sex characteristics in general appear to be normal (71,74), although a somewhat delayed sequence of pubertal events may be present in girls (71).

Menstrual Disorders

Many patients with treated classical CAH have regular menses after menarche (72,73,87). However, data from various clinics suggest that menarche was significantly delayed in patients treated with glucocorticoids, especially when those patients who were not menstruating after 16 years of age were included. Data regarding menarche were not observed in untreated patients (71–74,88,89).

Menstrual irregularity and secondary amenorrhea with or without hirsutism are not uncommon complications in postmenarchal girls (6,73,74,88,90,91). These menstrual abnormalities have been found frequently in patients with inadequately controlled disease (Table 3) (71,72,74,87,88,91). Several studies subsequently reported menarche or the normalization of the menstrual cycle following adequate suppression of adrenal sex steroids with long-acting and more potent glucocorticoid treatment (88,91,92). Delayed menarche or even primary amenorrhea may result from poor treatment or overtreatment. In poorly treated patients, the mechanism for delayed menarche may be interference by adrenal sex steroids in the cyclicity of the hypothalamic–pituitary–ovarian axis (71,72). The delayed menarche in patients who are overtreated may be related to the delay in bone age and general maturation known to occur with excessive glucocorticoid treatment (71).

Many treated women have had successful pregnancies with the delivery of a normal, healthy, full-term infant (72,93,94). A recent study reports successful pregnancy outcomes in four women with

Table 3 Pubertal Disorders in 21-Hydroxylase Deficiency Congenital Adrenal Hyperplasia

Puberty	Classic: abnormal (poorly treated or untreated)	Classic: normal	Nonclassic: abnormal	Cryptic: normal
Girls	No thelarche; no menarche; secondary amenorrhea or menstrual irregularity; cystic ovaries, anovulation, infertility	None reported	Precocious adrenarche, hirsutism, cystic acne; amenorrhea or menstrual irregularity; anovulation, infertility, cystic ovaries	No abnormalities
Boys	Small testes[a] Decreased spermatogenesis	Normal testicular size Spermatogenesis	Precocious adrenarche Cystic acne	No abnormalities

[a]Adolescent males may have nodular testes as a result of adrenal rest tumor.

classical CAH (one simple virilizing and three salt-wasting) (95). All four glucocorticoid-treated mothers delivered unaffected and non-virilized females. Additionally, a retrospective survey of fertility rates in a large group of women affected with 21-hydroxylase deficiency showed that simple virilizers were more likely than salt-wasters to become pregnant and carry the pregnancy to term (96). Adequacy of glucocorticoid therapy is probably an important variable with respect to fertility outcome (97). Among all patients questioned, only 50% reported that the vaginal introitus was adequate for intercourse, 5% reported homosexual preference, and 38% had no sexual experience. Based on these data, it seems prudent to perform early surgical correction of clitoromegaly but to delay vaginoplasty until adolescence (when the patient can be expected to assume responsibility for vaginal dilatation and strict adherence to medical therapy).

The clinical observation of gonadal function as described earlier clearly suggests that excess adrenal sex steroid production is the major contributing factor to gonadal dysfunction, menstrual disorders, anovulation, and infertility in girls with classical CAH. The generally accepted theory is that the excessive adrenal androgens may disrupt gonadotropin secretion, leading ultimately to hypogonadism (74,77,85,89).

Male Reproductive Function

Several long-term studies indicate that in a majority of successfully treated male patients with CAH, pubertal development, normal testicular function, and normal spermatogenesis and fertility occur (6,73,74,98–100). However, complications of small testes and aspermia have been reported in some patients with inadequately controlled disease (6,73,86,101). In contrast to this observation, some investigators have reported normal testicular maturation and normal spermatogenesis and fertility in patients who had never received glucocorticoid treatment (6,84,99,102,103) or in those whose glucocorticoid therapy was discontinued for several years (6,99). Thus, male patients with CAH and excessive adrenal androgens may have either normal gonadal function or hypogonadism. The factors resulting in such a disparities in puberty among patients with the same disorder are not known. Some patients with normal gonadal function may have nonclassical rather than classical CAH (see later). Hormonal studies in untreated classical patients with normal sexual maturation have shown either normal or increased gonadotropin production (104) or concentrations and follicle-stimulating hormone excretion (6,99,104). Of great interest is that in these male patients, excess adrenal sex steroids or their precursor steroids did not seem to affect gonadotropin secretion. Adrenal androgen levels in untreated boys with normal gonadal function did not appear to be lower than those patients with gonadal dysfunction in poor control (6,73,101). This suggests that adrenal androgens alone have no effect on gonadotropin secretion via a negative feedback mechanism in male patients.

Another frequently reported complication in post-pubertal boys with inadequate control of CAH is hyperplastic nodular testes. Almost all patients with such complications were found to have adenomatous adrenal rests within the testicular tissue, as indicated by the presence of specific 11β-hydroxylated steroids in the blood from gonadal veins (105). These tumors have been reported to be ACTH-dependent and to regress following adequate steroid therapy (106–112).

GENETICS

Studies of families carrying 21-hydroxylase deficiency have demonstrated that the disease locus is situated in the HLA major histocompatibility complex on the short arm of the sixth chromosome (113,114). Both classical and nonclassical 21-hydroxylase deficiency are transmitted as recessive traits. Characteristic combinations of HLA alleles, or HLA haplotypes, are associated with different forms of 21-hydroxylase deficiency. In general, the phenotype of classical 21-hydroxylase deficiency results from the presence of two severely affected alleles, and nonclassical 21-hydroxylase deficiency results from the presence of either two mild 21-hydroxylase deficiency alleles or one severe and one mild allele (115).

Based on estimates of its frequency among Ashkenazi Jews (3%) and all ethnic whites (individuals of mainly European descent) (1%) (49), it is apparent that nonclassical 21-hydroxylase deficiency is among the most frequent human autosomal recessive disorders. Molecular genetic studies have demonstrated that the gene encoding the cytochrome P_{450} enzyme specific for 21-hydroxylation (P450c21) is located in the HLA complex between the genes encoding the transplantation antigens, HLA-B and HLA-DR. This gene, *CYP21*, and an inactive homolog or pseudogene, *CYP21P*, are immediately adjacent to the *C4B* and *C4A* genes encoding the fourth component of serum complement (Fig. 4) (116,117). The protein-encoding sequence of *CYP21P* is 98% homologous to that of *CYP21*. The high degree of homology permits two types of mutation causing recombination events: (1) unequal crossing over during meiosis that results in

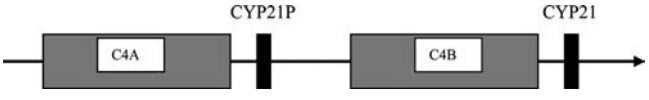

Figure 4 Diagram of *CYP21* region on chromosome 6p21.3. The arrow indicates the direction of transcription. *Abbreviations*: *CYP21P*, 21-hydroxylase pseudogene; *C4A* and *C4B*, genes encoding fourth component of serum complement.

complementary deletions/duplications of *CYP21* (118,119) and (2) non-correspondences between the pseudogene and the coding gene that, if transferred by gene conversion, result in deleterious mutations (120).

Approximately 25% of classical 21-hydroxylase deficiency alleles result from deletions of *CYP21* (121–123). The remaining three-quarters of classical alleles are caused by smaller mutations in *CYP21*, some of which are de novo point mutations resulting in amino acid substitutions (124–127) that significantly disrupt synthesis of the protein. Nonclassical 21-OHD is associated with conservative (or mild) amino acid substitutions in highly conserved portions of the gene encoding the active 21-hydroxylase (128–130).

In studies evaluating the phenotype–genotype relationship, there is generally a good correlation between the severity of the clinical disease and the discrete mutations observed (124,131). Recent studies, however, have demonstrated that there is often a divergence in phenotypes within mutation-identical groups, the reason for which requires further investigation (132–134).

EPIDEMIOLOGY

Screening studies indicate that the worldwide incidence of classical 21-OHD is 1:14,199 live births (4), of which approximately 75% are salt-wasters. The frequency of nonclassical 21-OHD is considerably higher; based on population genetic studies this allelic variant occurs in 1:100 persons in the general white population and in higher frequency among selected ethnic groups, most notably Ashkenazi Jews (49). The frequency of 11β-OHD is approximately 1:100,000 live births (135), however, among Sephardic Jews of Northern Morocco the incidence is estimated to be between 1:5000 and 1:6000 births (136).

DIAGNOSIS

CAH must be suspected in infants born with ambiguous genitalia. The physician is obliged to make the diagnosis as quickly as possible, to initiate therapy, and to arrest the effects of the enzyme disorders. The diagnosis and a rational decision of sex assignment must rely on the determination of genetic sex, the hormonal determination of the specific deficient enzyme, and an assessment of the patient's potential for future sexual activity and fertility. Physicians are urged to recognize the physical findings of ambiguous genitalia characteristic of CAH in newborns and to refer such cases to appropriate clinics for full endocrine evaluation.

As indicated in Table 1, each form of CAH has its own unique hormonal profile, consisting of elevated levels of precursors and elevated or diminished levels of adrenal steroid products (23,24). Traditionally, laboratory tests have measured the urinary excretion of adrenal hormones or their urinary metabolites (e.g., 17-ketosteroids). Collection of 24 hour urine excretion may be difficult, however, and the results in neonates may often be misleading (24). The development of simple and reliable radioimmunoassays for circulating serum levels of adrenal steroids is a significant advance in laboratory diagnostic techniques (23). The direct serum measurement of accumulated precursors and oversecreted adrenal steroids, such as 17-hydroxyprogesterone, Δ^4-androstenedione, and dehydroepiandrosterone is now possible, and more exact hormonal profiles of the different forms of CAH have been established (Table 1).

HORMONAL STANDARDS FOR DIAGNOSING 21-HYDROXYLASE DEFICIENCY

In our experience, the best diagnostic hormonal test for 21-hydroxylase deficiency has proven to be an ACTH (Cortrosyn, 0.25 mg) stimulation test measuring the serum concentration of 17-OHP at 0 to 60 minutes after intravenous bolus ACTH administration (137). The same dose of Cortrosyn is used for all age groups, including newborns and premature infants. The nomogram (Fig. 5) provides hormonal standards for assignment of the 21-hydroxylase genotype, i.e., patients whose hormonal values fall on the regression line within a defined group are assigned to this group. Because of the diurnal variation in l7-OHP, an early morning serum concentration of l7-OHP may be useful as a screening test for genotyping 21-OHD. In addition, early morning

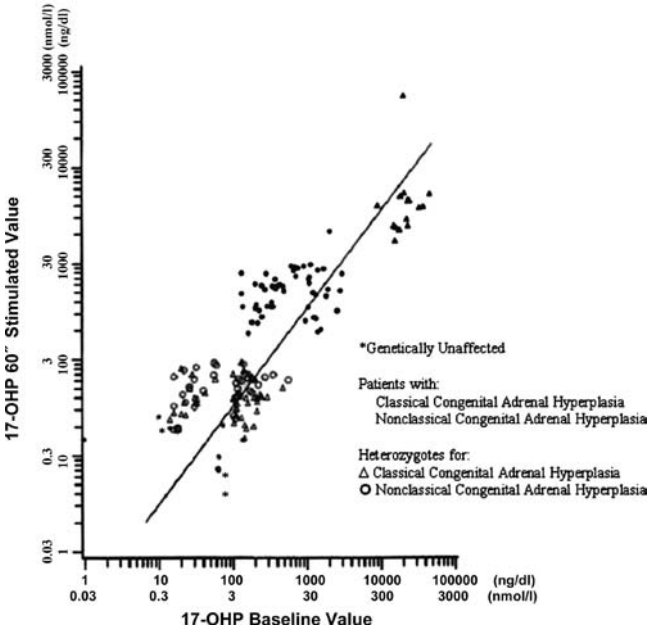

Figure 5 Nomogram relating baseline to ACTH-stimulated serum concentrations of 17-hydroxyprogesterone (17-OHP). The scales are logarithmic. A regression line for all data points is shown.

salivary 17-OHP has proven to be an excellent screening test for the nonclassical form (138). ACTH stimulation, however, remains the most definitive diagnostic test (64,138,139). It is important to note that the ACTH stimulation test should not be performed during the initial 24 hours of life as samples from this period are typically elevated in all infants and may yield false-positive results.

Newborn Screening

Diagnosis of 21-hydroxylase deficiency can also be made by microfilter paper radioimmunoassay for 17-hydroxyprogesterone; this has been useful as a rapid screening test for CAH in newborns (22). This convenient test requires only 20 μL blood, obtained by heel prick and blotted on microfilter paper, to provide a reliable diagnostic measurement of l7-hydroxyprogesterone, a cortisol precursor that accumulates in elevated concentrations in 21-hydroxylase deficiency. The simplicity of the test and the ease of transporting microfilter paper specimens through the mail have facilitated the implementation of many CAH newborn screening programs in the United States and worldwide (140).

Potential benefits of newborn screening include reduction in incorrect sex assignment, minimizing adrenal and salt-wasting crises along with their associated morbidity and mortality (particularly in males with normal genitalia), and early institution of treatment to prevent future complications.

There are, however, certain limitations to the screen (140–143). First, it is not always possible, to determine the subtype of CAH solely through the screening 17-hydroxyprogresterone; therefore, once a diagnosis of CAH has been made, genotyping is recommended in order to aid in classifying the subtype. Second, in order to maintain good specificity, many cases of the mild nonclassical form will be missed. Third, the cutoff values for a positive screen vary among different countries and among the different states within the United States. Fourth, preterm infants have higher 17-hydroxyprogesterone levels due to immaturity of the adrenal cortex (144) and may present a challenge in determining what is normal. Cutoff values in the United States, Canada, and New Zealand are typically based on birth weight, although reports from screening programs in other countries indicate that gestational age, despite being less reliable than birth weight, may be a better predictor (142,143).

Finally, 17-hydroxyprogesterone levels rapidly decline after birth, and interpretation of the screening value depends on when the sample was drawn. It has been suggested that using a multi-tiered method utilizing both birth weight and age can improve the positive predictive value of the screen (141). Normal 17-hydroxyprogesterone levels for birth weight and gestational age are given in Tables 4 and 5, respectively (142,143). Once a positive screen is identified, our suggested approach for evaluation is outlined in Figure 6 .

Table 4 Percentile Values of 17OHP by Gestational Age

Gestational age in weeks	17OHP (nmol/L)[a]	
	50th percentile	99th percentile
23–26	89.9	136.8
27–28	55.9	158.7
29–30	43.8	149.7
31–32	21.6	129.7
33	25.2	90.2
34	20.3	77.8
35	19.6	68.2
36	16.4	49.8
≥37	12.0	30.3

Samples drawn between third and fifth days of life.
[a]To convert to ng/mL, multiply by 0.33.
Abbreviation: OHP, hydroxyprogesterone.
Source: From Ref. 141.

Despite some issues that still need to resolved, overall, the newborn screening program for CAH has been widely successful in achieving the goals of an ideal screen: a relatively easy and efficient test, high morbidity and mortality if untreated, relatively high disease incidence, and established effective treatment to minimize life-threatening complications after diagnosis.

Prenatal Diagnosis and Prenatal Treatment

Prenatal Diagnosis

Since the report by Jeffcoate et al. of the successful identification of an affected fetus by elevated concentrations of 17-ketosteroids and pregnanetriol in the amniotic fluid, investigators in the past have undertaken prenatal diagnosis for CAH by measurements of hormone levels in pregnancy (145–147). The most specific hormonal diagnostic test for 21-hydroxylase deficiency is elevated 17-OHP in the amniotic fluid (40,148–151); Δ^4-androstenedione may be employed as an adjunctive diagnostic assay (150). It has been suggested that elevated amniotic fluid 21-deoxycortisol may also be a marker for 21-hydroxylase deficiency (152). Amniotic fluid

Table 5 Median Values of 17OHP by Birth Weight

Birth weight (g)	17OHP (nmol/L)[a]	
	Median	Range
< 1000	86	9–603
1000–1499	57	7–573
1500–1999	37	3–594
2000–2499	26	2–247
2500–2999	19	1–225
3000–3499	18	2–179
3500–3999	17	1–99
≥4000	17	1–45

Samples drawn between fifth and seventh days of life.
[a]To convert to ng/mL, multiply by 0.33.
Abbreviation: OHP, hydroxyprogesterone.
Source: From Ref. 142.

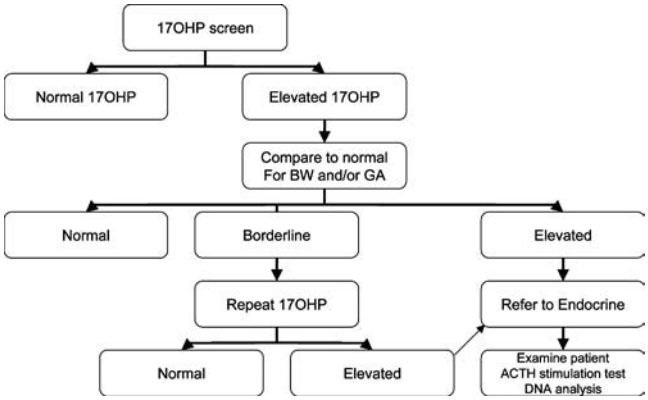

Figure 6 Algorithm depicting evaluation of infants with positive newborn screen.

testosterone levels may not be outside the normal range in an affected male (153). Measuring hormone levels in amniotic fluid, however, only detects severe cases, and has for the most part been replaced by molecular diagnosis.

HLA genotyping of amniotic fluid cells is another possible means of diagnosis but has now been superseded by direct molecular analysis of the 21-hydroxylase locus. With the advent of chorionic villus sampling, evaluation of the fetus at risk is now possible in the first trimester at 9 to 11 weeks gestation. The fetal DNA is used for specific amplification of the *CYP21* gene utilizing polymerase chain reaction and Southern blotting, which has the advantage of requiring small amounts of DNA (132). Because of improved accuracy and earlier diagnosis, molecular analysis of fetal DNA is now the method of choice for prenatal diagnosis.

Prenatal Treatment

Treatment with dexamethasone can be employed in pregnancies at risk for 21-hydroxylase deficiency (154–158). When properly administered, dexamethasone is effective in preventing ambiguous genitalia in the affected female. An algorithm for the diagnostic management of potentially affected pregnancies is given in Figure 7. The current recommendation is to treat the mother with a pregnancy at risk for 21-hydroxylase deficiency with dexamethasone in a dose of 20 mg/kg divided into two or three doses daily (158).

Adrenocortical steroidogenesis begins around sixth week of gestation, and differentiation of the external genitalia and urogenital sinus begins around week 9 (159). Institution of dexamethasone therapy is recommended as soon as pregnancy is confirmed and no later than nine weeks after the last menstrual period in order to effectively suppress adrenal androgen production and allow normal separation of the vaginal and urethral orifices, in addition to preventing clitoromegaly. The need to initiate at such an early date means that treatment is blind to the status of the fetus. If the fetus is determined to be a male upon karyotype or an unaffected female upon DNA analysis, treatment is discontinued. Otherwise, treatment is continued to term.

Between 1978 and 2002, prenatal diagnosis and treatment of CAH due to 21-OHD was carried out in 595 pregnancies at The New York Presbyterian Hospital-Weill Medical College of Cornell University,

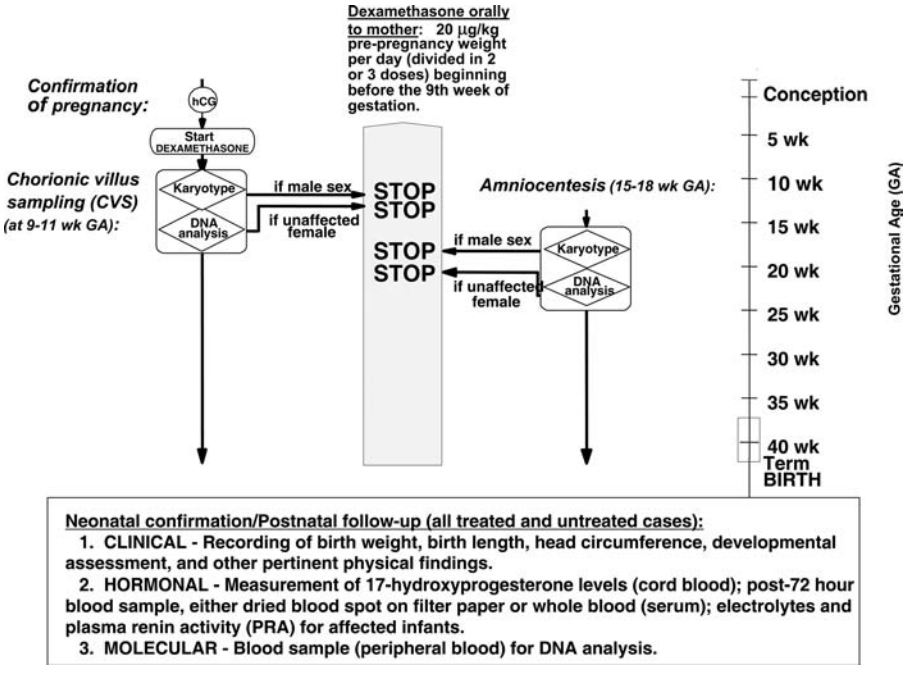

Figure 7 Algorithm depicting prenatal management of pregnancy in families at risk for a fetus affected with 21-hydroxylase deficiency. *Source:* From Ref. 147.

Table 6 Glucocorticoid Dose Equivalencies

Medication	Glucocorticoid/ anti-inflammatory (mg)	Mineralocorticoid (mg)	Growth-suppressing (mg)
Cortisone acetate	100	100	100
Hydrocortisone	80	80	80
Prednisone	20	100	16
Prednisolone	20	100	5
Methylprednisolone	16	No effect	10
Triamcinolone	16	No effect	
9-α-Fluorocortisone	5	0.2	
Betamethasone	3	No effect	
Dexamethasone	2	No effect	1

of which 108 babies were affected with classical 21-OHD. Of these, 64 were females, 51 of whom were treated prenatally with dexamethasone. Dexamethasone administered at or before nine weeks of gestation was effective in reducing virilization, thus avoiding postnatal genitoplasty (160,161). No significant or enduring side effects were noted in either the mothers (other than greater weight gain and a higher incidence of striae and edema than untreated mothers) or the fetuses. There was no statistically significant difference between treated and untreated mothers in hypertension or gestational diabetes. All mothers who took partial or full treatment stated that they would take dexamethasone again in the event of a future pregnancy. In our report and in others, no cases have been reported of cleft palate, placental degeneration, or fetal death, which have been observed in a rodent model of in utero exposure to high-dose glucocorticoids (162,163). Another study, in contrast, noted some significant maternal side effects, including excessive weight gain, Cushingoid facial features, severe striae resulting in permanent scarring, and hyperglycemic response to oral glucose administration (164).

A long-term follow-up study of 44 children treated prenatally in Scandinavia demonstrated normal pre- and postnatal growth compared to matched controls (165). In our study, prenatally treated newborns also did not differ in weight, length, or head circumference from untreated, unaffected newborns (160,161). Moreover, a survey of 174 prenatally dexamethasone-exposed children, ages 1 month to 12 years (including 48 with CAH), compared to 313 unexposed children (including 195 with CAH) found no differences in cognitive or motor development between the two groups (166). Therefore, we believe that proper prenatal treatment of fetuses at risk for CAH can be considered effective and safe. Long-term studies on the psychological development of patients treated prenatally are currently underway.

The efficacy of prenatal treatment in 11β-OHD CAH has been shown as well. In 1999, Cerame et al. (167) reported the first prenatal diagnosis and treatment of an affected female with 11β-OHD CAH. The treatment was successful, as the newborn had normal female external genitalia.

TREATMENT

In ambiguous genitalia caused by CAH, appropriate surgical repair may be made once a sex assignment has been made based on a reliable diagnosis of the underlying enzyme disorder. In female pseudohermaphroditism caused by 21- or 11β-hydroxylase deficiency, the aim of surgical repair should be to remove the redundant erectile tissue, preserve the sexually sensitive glans clitoris, and provide a normal vaginal orifice that functions adequately for menstruation, intromission, and delivery (168). Because of the normal internal genitalia in these patients, normal puberty, fertility, and childbearing are possible when there is early therapeutic intervention.

The aim of endocrine therapy is to replace the deficient hormones. In 21- and 11β-hydroxylase deficiencies, replacing cortisol both corrects the deficiency in cortisol secretion and suppresses ACTH overproduction. Proper replacement reduces stimulation of the androgen pathway, thus preventing further virilization and allowing normal growth and normal onset of puberty. Available pharmacologic forms of glucocorticoids and their relative potencies are shown in Table 6. Some synthetic steroids, such as dexamethasone and prednisolone, are substantially more growth-suppressing even at the same glucocorticoid equivalency and if possible should be avoided in growing children (169–171). Liquid preparations of hydrocortisone are not reliable and should not be used. The usual requirement of hydrocortisone (or equivalent) for the treatment of CAH is about 10 to 15 $mg/m^2/day$ divided into two or three doses. The daily dose should be titrated to adequately suppress 17-hydroxyprogesterone levels to less than 1000 ng/dL and to maintain androgen levels at age- and sex-appropriate levels. At the same time, overtreatment should be avoided because it can lead to growth suppression and Cushing syndrome. Depending on the severity, stress dose coverage may require doses of up to 50 to 100 $mg/m^2/day$ (Table 7 for recommended hydrocortisone during surgery).

Better understanding of the role of the renin-angiotensin system in CAH has made better therapeutic control of this condition possible. In addition to hypothalamic–pituitary regulation of adrenal

Table 7 Guidelines for Managing the Perioperative Congenital Adrenal Hyperplasia Patient

	Infant	Child	Adult
MN Pre-op	HC 25 mg po	HC 50 mg po	HC 100 mg po
On call to OR	HC 25 mg IM	HC 50 mg IM	HC 100 mg IM
During procedure	HC 25 mg IVSS	HC 50 mg IVSS	HC 100 mg IVSS
1st 24 hrs post-op	HC 10 mg IV q6	HC 20 mg IV q6	HC 40 mg IV q6
Post-op Day 1	Decrease by 50%, assuming no complications (may switch to po)		
Post-op Day 2	Decrease 50% and change frequency to q8		
Post op Day 3	Resume regular regimen		

steroidogenesis, the renin–angiotensin system exerts a primary influence on the adrenal secretion of aldosterone. The juxtaglomerular apparatus of the kidney secretes the enzyme renin in response to the state of electrolyte balance and plasma volume. Renin initiates a series of reactions that produce angiotensin II, a potent stimulator of aldosterone secretion (172).

Patients with salt-wasting 21-hydroxylase deficiency have elevated PRA in response to the sodium-deficient state, and they require treatment with the salt-retaining steroid 9α-fludrocortisone acetate. In addition to 9α-fludrocortisone acetate, infants with salt-wasting CAH require supplemental sodium of about 8 to 10 meq/kg/day because the sodium content in formula and breastmilk is extremely low. The total daily dose of sodium chloride can be dissolved in water, then divided into four doses and added to small amounts of formula or breastmilk for administration. Thereafter, dietary salt is usually sufficient for the 9α-fludrocortisone acetate to be effective.

Although aldosterone levels are not deficient in the simple virilizing form of 21-hydroxylase deficiency, PRA is commonly elevated as it is in the salt-wasting form (173). Despite elevated PRA, it has not been customary to supplement conventional glucocorticoid replacement therapy with the administration of salt-retaining steroids in simple virilizing 21-hydroxylase deficiency. However, Rosler et al.(174) have demonstrated that adding salt-retaining hormone to glucocorticoid therapy in patients with classical simple virilizing CAH with elevated PRA in fact improves the hormonal control of the disease. When PRA was normalized by the addition of 9α-fludrocortisone acetate, the ACTH level also fell and excessive androgen secretion decreased. The addition of salt-retaining steroids to the therapeutic regimen often made possible a decrease in the glucocorticoid dosage. Normalization of PRA also resulted in improved statural growth. Monitoring of PRA is also a useful index of hormonal control in other forms of CAH, particularly 11β-hydroxylase and 17α-hydroxylase deficiencies.

Steroid radioimmunoassay methods have been an asset not only for the initial diagnosis of CAH, but also for improved monitoring of hormonal control once therapy has been instituted. Studies indicate that serum 17-hydroxyprogesterone and androstenedione levels provide the most sensitive index of biochemical control. In girls and pre-pubertal boys (but not in newborn and pubertal boys), the serum testosterone level is also a useful index (23). The combined determination of PRA, 17-hydroxyprogesterone, and serum androgens, as well as the clinical assessment of growth and pubertal development, all must be considered in adjusting the dosage of glucocorticoid and salt-retaining steroid. 3α-Androstenediol (3AG) has been proposed as a useful serum metabolic marker of integrated adrenal androgen secretion in CAH patients (175). Both in our clinic and in others, combinations of hydrocortisone and 9α-fludrocortisone acetate have proven to be highly effective treatment modalities (176).

NEW TREATMENT STRATEGIES

For the past 50 years, glucocorticoid replacement has been an effective treatment for CAH and remains its key therapy; however, the management of these patients can be a challenge because complications arise from both undertreatment and overtreatment. Undertreatment leads to hyperandrogenic symptoms, early epiphyseal fusion, and infertility, whereas overtreatment leads to poor growth and Cushing syndrome.

Data from numerous groups have shown that patients with CAH are about 10 cm shorter than their parentally based target height (97,177–181). Lin-Su et al. (83) demonstrated that the combination of growth hormone and LHRH analog improved final adult height by 8 cm when compared to CAH subjects treated only with glucocorticoid and mineralocorticoid therapy.

Some preliminary data show that children may be treated with lower doses of hydrocortisone if an androgen receptor antagonist (flutamide) and an aromatase inhibitor (testolactone) are utilized (182). Likewise, anti-androgen treatment may be useful in adult women with persistent hyperandrogenic signs (183).

Bilateral adrenalectomy has been reported to improve symptoms in a few patients who were extremely difficult to control with medical therapy

alone (184–186). An adult female with CAH who had long-term amenorrhea resumed regular menses following bilateral adrenalectomy (187). Because this approach renders complete adrenal insufficiency, however, it should be reserved for extreme cases, and is not a good treatment option for patients who have a history of poor compliance with medication.

Gene therapy is on the horizon and may represent the most promising treatment strategy of the future (183,188,189).

PSYCHOLOGICAL ASPECTS OF CAH

Several studies have shown that compared to their unaffected sisters, girls with CAH tend to prefer more male-typical toy choices, such as construction and transportation toys, and more male-typical play behavior, such as rough outdoor play (190,191). Meyer-Bahlburg et al. (192) demonstrated that CAH girls aged 5 to 12 years have masculinization of gender-related behavior, but no gender identity issues. Adult women with CAH have also been demonstrated to have masculinized behavior, being most pronounced in salt-wasting (SW-CAH) (193), slight but demonstrable in simple virilizing, and questionable in nonclassical CAH. For the majority of the adult women with CAH, gender identity was clearly female; however, gender dysphoria was identified in 3 out of 42 women with SW-CAH. Long et al. (194) also demonstrated that CAH girls preferred male-typical toys and male playmates, but found that masculinity decreased across developmental stages, such that by adulthood, there was no significant difference in masculinity between controls and women with CAH.

Male predominance of left-handedness has been attributed to early androgen exposure. In support of this theory, girls with CAH have been found to be more left-handed biased than their unaffected sisters (195). There is also some evidence that CAH women have a post-pubertal spatial advantage (196).

In both children and adults, psychological adjustment does not appear to be compromised in females with virilized genitalia who are treated early in life and reared as females (197). However, there are reports of less satisfaction with sexual function in women with classical CAH, particularly in those with the salt-wasting form (191,198–200).

CONCLUSION

Abnormalities of sexual differentiation and development, often in combination with hypertension (as in 11β-hydroxylase deficiency) or severe salt-wasting (associated with 21-hydroxylase and 3β-hydroxysteroid dehydrogenase deficiency), are clinical hallmarks of CAH. The pathophysiology can be traced to discrete, inherited defects in the genes encoding enzymes for adrenal steroidogenesis. Treatment of CAH is targeted to replace the hormones that are produced in insufficient quantity. With proper hormone replacement therapy, normal and healthy development may often be expected. Radioimmunoassay of serum and urinary steroid levels permit reliable diagnosis of the various forms of CAH. Prenatal diagnosis and therapy are possible in 21-hydroxylase deficiency, and recently has been shown to be successful in 11β-hydroxylase deficiency as well.

The most common form of CAH, 21-hydroxylase deficiency, has served as a prototype for examination of the molecular genetic basis of phenotypic diversity. Similar studies in other enzymatic defects are now in progress.

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10

Disorders of the Adrenal Medulla-Catecholamine Producing Tumors in Childhood

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CHROMAFFIN CELL BIOLOGY

Developmental Biology of the Chromaffin Cell

Catecholamine-producing tumors, including pheochromocytomas, paragangliomas, neuroblastomas, ganglioneuroblastomas and ganglioneuromas, develop from neural crest-derived progenitors and chromaffin cells of the adrenals and paraganglia. The sympathetic ganglia are formed during embryogenesis after migration of thoracic neural crest progenitors, sympathogonia, to locations along the aorta to form the sympathetic chain (1). The adrenals are first noticeable during the sixth week of gestation as an accumulation of mesodermal cells that gives rise to the adrenal cortex. Sympatho-chromaffin cells from adjacent ganglionic masses then invade the cortex to form the adrenal medulla.

The developing adrenal medulla consists of three types of cells. The sympathetic cells (sympathogonia) are small cells with hyperchromatic nuclei and scanty cytoplasm. Pheochromoblasts are larger with more prominent nuclei, one or two prominent nucleoli and abundant cytoplasm. Pheochromocytes have only one nucleolus and show a characteristic positive chromaffin reaction (staining with chromaffin salts) at the 50 mm stage or later. The sympathetic cells and the pheochromoblasts show a negative chromaffin response. While the pheochromocytes increase in number by division, the number of primitive cells (sympathogonia and pheochromoblasts) gradually decreases by apoptosis.

In humans substantial amounts of chromaffin tissue also develop in extra-adrenal locations, particularly as para-aortic tissue, such as the organ of Zuckerkandl. Most extra-adrenal chromaffin tissue regresses after birth leaving only remnants, such that in the adult most chromaffin cells are confined to the adrenal medulla. The sympathoadrenal system thus develops as a consequence of a complex interplay of migration, mitotic activity and differentiation of neural crest precursors from primitive sympathogonia to neuroblasts and pheochromoblasts. The final mature sympathetic ganglion and chromaffin cell components are determined in a number of steps by multiple neurotrophic environmental signals promoting survival and differentiation of some cells and apoptosis of others (2–4).

Understanding the processes that contribute to the development of the sympathoadrenal system may be important for understanding differences in the biology, behavior and presentation of the various types of catecholamine-producing tumors. Such understanding may also have implications for diagnosis, management and treatment of these tumors. For example, the arrested development of neuroblasts with failure of apoptosis is presumed to lead these particular cells to develop into neuroblastomas (5). Furthermore, differences in the clinical course and prognosis of neuroblastomas appear linked to differences in stromal and morphological differentiation of cells, which in turn may depend on the particular stage at which sympathoadrenal progenitor cells are arrested in their development (6). Even in pheochromocytoma, more common in adults than in children, there are now indications that rather than developing from mature chromaffin cells, at least some of these tumors develop from neural crest progenitor cells arrested at different stages of

development (7). Such cells may be present from before birth until full development of tumors later in life, with differences in clinical and biochemical phenotypes of tumors dependent on particular mutations influencing the stage at which progenitor cells are arrested in their development.

Pathways of Catecholamine Synthesis and Metabolism

Excessive production of catecholamines—dopamine, norepinephrine (NE), and epinephrine (EPI)—is a feature of most chromaffin cell tumors crucial for their diagnosis. The nature of the particular catecholamines produced in excess and the pattern of accompanying increases in catecholamine metabolites depends on the presence of particular enzymes in the catecholamine biosynthetic pathway and of other components responsible for the storage and metabolism of catecholamines (Fig. 1). Differing cellular expression of these elements results in considerable differences in biochemical profiles of the various types of chromaffin cell tumors.

Tyrosine hydroxylase, an enzyme confined to catecholamine-producing cells, catalyzes the rate-limiting step in catecholamine biosynthesis, conversion of tyrosine to 3,4-dihydroxyphenylalanine (DOPA). DOPA is converted to dopamine by L-aromatic amino acid decarboxylase, an enzyme with a wide tissue distribution. The dopamine produced within central nervous system noradrenergic nerves, peripheral sympathetic nerves and adrenal chromaffin cells is converted to NE by dopamine β-hydroxylase, an enzyme localized within chromaffin storage vesicles. Most NE in the body, including that present in plasma and urine, is derived from synthesis within sympathetic nerves, where the amine is stored in vesicular granules awaiting exocytotic release. Further conversion of NE to epinephrine requires the presence of phenylethanolamine *N*-methyltransferase (PNMT), an enzyme largely localized to chromaffin cells of the adrenal medulla (9,10). As a consequence, over 90% of the EPI present in plasma and urine is produced by the adrenal medulla. Because PNMT is a cytoplasmic enzyme, synthesis of epinephrine depends on leakage of NE from chromaffin granules with subsequent metabolism and repackaging of epinephrine into granules for exocytotic release.

Dopamine in sympathetic nerves and adrenal chromaffin cells is largely produced as an intermediate in the subsequent production of NE and EPI and is therefore stored in these cells in relatively minor amounts. As a consequence, sympathoneural and adrenalmedullary cells represent a relatively minor source of circulating and urinary dopamine and dopamine metabolites. A substantially larger source is provided by gastrointestinal tissues, but because of local metabolism and highly efficient hepatic extraction only small amounts of dopamine produced in these tissues escape into systemic plasma (11). Most dopamine is metabolized locally by sulfate conjugation to dopamine-sulfate or by O-methylation and deamination to homovanillic acid. Consequently, levels of free dopamine in systemic plasma are usually much lower than levels of NE and EPI. A reverse pattern is present in urine, with higher urinary levels of dopamine than of NE and EPI. The dopamine present in urine is derived largely by renal extraction of circulating DOPA and local conversion to dopamine by L-aromatic amino acid decarboxylase (12).

Catecholamines undergo metabolism by multiple pathways involving differing series of several enzymes with differing expression in various cells and tissues (Fig. 2). In contrast to the views espoused in many textbooks, where catecholamine metabolism is suggested to mainly occur after exocytotic release, most metabolism actually occurs within the same cells where catecholamines are synthesized, with most of

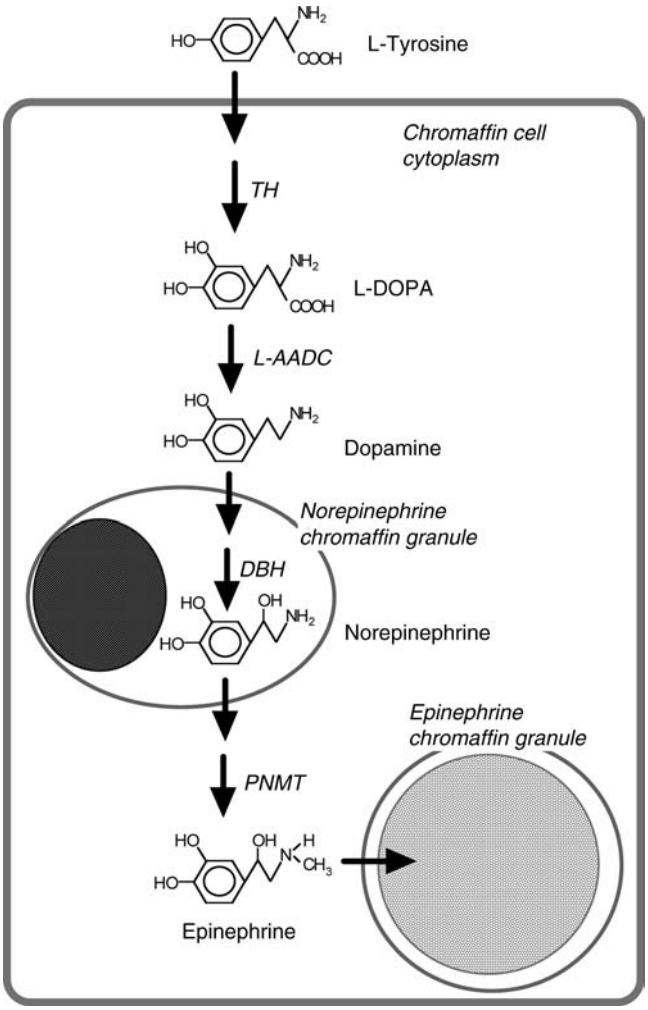

Figure 1 Pathway of catecholamine biosynthesis in an adrenal chromaffin cell. *Abbreviations*: TH, tyrosine hydroxylase; l-AADC, L-aromatic amino acid decarboxylase; DBH, dopamine B-hydroxylase; PNMT, phenylethanolamine *N*-methyltransferase. *Source*: From Ref. 8.

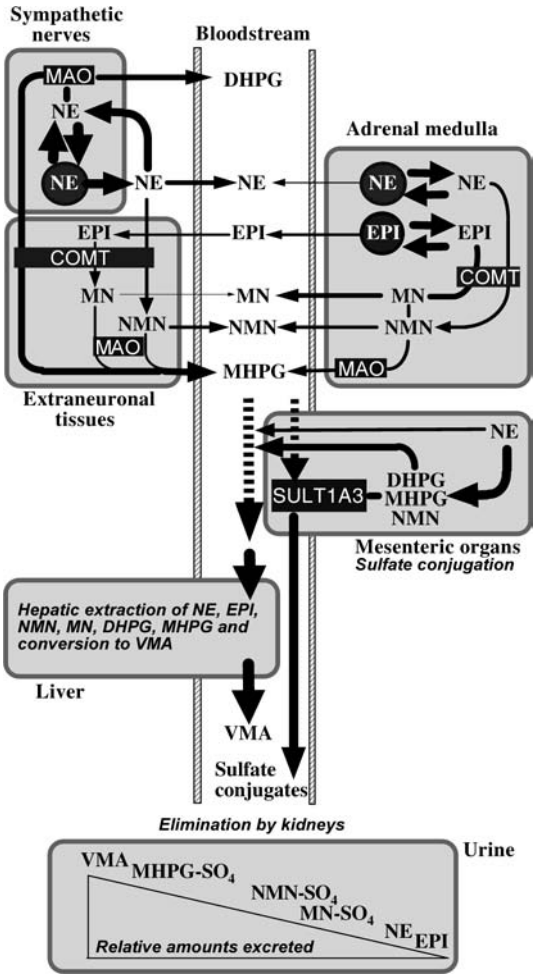

Figure 2 Model illustrating the regional pathways of NE and EPI metabolism. Most NE is released and metabolized within sympathetic nerves, including up to a half produced in sympathetic nerves of mesenteric organs. Sulfate conjugation of catecholamines and catecholamine metabolites, particularly MHPG, occurs mainly in mesenteric organs, whereas production of VMA occurs mainly in the liver. *Abbreviations*: MAO, monoamine oxidase; COMT, catechol-O-methyltransferase; SULT1A3, sulfotransferase type 1A3; NE, norepinephrine; EPI, epinephrine; NMN, normetanephrine; MN, metanephrine; DHPG, 3,4-dihydroxyphenylglycol; VMA, vanillyl mandelic acid; NMN-SO_4, normetanephrine sulfate; MN-SO_4, metanephrine sulfate; MHPG-SO_4, 3-methoxy-4-hydroxyphenylglycol sulfate. *Source*: From Ref. 8.

this is dependent on leakage of the amines from storage vesicles into the cytoplasm (8). In sympathetic nerves the presence of monoamine oxidase (MAO) leads to conversion of NE to 3,4-dihydroxyphenylglyocol (DHPG). The DHPG so produced is then largely metabolized by catechol-O-methyltransferase (COMT) in extraneuronal tissues to 3-methoxy-4-hydroxyphenylglycol (MHPG). Vanillylmandelic acid (VMA), the major end-product of NE and epinephrine metabolism, is produced almost exclusively in the liver, this dependent on the hepatic localization of alcohol dehydrogenase, an enzyme required for conversion of MHPG to VMA.

In adrenal chromaffin cells, the additional presence of COMT leads to metabolism of NE to normetanephrine and of EPI to metanephrine (8). Because the intraneuronal deamination pathway far predominates over the extraneuronal O-methylation pathway of catecholamine metabolism, normetanephrine and metanephrine represent relatively minor products of catecholamine metabolism. As a consequence the adrenal medulla represents the single largest tissue source of normetanephrine and metanephrine, accounting for 24% to 40% of the former and over 90% of the latter (13,14). The metanephrine and normetanephrine produced in the adrenal medulla or in extraneuronal tissues—the former from catecholamines leaking from storage vesicles and the latter from catecholamines release from sympathoadrenal sources and localized extraneuronally—may be deaminated to MHPG and then converted to VMA in the liver or may be sulfate conjugated by a sulfotransferase enzyme expressed mainly in the gastrointestinal tissues. Sulfate conjugated normetanephrine and metanephrine are cleared much more slowly from the circulation, than the free metanephrines, and almost exclusively by renal elimination in the urine. As a consequence, sulfate-conjugated normetanephrine and metanephrine are present in plasma at 20- to 40-fold higher concentrations than free normetanephrine and metanephrine and represent the main forms excreted in urine.

PHEOCHROMOCYTOMA

Pheochromocytomas are chromaffin cell tumors that produce, store, metabolize, and secrete catecholamines (15). Nearly 80% to 85% of pheochromocytomas arise from the adrenal medulla while about 15% to 20% arise from extra-adrenal chromaffin tissue called paragangliomas (16–20). Paragangliomas are divided into two groups: those that arise from parasympathetic-associated tissues, most commonly along cranical and vagus nerves (e.g., glomus tumors, chemodectomas, carotid body tumors) and those that arise from sympathetic-associated chromaffin tissue (often designated extra-adrenal pheochromocytomas).

Extra-adrenal pheochromocytomas arise mainly from chromaffin tissue adjacent to sympathetic ganglia of the abdomen (75%), urinary bladder and pelvic area (12%), and less commonly from the thorax (10%), and rarely from the head (3%), and the neck (2%) (21–23). Abdominal paragangilomas are usually positioned along great vessels, most commonly around the aorta below the diaphragm and at the origin of the inferior mesenteric artery (organ of Zuckerkandl) (22,24). Other less common extra-adrenal sites include the sympathetic chain anywhere from the base of the skull to the pelvis; and more rarely, in the urinary bladder, spermatic cord and vagina. Pheochromocytomas in childhood are extremely rare, accounting for well under 1% of all neoplasms seen in large pediatric

centers but they represent the most common pediatric endocrine tumor (17,18). They account for only 5% to 10% of all pheochromocytomas with the incidence 2 per million (25,26). In children, pheochromocytomas are more frequently familial (9–50%), extra-adrenal (8–43%), bilateral adrenal (7–53%) and multifocal (27,28).

Pheochromocytoma usually presents in older children but rarely it has been reported in infants (16,17). Childhood pheochromocytomas peak at 10 to 13 years with a male predominance before puberty (27–29). Less than 10% of pediatric pheochromocytomas are malignant (27,28,30,31) with reported mean survival rates of 73% at three years and 40% to 50% at five years after diagnosis (32,33). Recurrent pheochromocytomas are unlikely in children but recurrent tumors may appear years after initial diagnosis, emphasizing the importance of close long-term follow-up (31).

Because pheochromocytoma is more common in adults than in children, most of the data available on its behavior and management have been based on adults, and thus data about pediatric pheochromocytoma remains obscure.

Clinical Presentations

The presentation of pheochromocytoma is highly variable, with tumors found in some patients who may be entirely asymptomatic (20,34). Signs and symptoms associated with pheochromocytoma may be present in other clinical conditions, therefore, pheochromocytoma is often referred to as the great mimic (35). Most but not all of the symptoms are due to the direct actions of secreted catecholamines.

Children more commonly present with signs and symptoms related to hypertension than adults. Sustained hypertension is found in only about 50% of cases of pheochromocytomas in adults compared to more than 70% to 90% in children (26,27,31) (Vol. 1; Chap. 13).

Pheochromocytoma is an underlying cause of 1% to 2% cases of pediatric hypertension and should be considered after exclusion of more common causes such as renal artery stenosis (28). Other differential diagnoses of pheochromocytoma in children include coarctation of the aorta, neuroblastoma, ganglioneuroblastoma, ganglioneuroma and less commonly panic/anxiety disorders, ''autonomic epilepsy,'' cluster or migraine headache, hyperthyroidism, and side effects of medications or dietary supplements (36–38).

Certain symptoms of pheochromocytoma such as sweating, visual complaints, nausea, vomiting, weight loss, polydipsia and polyuria have been reported more often in children than in adults (28,39). In addition, children may present with palpitations, anxiety, and hyperglycemia (23). Pheochromocytomas secreting epinephrine may present with hypotension, particularly postural hypotension (40,41). Other signs of catecholamine excess consist of pallor and flushing (23). As summarized by Manger and Gifford (23) occasionally some children exhibit a reddish blue mottling of the skin and puffy red and cyanotic appearance of the hands. Less frequent clinical manifestations are fever, constipation and seizures. The presence of the triad of headache, palpitations and sweating in combination with hypertension should immediately raise suspicion of pheochromocytoma. Spells may last from a few seconds to several hours, with intervals between attacks varying widely and as infrequent as once every few weeks or months. The occurrence of attacks is unpredictable because they often occur at rest, however, some are always associated with physical activity, trauma, by direct stimulation of tumor (e.g., urinary bladder distension), or after using certain drugs or taking food (e.g., tyramine in chocolate). Unusual symptoms related to paroxysmal blood pressure elevation or sudden arrhythmia during diagnostic procedures (e.g., endoscopy, catheterization), or anesthesia should promptly arouse a suspicion of pheochromocytoma. However, rarely pheochromocytomas with predominance of dopamine secretion do not present with cardiovascular symptoms, posing a significant diagnostic challenge (42–46). Therefore, it is not surprising that in these cases patients may eventually exhibit symptoms reflecting local invasion of a tumor or with metastatic spread involving bone and soft tissue organs (44).

Biochemical Diagnosis of Pheochromocytoma

Biochemical diagnosis of pheochromocytoma relies principally on demonstration of excessive production of catecholamines. Because pheochromocytomas express COMT, these tumors produce excessive amounts of O-methylated catecholamine metabolites, this production dependent on leakage of catecholamines from chromaffin granules into the cytoplasm (47). Production of metanephrines—normetanephrine from NE and metanephrine from EPI—within tumors is continuous and independent of variations of catecholamine release. Consequently, patients with pheochromocytoma have more consistent and relatively larger increases in plasma free metanephrines than increases in plasma and urinary catecholamines (48,49).

Studies from three independent groups have confirmed that measurements of plasma free metanephrines provide superior diagnostic sensitivity for detection of pheochromocytoma than other tests (50–52). Measurements of 24-hour urinary excretion of fractionated metanephrines provide another sensitive test for detection of pheochromocytoma, but this test is less specific than measurement of plasma free metanephrines (51). Possibly, the relatively poor specificity of the urinary test compared to the plasma test may reflect differences in the nature of the metabolites. Metanephrines in urine are usually measured after a deconjugation step and thus largely reflect sulfate-conjugated metabolites formed by an enzyme present mainly in gastrointestinal tissues.

Table 1 Characteristics of Biochemical Tests for the Detection of Pheochromocytoma in Children

Biochemical test	Sensitivity	Specificity
Percentage (number/total number)		
Plasma normetanephrine and metanephrine	100 (12/12)	94 (31/33)
Plasma norephinephrine and epinephrine	92 (11/12)	91 (30/33)
Urinary normetanephrine and metanephrine	100 (5/5)	95 (21/22)
Urinary norephinephrine and epinephrine	100 (10/10)	83 (25/30)

Source: From Ref. 53.

Data on biochemical test performance in children are limited, but where available have also indicated higher diagnostic sensitivity of measurements of plasma free and urinary fractionated metanephrines over measurements of plasma or urinary catecholamines (Table 1) (53).

Because missing the diagnosis of pheochromocytoma can have catastrophic consequences, the most important consideration for choice of initial test is a high level of reliability of a positive result in that rare patient with the tumor. This also provides confidence that a negative test result reliably excludes the tumor, thereby avoiding the need for further time-consuming and costly diagnostic tests. Initital testing of the tumor in both adults and children should therefore include a suitably sensitive diagnostic test—either or both measurements of plasma free metanephrines or urinary fractionated metanephrines. Diagnosis should be based on reference intervals that provide optimal diagnostic sensitivity, with specificity a secondary consideration.

Importantly, biochemical diagnosis of pheochromocytoma in children requires use of age-appropriate reference intervals. Reference intervals for urinary tests show considerable differences between adults and children, particularly in young children in who urinary outputs of catecholamines and metanephrines can be less than a third those of adults. For plasma tests the differences are less striking but still require consideration (Fig. 3). Upper limits of reference intervals are higher for plasma concentrations of EPI and metanephrine and lower for plasma NE and normetanephrine in children than in adults (53). Furthermore, boys demonstrate 45% higher plasma concentrations of EPI and 30% higher concentrations of metanephrine than girls. In contrast, plasma concentrations of NE and normetanephrine do not appear to differ according to gender.

Although age-dependent variations in reference intervals may be more troublesome for interpretation of urinary than plasma test results, there are other disadvantages to plasma tests that may limit their utility (Table 2). A disadvantage of plasma tests is that these require a needle stick, which in children can be particularly stressful, leading to emotion-induced elevations of plasma catecholamines and catecholamine metabolites that may confound interpretation of a positive result. Standard phlebotomy procedures are therefore innappropriate. Blood sampling should

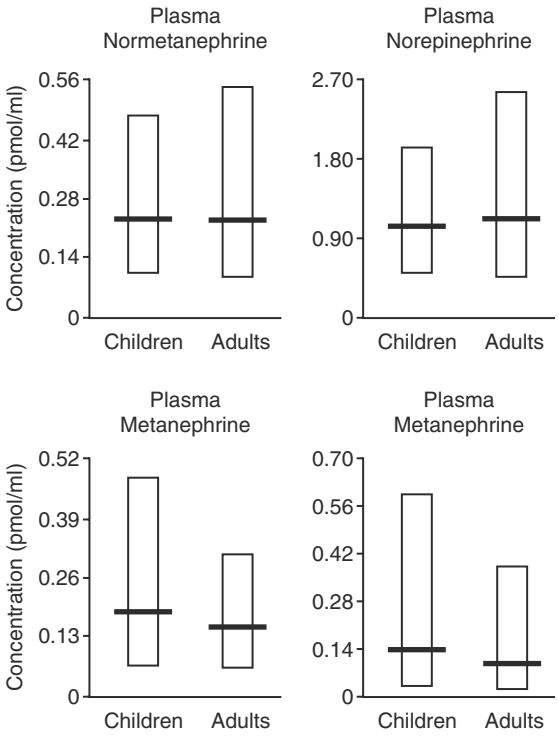

Figure 3 Comparison of pediatric ($n = 86$, 43 boys) and adult ($n = 158$, 85 men) reference ranges for plasma free metanephrines (normetanephrine and metanephrine) and catecholamines [norephinephrine (NE) and epinephrine (EPI)]. (*Horizontal line*), Median; *box*, 95% confidence intervals as calculated from the logarithmically transformed data. Children had higher ($p < 0.01$) plasma concentrations of EPI and metanephrine than adults, whereas plasma NE and normetanephrine levels did not differ significantly between age groups. *Source*: From Ref. 53.

be carried out using an indwelling intravenous cannula, with the subject resting comfortably in the supine position for at least 20 minutes after insertion of the cannula and before the sample is taken. Despite the difficulties associated with such blood sampling, it should also be considered that reliable 24-hour urine collections can be difficult in children. Such collections should therefore always include measurements of urinary creatinine output to check and correct for incompleteness of the 24-hour urine collection. Dependence of urinary creatinine output on diet (54), muscle mass (55), physical activity (56) and diurnal variation (57) may, however, further confound interpretation of urinary test results.

Despite the above potential difficulties in test result interpretation, most pheochromocytomas, including those in children, are easily diagnosed by large increases in plasma or urinary metanephrines, well above what can be expected in any subject without the tumor. If plasma concentrations of free normetanephrine, metanephrine or both are four times or more above the upper reference limits (typical in about 80% of patients with the tumor), the diagnosis of pheochromocytoma is essentially

Table 2 Relative Advantages and Disadvantages of Measurements of Urinary Fractionated and Plasma Free Metanephrines for Diagnosis of Pheochromocytoma

Urinary fractionated metanephrines	Plasma free metanephrines
Well established widely available test	Relatively new test with increasing availability
Urinary concentrations (200–2000 nmolar) make analysis relatively easy	Plasma concentrations (0.1–0.5 nmolar) can make analysis difficult
Easy for clinicians to implement with minimal expenditure of time and effort	Blood collections requiring some time and effort by medical staff
Twenty-four hour collections can be inconvenient for patients	Blood sampling relatively more convenient for patients
Potential problems with reliability of incomplete timed urine collections	Collection and handling of blood samples can be carried out reliably
Difficult to control for daily life influences on sympathoadrenal function or diet	Influences of diet and sympathoadrenal function more easily controlled for
In children 24-hour collections are difficult with results difficult to interpret without age-appropriate reference intervals	In children blood sampling may be stressful, but results are more easily interpreted without age-appropriate reference intervals
Test not useful in patients with renal failure	Test can be used in patients with renal failure

Source: From Ref. 58.

confirmed (this assumes no analytic interference), and the next priority is to localize the tumor.

Difficulty in biochemical diagnosis occurs with small tumors that produce relatively small increases in plasma or urinary metanephrines that can be difficult to distinguish from false-positive results due to sympathoadrenal activation or other causes. Confirmatory biochemical tests are invariably necessary in such cases. Patterns of increases in follow-up measurements of urinary normetanephrine and metanephrine can be useful for confirming the validity of patterns of increases in plasma metanephrines, and vice versa for use of plasma tests to confirm initial urinary tests. Such cross-validation may be particularly useful when there is concern about analytical interference. Nevertheless, no matter how many of these supplementary tests are carrried out; there still may remain difficulty in distinguishing positive results due to a small tumor from those due to sympathoadrenal activation. In cases that include small increases in plasma free normetanephrine the clonidine suppression test may be useful (59).

Clonidine given orally at a dose of 0.3 mg/70 kg (4.3 mg/kg) suppresses sympathoneural release of NE by activating α_2-adrenoceptors in the brain and on sympathetic nerve endings. Patients without pheochromocytoma therefore show a decrease in plasma NE concentrations after clonidine. Because the drug does not suppress NE release from pheochromocytomas, lack of a clonidine-induced decrease in plasma concentrations of NE provides strong evidence for a tumor. A limitation of the clonidine-suppression test is that it is not reliable in patients with normal or only mildly increased plasma catecholamine levels (60–68). In these patients there may be normal suppression of plasma NE after clonidine despite the presence of a pheochromocytoma. Presumably, normal suppression occurs because much of the circulating NE is derived from sympathetic nerves and remains responsive to clonidine. The above limitation is largely overcome by additional measurements of plasma normetanephrine, which usually show much greater elevations above normal than the parent amine. Suppression of plasma normetanephrine by 40% or more or to below the upper reference limit excludes the diagnosis of pheochromocytoma with high specificity, whereas lack of suppression provides near certain proof of the tumor (69).

The glucagon stimulation test provides a procedure advocated as useful in patients with a very high suspicion for having pheochromocytoma but normal or mildly elevated plasma catecholamines or in patients in whom clonidine-suppression testing yields equivocal results (66). Glucagon is administered intravenously and blood samples are taken before and at two minutes after administration of the hormone. A threefold increase in plasma concentrations of NE provides reasonably conclusive evidence of a pheochromocytoma. A problem with the glucagon stimulation test is its low sensitivity (i.e., many patients with pheochromocytoma do not respond to the hormone).

Because of possible severe hypotension during the clonidine test and hypertension during the glucagon test, and due to the complexities of implementation and interpretation, both tests are best carried out by clinicians experienced in their use. The value of these tests, when judiciously implemented and appropriately interpreted, is that they can indicate a pheochromocytoma with high specificity.

In case of high suspicion for pheochromocytoma with predominant dopamine production, when sole measurement of serum free metanephrines would fail to detect the disease, we recommend measurement of plasma free methoxytyramine (dopamine metabolite) or dopamine. Urinary dopamine is less reliable due to derivation of the urinary amine mainly from circulating DOPA, but not dopamine (44).

Similar to adults, metastatic pheochromocytomas in children can be characterized by high plasma and urinary levels of DOPA or dopamine (68,70–74). If they are accompanied by elevations in plasma NE or other clinical evidence of pheochromocytoma, such elevations should arouse immediate suspicion of metastatic disease.

In summary, standardized collections of blood for measurements of plasma free metanephrines or 24-hour urine collections for measurements urinary fractionated metanephrines—with interpretation of results using appropropriate of age specific reference intervals provide the biochemical tests of choice for detecting childhood pheochromocytoma. Based on our experience we recommend biochemical diagnosis using plasma free metanephrines, with an algorithm that considers not only whether the test is positive or negative, but also the extent of increase in cases of positive test results (Fig. 4).

Localization of Pheochromocytoma

A variety of conventional imaging techniques are available for localization of a pheochromocytoma (75–77). These techniques include ultrasound (U/S), computed tomography (CT), magnetic resonance imaging (MRI), and scintigraphy after administration of ^{123}I-labeled meta-iodobenzylguanidine (MIBG). In children, more than 90% of pheochromocytomas are localized to the abdomen; therefore, imaging studies should first be directed to this part of the body. Although CT localizes about 85% to 95% of pheochromocytomas, MRI or U/S is the preferred imaging modality in children to avoid radiation exposure.

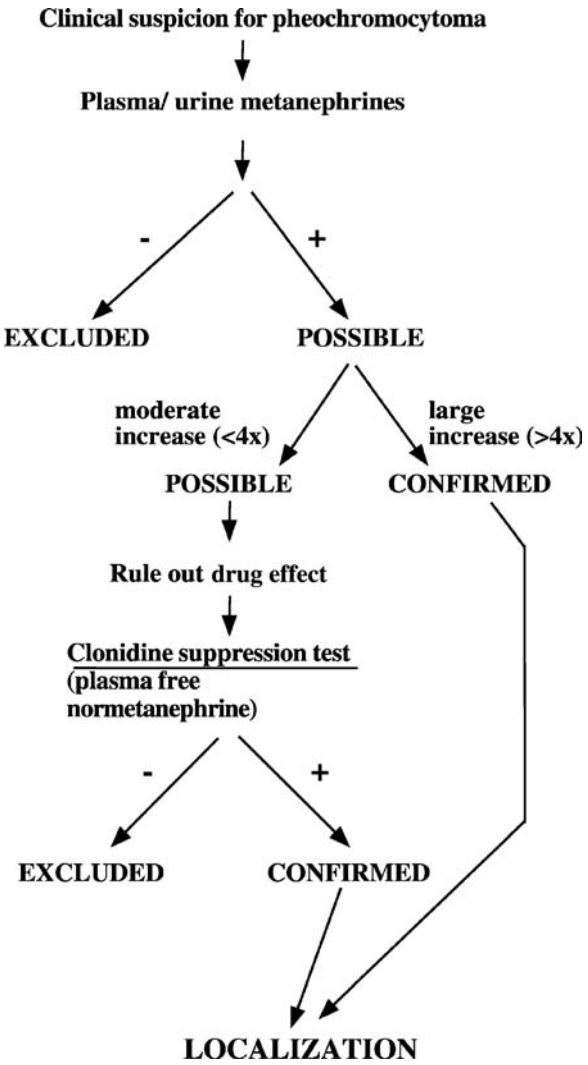

Figure 4 Diagnostic algorithm for biochemical diagnosis of pheochromocytoma. *Source*: From Ref. 75.

On U/S imaging, pheochromocytomas are usually seen as well defined, round or ovoid masses that demonstrate low echogenicity and homogenous consistency (78,79). Large tumors frequently undergo hemorrhage or necrosis, and in this case homogeneity is lost (79). The sensitivity of U/S in evaluating pheochromocytoma has been assessed in relatively small numbers of patients and has been reported to be 83% to 89% (80,81). Extensive studies on the specificity of modern U/S techniques in the diagnosis of pheochromocytoma have not been performed, but it is believed to be low with early studies indicating the specificity for adrenal tumors was about 60% (82).

MRI provides excellent contrast resolution without radiation exposure but does involve a longer scan period and may be less accessible than CT. The hypervascularity of pheochromocytoma makes it appear characteristically bright, with a high signal on T2 sequence and no signal loss on opposed phase images. More particularly, almost all pheochromocytomas have a more intense signal than that of the liver or muscle and often more intense than fat on T2-weighted images (83–85). However, such intense signals can be elicited by hemorrhages or hematomas, adenomas, and carcinomas, so an overlap with pheochromocytoma must be considered and specific additional imaging is needed to confirm that the tumor is pheochromocytoma (86–88).

MRI is an excellent imaging modality for the detection of intracardiac, juxtacardiac, and juxtavascular, because it reduces cardiac and respiratory motion-induced artifacts. The use of T2 sequences enables better differentiation from surrounding tissues MRI offers the possibility of multiplanar imaging and superior assessment of the relationship between a tumor and its surrounding vessels (the great vessels in particular) compared with CT, rendering this modality of utmost importance in the evaluation of patients with pheochromocytoma in these areas, especially to rule out vessel invasion. For all of the above listed reasons MRI may be preferable as the initial imaging method for pheochromocytoma in children.

CT of the abdomen, either with or without contrast, usually provides the initial method of localizing pheochromocytoma. It is easy and widely available, and relatively inexpensive imaging technique. It continues to be the primary imaging tool for the diagnosis of most pediatric solid tumors, despite obvious disadvantages of radiation exposure and limited contrast

resolution. CT can be used to localize adrenal tumors 1 cm or larger and extra-adrenal tumors 2 cm or larger. Sensitivity varies between 85% and 98% for adrenal pheochromocytomas (89–92). Sensitivity for detecting extra-adrenal pheochromocytoma is around 90% (89,93,94), but may decrease to around 77% for detection of local recurrences due to post-operative changes that may confound interpretation of images (94,95). For lesions limited to the adrenal glands, unenhanced CT followed by contrast-enhanced and delayed contrast-enhanced CT imaging yields a sensitivity of 98% (96,97). CT shows the structures surrounding a pheochromocytoma and permits exact localization of the tumor, although intraabdominal foreign bodies such as surgical clips may distort imaging findings (98,99). Spiral CT is preferred for small thoracic tumors (100–103).

Pheochromocytomas may be homogenous or heterogenous, solid or cystic complex masses or may show calcifications. Smaller tumors tend to have more uniform attenuation. CT attenuation values have proved to be helpful for characterization of adrenal tumors. Different image presentation of adrenal adenomas is due to intracellular fat content with resultant low attenuation of less than 10 Hounsfield units (HU) (96,104,105). Most pheochromocytomas have attenuation higher than 10 HU on noncontrast CT. They rarely present as lesions of less than 10 HU (106). Pheochromocytomas typically enhance on contrast CT but they may also present as heterogenous lesions due to cystic changes, with areas that do not enhance. Washout characteristics of adrenal masses are also helpful when it comes to differential diagnosis. Adenomas have a relative washout rate of more than 40% when delayed images are obtained 15 minutes after contrast administration. The washout rate of malignant lesions is less than 40% (96). None of the methods is perfect, therefore, may be confusing (96,106,107).

In some patients with pheochromocytoma, CT may be negative or have equivocal findings while MRI exams are positive (108,109) but these cases are rare, especially in patients with no history of previous operation.

Extra-adrenal pheochromocytomas are located most commonly in the abdomen. Therefore, we suggest that CT of the abdomen, including pelvis, to be done first. This should be followed by chest and neck imaging if abdominal CT is negative.

If pheochromocytoma is highly suspected by either positive biochemical results or by both imaging studies and positive biochemistry, MIBG scintigraphy (MIBG scan) may be used to locate and confirm pheochromocytoma and rule out metastatic disease. The specificity of MIBG is about 95% to 100% but this technique offers suboptimal sensitivity (78–83%) with the ^{131}I-labeled agent.^{123}I-MIBG scintigraphy provides superior image quality and seems to be especially useful for detecting recurrent or metastatic pheochromocytoma, tumors with fibrosis or distorted anatomy, and tumors in unusual locations (110–112). Additionally, the biological half-life of ^{123}I is shorter than of ^{131}I resulting in a favorable decrease of radiation exposure. Situations where ^{123}I-MIBG imaging may not be necessary include unilateral adrenal masses of less than 5 cm associated with an elevation of plasma metanephrine or EPI. This is because practically all epinephrine-producing pheochromocytomas are found in the adrenal gland. The likelihood of an extra-adrenal epinephrine-producing pheochromocytoma is close to zero.

Other imaging modalities that can be used to locate pheochromocytoma include octreotide scintigraphy (octreoscan) (113) and positron emission tomography (PET), using [^{18}F]fluorodeoxyglucose (FDG), [^{11}C]hydroxyephedrine (114), [^{11}C]epinephrine, [^{18}F]-DOPA (115) and [^{18}F]fluorodopamine (116–118). These functional imaging modalities should be considered in cases with high suspicion of a tumor when ^{123}I-MIBG scintigraphy is negative. The high costs of radioligands and necessary equipment are disadvantages of PET, but the high quality of images obtained by PET, lack of requirement for thyroid blockade, and relatively low radiation exposure offer advantages over MIBG imaging.

The main technical advantage of PET is that PET radioligands can be designed to target specific processes unique to certain tumors. For pheochromocytomas such processes include the catecholamine biosynthetic pathway and catecholamine transporter systems. [^{18}F]fluorodopamine is more specific substrate for NE transporter compared to most other amines (119). Recent studies showed that [^{18}F]fluorodopamine PET scanning can detect and localize pheochromocytoma not only as a primary tumor in the adrenal gland but also as a recurrent extra-adrenal or metastatic tumor and it is superior to MIBG scintigraphy (77).

In contrast to [^{18}F]fluorodopamine, imaging with [^{18}F]-DOPA does not depend on uptake by the cell membrane NE transporter system. Uptake and retention appears dependent on the presence of other components of the amine uptake and decarboxylation pathway common to many neuroendocrine cells. This may make [^{18}F]-DOPA less specific as an imaging agent than [^{18}F]fluorodopamine. Nevertheless, results for patients with adrenal pheochromocytomas and paragangliomas have been encouraging (115,120).

The uptake of glucose labeled with FDG should be useful in the imaging of malignant pheochromocytomas with increased metabolic rate (117). This diagnostic modality is important especially in rapidly progressing tumors when other functional imaging may be negative (121). Nevertheless FDG cannot distinguish malignant from benign disease. It should be noted that the use of FDG is not recommended in the initial diagnostic localization of pheochromocytoma. This radiopharmaceutical is nonspecific for pheochromocytoma.

Based on most current data algorithm for localization of pheochromocytoma in patients in whom

pheochromocytoma was proven based on biochemical test results should include: anatomical imaging methods (either CT or MRI) but MRI is preferable and U/S may also be considered in children. Except in few situations as described earlier, the presence of pheochromocytoma should always be ruled out or confirmed with functional imaging. The functional imaging test of choice at present is [^{123}I]-MIBG scintigraphy. If MIBG scintigraphy is negative, PET studies should be performed with specific ligands, preferably 6-[^{18}F] fluorodopamine. If this is also negative, pheochromocytoma is most likely dedifferentiated (commonly seen in malignant tumors). In such a situation, FDG PET or Octreoscan should be carried out. If a tumor is not found despite positive biochemical results, repeated noninvasive localization work-up after two to six months is recommended (Fig. 5). Concluding pheochromocytoma is an imaging chameleon and that is why multiple imaging techniques need to be used in its diagnostic process.

Treatment of Pheochromocytoma

The first line treatment of pheochromocytoma in children, as in adults is surgical excision of the tumor. To prevent perioperative complications due to massive outpouring of catecholamines from the tumor, preoperative pharmacological blockade of catecholamine effects and synthesis is required (122–126). Routine used pharmacological agents include phenoxybenzamine, an α1 and α2 -adrenoreceptor noncompetitive antagonist, that opposes catecholamine-induced vasoconstriction and propranolol, atenolol, or metoprolol as ß-adrenoceptor blockers that oppose catecholamine-induced arrhythmia and the reflex tachycardia (28,31,39,76,127). β-blockade alone is contraindicated. It can actually augment effects of catecholamines at α-adrenoceptors resulting in hypertensive crisis.

The average oral dose of phenoxybenzamine in children is between 20 and 50 mg/day given at six to eight hour intervals. To reach adequate preoperative blockade, phenoxybenzamine is suggested to be increased until orthostatic hypotension is present or mean arterial pressure is normalized (128,129). However, several reports questioned this approach finding no correlations between duration of preoperative treatment and the dosage of α blockers and intraoperative cardiovascular instability (130). The average dose of atenolol in children is 20 to 60 mg a day divided into two or three doses. Side effects of phenoxybenzamine include postural hypotension, reflex tachycardia, nasal congestion, sedation, tiredness and retrograde ejaculation. Atenolol can cause bradycardia and sedation. If hypertensive crisis occurs, similar to adult patients, an intravenous bolus of 2 to 5 mg phentolamine (Regitine) is the treatment of the choice. Phentolamine has a very short half-time, therefore, if necessary, the same dose can be repeated every two minutes until hypertension is adequately controlled or alternatively phentolamine may given as continuous infusion (100 mg of phentolamine in 500 ml of 5% dextrose in water). α-Methyl-*para*-tyrosine (metyrosine, Demser^{TM}), a competitive inhibitor of tyrosine hydroxylase is routinely administered preoperatively in some institutions (the starting dose is usually 250 mg twice to four times a day) and may be tried in patients in whom elevated blood pressure and arrhythmia cannot be controlled by using α and β blockade. At some other centers, calcium channel blockers are used as a primary drug to control hypertension (28,40,131). Significant hypovolemia should be corrected by perioperative administration of intravenous fluids to avoid hypotension. Because children have a higher risk of catecholamine-induced pulmonary edema, fluid replacement should not exceed 10 mL/kg/hr (129). Risk of excessive hypotension and hypovolemia can be minimized by the increase of salt and fluid intake in the preoperative phase as well. The additional advantage of this approach is that it significantly reduces the risk of postoperative hypotension. It should be taken into

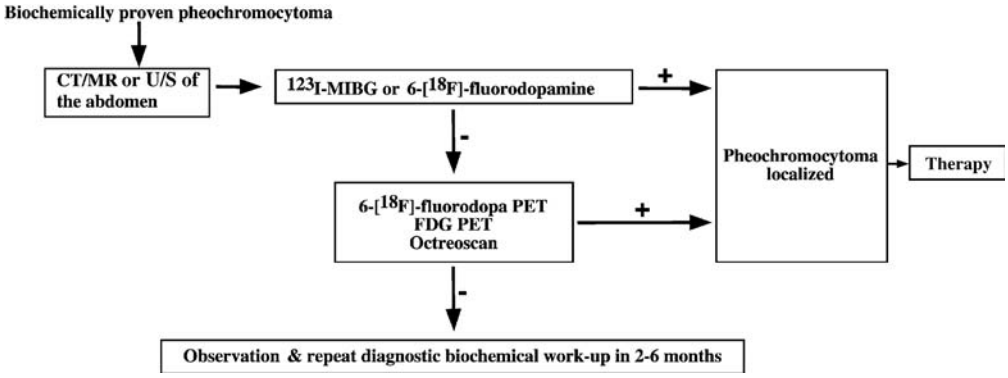

Figure 5 Diagnostic algorithm for pheochromocytoma localization in children.

consideration that pressor agents are not usually effective in the presence of severe and persistent hypovolemia and that normovolemia should be ensured by close monitoring of valume status. Children should also be watched for hypoglycemia up to 48 hours after surgery (catecholamines block insulin release via α-adrenoceptors).

Surgical removal of intra-adrenal pheochromocytomas in children is usually successfully carried out by laparoscopy, a procedure that minimizes catecholamine-induced hemodynamic changes during operation, postoperative morbidity, hospital stay, and expenses compared to conventional transabdominal adrenalectomy (132–141). In children with familial pheochromocytoma that carries higher risk for bilateral adrenal involvement prophylactic adrenalectomy of the contralateral side is not recommended. Adrenal-sparing surgery should be considered in any patient with bilateral tumors or a tumor in solitary adrenal gland. This is because of significant morbidity associated with bilateral adrenalectomy and life long steroid replacement. Given the lifelong high risk of occurence of contralateral disease adrenal-sparing surgery might be an option in all pediatric cases (28,141–143). However risk of tumor reoccurrence is 10% to 30% especially in familial pheochromocytomas (141–143).

After surgical resection yearly follow-up evaluation for assessment of pheochromocytoma recurrence is recommended for at least five years and should include measurements of plasma free metanephrines. Children with familial pheochromocytoma or familial predisposition to develop this tumor should undergo annual screening indefinitely.

In the presence of metastatic and therefore incurable disease, drug therapy is the first line treatment with the goal to control cardiovascular effects and complications related to catecholamine excess. Often more aggressive treatment including chemotherapy, MIBG and octreotide therapy, external radiation, and in some cases embolization of the tumor is required. However, less than 30% of patients with metastatic pheochromocytoma respond (mostly partial remission) to these currently used therapeutic modalities such as MIBG or chemotherapy. Therefore, most children are only treated when the quality of their life is affected by catecholamine excess or metastatic lesions that are aggressive and affect local surrounding tissue. Clinicians using the above therapies, particularly chemotherapy, should be aware of potentially fatal complications arising from excessive catecholamine release as tumor cells are destroyed (usually within the first 24 hours). With use of ^{131}I-MIBG, a major complication is bone marrow suppression (mainly thrombocytopenia) seen usually four weeks after initiation of therapy.

Genetics of Pheochromocytoma

According to recent studies, up to one-third of pheochromocytomas are inherited (144–148) Hereditary pheochromocytoma is associated with multiple endocrine neoplasia type 2 (MEN 2A or MEN 2B), von Recklinghausen's neurofibromatosis type 1, von Hippel–Lindau (VHL) syndrome, and familial paraganglioma due to germ line mutations of genes encoding succinate dehydrogenase (SDH) subunits B,C, and D (Table 3) (Vol. 2; Chap. 27). In general, the traits are inherited as an autosomal dominant.

Genetic testing should be performed in every child with pheochromocytoma. This is because the knowledge of these results usually implies early diagnosis of associated pathologies and allows taking preventive measures. That to our best knowledge should include detailed imaging to look for hemangioblastoma, pancreatic tumors and, renal cancer in VHL (149), total thyroidectomy by age six months in MEN 2B and before age five years in MEN 2A (150) and perhaps thyroid U/S in SDHB and SDHD carriers. Hopefully with better understanding of phenotype and genotype correlation future diagnostic work up will be tailored to each individual patient, therefore, limiting unnecessary tests and expenditure.

Multiple Endocrine Neoplasia 2A (Sipple Syndrome)

MEN 2A was first described in 1961 by Sipple and is defined by the occurrence of medullary thyroid carcinoma (MTC), pheochromocytoma (affecting about 50% of patients), and hyperparathyroidism caused by parathyroid gland hyperplasia (affecting about 20% of patients) (151–154). There is also familial MTC characterized by hereditary MTC without other associated endocrinopathies (although adrenomedullary hyperplasia secondary to a germline *RET* mutation may still be present but undiagnosed). Familial MTC belongs genotypically to MEN 2A. Rare variants of MEN 2A represent MEN 2A associated with cutaneous lichen amyloidosis and MEN 2A or familial medullary thyroid carcinoma associated with Hirschprung's disease (Vol. 2; Chap. 25) (155–158).

MEN 2A accounts for the majority of MEN 2 cases. In general, MEN 2 affects about 1 in 40,000 individuals, and there are less than 1000 kindreds worldwide. The gene responsible for MEN 2 is a proto-oncogene called *RET* (159). In contrast to MEN 1, *RET* is specifically expressed in neural crest-derived cells, such as the calcitonin-producing C-cells in the thyroid gland and the catecholamine-producing chromaffin cells in the adrenal gland. Whether it is also expressed in the parathyroid glands, remains to be ascertained, especially when considering the low rate of hyperparathyroidism in patients with MEN 2A and the lack of hyperparathyroidism in MEN 2B, although both conditions are caused by mutations in the *RET* gene. *RET* plays a role in normal gastrointestinal neuronal and kidney development as exemplified by the *RET* knockout mouse which has a Hirschprung-like phenotype and renal dysgenesis or agenesis (160,161). *RET* is located on chromosome

Table 3 Familial Pheochromocytoma

Syndrome	Genetic abnormalities	Phenotypic abnormalities
Multiple endocrine neoplasia syndromes		
Mutliple endocrine neoplasia type 2A (Sipple's syndrome)	Chromosome 10 (10q11.2) RET proto-oncogene mutations affect tyrosine kinase ligand-binding domain	Medullary ca. of the thyroid Hyperparathyroidism
Mutliple endocrine neoplasia type 2B	Chromosome 10 (10q11.2) RET proto-oncogene mutations affect tyrosine kinase ligand-binding domain kinase catalytic site	Medullary ca. of the thyroid Mucosal neuromas Intestinal ganglioneuroma Megacolon Marfanoid habitus
Neuroectodermal syndromes		
Neurofibromatosis (von Recklinghausen's disease) type (NF-1)	Chromosome 17 (17q11) mutations affect NF-1, tumor suppressor gene	Retinal angiomas
Cerebelloretinal hemangioblastomatosis (VHL) type 2	Chromosome 3 (3p25–26) missense mutations affect VHL, tumor suppressor gene	Cerebellar and spinal cord hemangioblastomas Renal cell cancer Pancreatic, renal, epididymal, and endolymphatic cysts/tumors
Succinate dehydrogenase gene family syndromes		
SDHB (Paraganglioma /PG/ type 2)	Chromosome 1 (1p36) missense, nonsense, frameshift mutations	Adrenal or extra-adrenal pheochromoctyoma, often metastatic; parasympathetic paraganglioma
SDHC PG type 3	Chromosome 1 (1q21)	Parasympathetic paraganglioma, extra-adrenal pheochromocytoma
SDHD PG type 1	Chromosome 11 (11q23) missense, nonsense, and frameshift mutations paternal transmission	Parasympathetic paraganglioma, extra-adrenal pheochromocytoma

Abbreviations: NF-1, neurofibromatosis type 1; VHL, von Hippel-Lindau syndrome.
Source: From Ref. 15.

10q11.2 and encodes a receptor tyrosine kinase, RET protein. As an oncogene, activation of *RET* leads to hyperplasia of target cells *in vivo*. Subsequent secondary events then lead to tumor formation (162–164). *RET* consists of 21 exons with six so-called ''hot spot exons'' (exons 10, 11, 13–16) in which mutations are identified in 97% of patients with MEN 2. *RET* germline mutation screening is commercially available (165) and has widely replaced the cumbersome provocative testing of calcitonin stimulation (with calcium and/or pentagastrin) which has been notoriously unreliable in children, because for this population the normal range of basal and stimulated calcitonin is still unknown. Similarly, the normal range for catecholamines including metanephrines in children with and without pheochromocytoma and/or adrenal medullary hyperplasia has only recently been elucidated (166,167).

Upon presentation in early childhood, there is usually no abnormal finding on physical examination or U/S in patients with MEN 2A. If *RET* germline mutation testing or the family history is positive for MEN 2, a neck computed or magnetic resonance tomogram (baseline) for evaluation of already developed metastases from MTC should be performed.

Before any surgery including prophylactic thyroidectomy in children with MEN 2, a search for pheochromocytoma should be undertaken by measuring plasma free metanephrines. Pheochromocytoma is the first manifestation in 10% to 30% and develops on the grounds of adrenomedullary hyperplasia secondary to a *RET* germline mutation, and becomes manifested (e.g., biochemically or on imaging) in about 50% of patients. The peak age is around age 40 but children as young as age 10 can be affected (168,169). Thus, annual surveillance for plasma free metanephrines, is recommended after age five (76). Patients with MEN 2-related pheochromocytoma lack hypertension (occurs only in about 50%) and have β-adrenergic symptoms including palpitations and tachycardia. In 50% of patients pheochromocytoma produces EPI, metanephrine, NE and normetanephrine with predominance of EPI (170). In another half of patients pheochromocytoma produces only EPI and metanephrine. When metanephrine levels are significantly increased CT and/or MRI plus MIBG scintigraphy should be performed (76). Although most MEN 2-related pheochromocytomas are located in the adrenal gland 50% to 80% are bilateral either simultaneously or at different times and rarely they may be found as extra-adrenal tumor. Malignancy rate is very low in MEN 2 and represents less than 5% (166,170,171). Therefore, the use of functional imaging, especially^{123} I-MIBG scintigraphy is not recommended in all cases. Based on our experience, we recommend using functional imaging in unilateral pheochromocytomas beyond 5 cm in diameter or when there is high suspicion

for bilateral adrenal disease or metastatic pheochromocytoma.

Multiple Endocrine Neoplasia 2B

MEN 2B represents about 5% of all MEN 2 cases and is defined by the presence of MTC, pheochromocytoma, and associated abnormalities including mucosal neuromas (within the lips, the gastrointestinal tract, on the tongue tip and eyelids), medullated corneal nerve fibers, and marfanoid habitus (152,172). In contrast to Marfan syndrome, however, patients with MEN 2B do not have lens or aortic abnormalities. Mucosal neuromas within the lips often give patients a ''blubbery/bumpy lip look.'' Physical examination is remarkable for these associated abnormalities. For instance, slit lamp examination may reveal medullated, hypertrophied corneal nerves. Often, patients have an acromegaloid appearance. Intestinal ganglioneuromatosis may cause diarrhea alternating with constipation or even obstruction.

In contrast to patients with MEN 2A, patients with MEN 2B do not have hyperparathyroidism, although MEN 2B is also caused by germline mutations of *RET* (173). These mutations affect exons 15 and 16, coding for the intracellular tyrosine kinase domain of RET protein. This molecular difference to MEN 2A supposedly leads to autophosphorylation without dimerization and may explain why patients with MEN 2B present at an earlier age with MTC and/or pheochromocytoma. It remains puzzling, however, why hyperparathyroidism only rarely occurs in MEN 2B. Authorities recommend total thyroidectomy and central lymph node dissection within the first six months of life for children with MEN 2B (*RET* germline mutations in codons 883, 918, and 922), since metastases from MTC may develop within the first year of life (174,175). Also, pheochromocytoma in patients with MEN 2B occurs at an earlier age than in MEN 2A. Clinical management of MTC and/or pheochromocytoma is otherwise identical in MEN 2A and MEN 2B. Importantly, about 7% of patients with apparently sporadic MTC have germline mutations in *RET*, making it reasonable to perform *RET* mutation analysis in all patients with MTC (176–178).

Von Hippel–Lindau Syndrome

VHL is another autosomal dominant inherited tumor syndrome with pheochromocytoma (VHL type II) or without pheochromocytoma (VHL type I) (179,180) that may occur in children. Apart from pheochromocytoma, major tumors in VHL disease include renal cell carcinoma, hemangioblastoma, neuroendocrine pancreatic tumors and cysts, endolymphatic sac tumors and epididymal cysts (181,182). All children with familial, multiple or early onset of pheochromocytoma should be examined for VHL disease which has variable expression and age and tumor-dependent penetrance. Pheochromocytoma occurs in about 10% to 20% of VHL patients and is the presenting manifestation in about 5% of cases (183,184). Mean age at presentation is 30 (149). However, there are large interfamilial variations with some families having pheochromocytoma as the most frequent complication of VHL disease (183,184). It is important that children with VHL syndrome who underwent unilateral adrenalectomy for pheochromocytoma need lifelong follow-up to timely diagnose recurrence or another pheochromocytoma on the contralateral side. In contrast to pheochromocytomas of children with MEN 2 (''adrenergic''), VHL-associated pheochromocytomas have a more ''noradrenergic'' phenotype, pointing to their developmental different stage, i.e., MEN 2-related pheochromocytomas frequently possess all the enzymes to synthesize catecholamines from tyrosine to EPI, whereas less differentiated pheochromocytomas including those in VHL disease lack the enzymes involved in the final catecholamine biosynthesis pathway (185). Thus, symptoms and signs of patients with VHL-related pheochromocytoma are related to NE excess including, e.g., hypertension. Metastatic pheochromocytoma is rare at about 5% or lower (186). The diagnostic algorithm is similar to patients with other familial adrenal pheochromocytomas (76), however, it is important to add that pheochromocytoma in VHL has often bilateral adrenal presentation, but occasionally may be multifocal with abdominal and thoracic localizations (184,185,187) and has noradrenergic biochemical phenotype.

The *VHL* tumor suppressor gene is located on chromosome 3p25–26. The cloned coding sequence comprises three exons. Most patients with VHL-associated pheochromocytoma have missense mutations (188–190). There are genotype-specific VHL phenotypes (182). Founder effects may explain regional prevalence rates, e.g., the Black Forest area in Southern Germany with the missense mutation tyrosine to histidine at codon 98 (Tyr98His) and subsequent high risk of pheochromocytoma (170,191,192). *VHL* missense mutations may have tissue-specific effects (191,192). A ''second hit'' is required in patients with *VHL* germline mutations, to develop pheochromocytoma (193).

The *VHL* gene product forms a stable complex with the highly conserved transcription elongation factors elongin B and elongin C, factors that regulate RNA polymerase II elongation. Formation of this heterotrimeric complex with elongin B and C appears to be the tumor suppressor function of the *VHL* gene, because the majority of tumor-predisposing mutations of *VHL* disrupt the formation of this complex (194–196). Normal VHL protein function leads to degradation of a proteasome complex (197).

Neurofibromatosis Type 1

NF1 is the most common (affects about 1 in 4000 individuals) familial cancer syndrome that predisposes to pheochromocytoma. However, the risk of it is small,

less than 5% overall (198,199). That is why routine screening for pheochromocytoma is generally not recommended. If pheochromocytoma occurs it is usually later in life around age 50 and is seen seldom in children. Only 12% of NF1 patients are diagnosed with bilateral and multifocal pheochromocytomas and less than 6% of patients have metastatic pheochromocytoma (200). Biochemical phenotype includes both secretion of NE and EPI and diagnostic algorithm is the same as for other familial adrenal pheochromocytomas.

NF1 is inherited in an autosomal dominant manner with variable expression. 50% of patients have new mutations and mutation analysis is cumbersome because of the large gene size (11 kb of coding sequence).

The NF1 gene is a tumor suppressor gene mapping to chromosome 17q11.2. Patients with NF1 associated pheochromocytoma show loss of the wild type allele (201) following Knudson's ''two-hit'' hypothesis. Neurofibromin, the NF1 gene product, bears homology to the ras/GTPase activating protein (GAP) (202) P21-ras/GAP increases the rate of intrinsic GTP hydrolysis in the small G proteins, the ras genes, thereby mediating the return of the G protein switch to the ''off'' guanosine diphosphate-bound form. By this mechanism, signal transduction is controlled via the ras pathways.

Familial Pheochromocytoma/Paraganglioma Syndromes—SDHX Mutations

In recent years convincing evidence has come up that the majority of familial paragangliomas and also a significant fraction of the nonfamilial tumors are associated with germline mutations in the three subunits of the SDH (203). The paraganglioma (PGL) syndromes include: type 1, PGL 1 with *SDH*D gene mutation, type 3, PGL3 with *SDH*C gene mutation and type 4, PGL4 with *SDH*B gene mutation, gene mutations, respectively (Table 3).

The *SDH* genes (*SDH*A, *SDH*B, *SDH*C, and SDHD) encode the four subunits of complex II of the mitochondrial electron transport chain. As a complex of the mitochondrial electron transport chain, the SDH protein is a major link between two important mechanisms: Krebs cycle and oxidative phosphorylation. This enzyme, coded completely in nuclear DNA, consists of four protein subunits, which are named SDHA, SDHB, SDHC and SDHD. Interestingly, of the five mitochondrial complexes I to V, complex II is the only one with no subunits encoded by the mitochondrial genome. Except for *SDH*A gene, responsible for catalytic part of the enzyme, mutations of all other *SDH* genes are associated with the presence of familial and nonfamilial extra-adrenal and nonfamilial extra-adrenal pheochromocytoma (SDHB, SDHD) and parasympathetic paraganglioma (SDHB, SDHC, SDHD).

The *SDH*B gene, encoding for the iron sulfur proteinprotein in mitochondrial complex II, consists of eight exons and is located on chromosome 1p35–36 (204). The *SDH*C gene encodes for the large subunit of cytochrome c, consists of eight exons and is located on chromosome 1q21 (205). The *SDH*D gene encodes for the small subunit of cytochrome c, consists of four exons and is located on chromosome 11q23 (206).

The mutations of *SDH*X genes are probably inactivating. Functional analysis confirmed that inactivation of cytochrome b activates the hypoxia pathway through increased expression of VEGF (148). *SDH* mutation might link to blockade of neuronal apoptosis and have a common pathway with RET and VHL (7,203).

Recent studies and observations suggest that *SDH*B germline mutation is associated with a high risk of malignancy and multiple tumors. Thus identification of this mutation requires careful management and follow up (146,207). Patients who develop SDHB related pheochromocytoma should be operated as soon as possible and then presymptomatic genetic testing should be offered to all first-degree relatives in order to reduce inherited cancer morbidity and mortality. Mutation carriers should be offered biochemical screening (preferably the measurement of plasma free metanephrine levels) on a yearly basis starting at age 5 to 10 (147,208,209). *SDH*D mutation carriers develop phenotype only if defect allele is inherited from the father (maternal genomic imprinting).

The biochemical phenotype of pheochromocytomas in patients with *SDH* mutations is currently unknown although our preliminary data suggest noradrenergic or dopaminergic phenotype (Pacak and Eisenhofer, unpublished observations). Complete SDH mutation phenotype is also not known. It has been suggested by H. Neumann et al. that extra-adrenal abdominal presentations, malignant disease, renal cell carcinoma, and thyroid carcinoma may suggest SDHB (147,210). Whether thyroid malignancies are components of SDHB- or SDHD- related disease is unclear at present.

Familial Nonsyndromic Pheochromocytoma

There are also descriptions of families with nonsyndromic pheochromocytoma without any detected mutations in *RET*, *VHL*, *SDH*X. These suggest that there are probably other undiscovered genes responsible for the disease or presence of large deletions of known genes like some missed by commonly used PCL techniques.

Other Pheochromocytomas and Associated Syndromes

Pheochromocytomas may also occur as part of Carney's triad, that is, gastric leiomyosarcoma, pulmonary chondroma, and extra-adrenal pheochromocytoma (211). The syndrome is very rare; less than 30 cases have been reported and only 25% of patients manifest all three parts of the triad. It has so far not been described as a familial problem (212). Other diseases associated with pheochromocytoma are tuberous sclerosis and Sturge–Weber syndrome.

NEUROBLASTOMA

Neuroblastomas are tumors that derive from primordial neural crest cells of the sympathetic system. They are the most common solid extra-cranial tumor in children and they account for 7% to 10% of all tumors diagnosed in children (213–215). Neuroblastomas have been found to have the highest percentage of spontaneous regression amongst tumors (216).

The annual incidence in the US is 1 per 100,000 children under the age of 15. It is predominantly a tumor of very young children: in 80% the tumor presents in children younger than five years, in 15% in children between the age of 5 and 10, and in 5% in children older than 10 (217). The median age at diagnosis is found to be between 18 and 24 months (218–220). Often two peaks can be distinguished in the incidence of neuroblastoma and each is associated with a distinct pattern of clinical behavior. Tumors detected in the first year of life (initial peak) have a more benign course than those diagnosed in older children (second peak in children around two years of age) (219,221,222). The tumor has a slight predominance in boys (with boys to girls ratio of 1.2:1) and is reportedly more common in white than in black children (214).

Most cases of neuroblastoma are sporadic although they may be associated with familiar syndromes such as NF type 1 (223,224) or Ondine–Hirschprung syndrome. The last one is linked with germ line mutation at chromosome 4p12 (the *PHOX2B* gene), which may be responsible for the rare synchronous occurrence of neuroblastoma and other neural crest derived cell disorders (225–227). The rare familial 1% to 2% of all cases might be conferred trough disruption of locus at 16p12–13 (228,229). In recent years, a rise in incidence over time has been noted (214). This rise may reflect increased physician awareness, improved diagnostic procedures or changes in reporting (230).

Pathogenesis

Although the influence of exposure to several environmental factors (such as parental occupation, use of medication, lifestyle's exposures) on the risk of developing neuroblastoma has been investigated, no unequivocal relationship has been demonstrated (219,221,231). Further investigation of some possible risk factors, such as parental exposure to metals, solvents or radiation and maternal use of hormones during pregnancy, appears warranted (232).

Numerous studies using a range of experimental techniques have been undertaken to discover genes involved in neuroblastoma development. It has become apparent that both inactivation of tumor suppressor genes and activation of tumor oncogenes are involved in neuroblastoma tumorigenesis. Chromosomal loci that have been found to show loss of heterozygosity (LOH), indicative for the presence of a tumor suppressor gene, include 1p (19–31%), 2q (30%), 3p (9–25%), 4p (14–19.5%), 9p (19–36%), 11q (5–44%), 14q (10–31%), and 18q (31–81%) (233–247). Although intensive research has been directed toward identifying candidate genes in the regions that show LOH no indisputable tumor suppressor gene has yet been identified (248,249).

Amplification of the *MYCN* tumor oncogene, located on the distal short arm of chromosome 2 (2p24) was first reported in 1983 (250,251). In recent years it has become apparent that gain of the long arm of chromosome 17q, occurring in 54% to 83% of primary tumors, is probably the most frequent chromosomal abnormality in neuroblastomas (233,242,243,252,253). At present, genetic abnormalities found in neuroblastomas seem to have more relevance for prognosis than insight into pathogenesis.

Clinical Presentation

The symptoms and signs at presentation depend largely on the size and the location of the primary tumor, and whether or not the tumor has metastasized (Table 4). Metastases can occur as a result of both lymphatic and hematogenous extension and are most common in lymph nodes, liver, skin, bone, bone marrow and soft tissues (254), metastasis to lungs and the central nervous system is rare. In case of lymph node involvement outside the cavity of origin, a child is considered to have disseminated disease (219). Thus, a patient can present with local, regional or disseminated disease. The proportion of patients with disseminated disease at diagnosis—about 50% is age-dependent (255). Metastasized disease is more commonly seen in older children. In 35% of patients with apparent localized disease regional lymph node metastases are found.

Table 4 Presentation

Disease	Tumor site	Possible symptoms
Localized	Abdomen	Abdominal discomfort, fullness, abdominal pain, rarely respiratory distress
	Pelvis	Bladder and bowel disorders as a result of compression
	Neck, thorax	Dyspnea, dysphagia, Horner's syndrome
Disseminated	Nonspecific	General malaise, low grade fever, weight loss
	Bone	Limp, swelling of joint, bone pain
	Paraspinal	Neurological symptoms
	Orbital area	Proptosis, periorbital ecchymoses (raccoon eyes)
Producing	Catecholamines VIP	Hypertension, sweating, flushing, tachycardia, paroxysmal phenomena; Chronic secretory diarrhea (Kerner–Morrison syndrome)

The most common site of tumor localization is intra-abdominal (65%), most commonly in the adrenal gland. Children older than one year have a higher incidence of adrenal tumors than infants (40% vs. 25%, respectively). In 20% of cases the tumor is localized in the chest, in 2% to 3% in the pelvis, in 1% to 5% in the neck and in 6% to 12% other localizations. In 1% the primary tumor is not found (219–221,254–256).

The tumor may mimic other diseases: for instance bone metastasis may resemble musculoskeletal diseases such as rheumatoid arthritis (257) and paraspinal tumors compressing nerve roots or extending into vertebral bodies may mimic neurological diseases. Furthermore, the tumor may be accompanied by paraneoplastic phenomena such as opsoclonus–myoclonus syndrome, which occurs in 2% to 3% of children with neuroblastoma (258) or secretory diarrhea if the tumor is producing vasoactive intestinal peptide (VIP).

Rarely, production of catecholamines or catecholamine metabolites contributes to symptoms and signs seen at presentation. Although 95% of tumors produce catecholamines or catecholamine metabolites such as VMA and HVA, this only rarely causes symptoms of catecholamine excess such as hypertension or paroxysmal phenomena like tachycardia and flushing (259). Compared to pheochromocytomas, neuroblastomas appear to have a paucity of storage granules and low levels of tissue catecholamines, which is suggestive for an ineffective storage mechanism. Furthermore, in neuroblastoma patients catecholamines are effectively metabolized by COMT and MAO to yield nonpressor catecholamine metabolites such as VMA and HVA, which are thereafter excreted in the urine. This may explain the absence of symptoms of catecholamine excess (259). No correlation has been found between the tumor size and the level of urine catecholamines excretion. A less differentiated tumor may predominantly secrete dopamine instead of VMA or HVA. In some cases elevated blood pressure may be caused by compression of the large retroperitoneal mass on the kidney and its vasculature.

At physical examination care should be given to the detection of palpable masses in the abdomen or flank and the presence of hepatomegaly. Skin including scalp and joints should be inspected for lesions, swelling and tenderness. A neurological examination, including assessment of pupil sizes of both eyes and strength in the lower limbs, should be part of the workup for neuroblastomas.

Histopathologically, the differential diagnosis includes other 'small blue round cell neoplasia of childhood such as rhabdomyosarcoma, Ewing's sarcoma, peripheral neuroepithelioma (or PNET: primitive neuroectodermal tumor), lymphoma, and leukemia (260). Wilms tumor, hydronephrotic kidney, enlarged spleen or liver, lymphoma, germ cell tumor and mesenteric cysts should also be considered in the differential diagnosis (220,261).

Diagnosis and Localization

In 1988, a set of international criteria was formulated for neuroblastomas with respect to diagnosis, staging and response to treatment (262). In 1993, these criteria were revised (260). Obtaining tumor tissue is critical for diagnosis and staging of neuroblastoma.

According to the International Neuroblastoma Staging System (INSS) criteria, a neuroblastoma is confirmed if either (i) an unequivocal pathologic diagnosis is made from tumor tissue by light microscopy, or (ii) a bone marrow aspirate or trephine biopsy contains unequivocal tumor cells (e.g., syncytia or immunocytologically positive clumps of cells), and increased urine or serum catecholamines or their metabolites. In case of equivocal histology and immunohistology, genetic features characteristic of neuroblastoma, such as 1p deletions or N-*myc* amplification, can support the diagnosis.

Seventy one to 95% of neuroblastomas excrete abnormally large amounts of dopamine, HVA, NE, and VMA (219,221,263). In patients with suspicion for neuroblastoma measurement of urinary catecholamine metabolites is mandatory. In 15% of the tumors urinary VMA is not elevated. In order to be considered elevated VMA or HVA levels normalized to milligrams of creatinine have to be above 3.0 SD for the mean for age (219,221). With this cut-off approximately 92% of biopsy-proven neuroblastoma patients will have elevated catecholamines at diagnosis. It is recommended that both VMA and HVA to be measured in a patient with suspected neuroblastoma.

For localization of the tumor several imaging modalities are available. Plain radiographs can detect the tumor if calcifications are present. US may yield good information and is the least expensive imaging technique. Other imaging techniques such as CT and magnetic MRI provide better anatomic detail including the tumor's relation to surrounding tissues. This information is important when operability and respectability are discussed. To evaluate the presence of bone metastases bone scintigraphy with ^{99m}Tc-diphosphates is most sensitive (264). If available ^{131}I-MIBG or ^{123}I-MIBG scintigraphy should be performed routinely in the evaluation of all patients suspected of harboring a neuroblastoma (260). Ninety one percent of the neuroblastomas concentrate ^{131}I-MIBG, which makes it a specific and very sensitive indicator for neuroblastoma comparable with urine analysis for catecholamine metabolites (264,265). Addition of single photon emission computed tomography has been found to be beneficial for the interpretation of the MIBG scan (266). Recently FDG PET has been a very promising imaging modality for both soft tissue and bone lesions of neuroblastoma (116,267–269). FDG PET has higher spatial resolution than MIBG scintigraphy. It may be better for identification of small lesions especially in liver where physiologic uptake of MIBG can prevent correct

localization of lesions. Other investigational modalities include radiolabeled monoclonal antibodies reactive with neural antigens (270–272).

The recommendations of the INSS for clinical testing include CT and/or MRI with three-dimensional measurements for the primary site and abdomen/liver. To assess bone marrow involvement bone marrow biopsy is required. Bone involvement should be assessed by MIBG scan or ^{99}Tc scan. At physical examination care should be taken to assess lymph node involvement. These findings should be confirmed with histology. Every patient should receive a chest radiograph and if this is found to be positive a chest CT or MRI should be performed. Imaging modalities are also important for staging and follow up.

Staging

In the INSS classification, four stages are distinguished (Table 5). In stages 1 to 3, the disease is localized and the stage depends on the extent of lymph node involvement and the presence of midline-extension of the disease. In stage 4, the disease is disseminated (260). Stage 4S is a special subcategory: it consists of infants with a distinct pattern of disseminated disease that is associated with spontaneous regression. With or without nonsurgical treatment, these infants have a high cure rate (273,274).

Prognosis

Over the years numerous factors have been studied in neuroblastomas for their capability to predict outcome. Stage, age and site of primary tumor are most important for the prognosis. Biological variables found to be associated with outcome can be divided in several categories: histopathological characteristics, serum markers and genetic features.

The International Neuroblastoma Pathology Committee developed an age-linked classification system based on morphologic features of neuroblastomas in relation to prognosis. Patients are classified in four different categories based on the differentiation grade of the neuroblastomas, their cellular turnover index and the presence or absence of Schwannian stromal development (261). Other factors relating to prognosis that are taken into account in this classification system are mitosis-karyorrhexis index, mitotic rate and calcification.

Serum markers known to have a correlation with outcome are serum ferritin, neuron specific enolase (NSE), circulating ganglioside$_{D2}$shed from neuroblastoma cell membranes, and serum lactic dehydrogenase (LDH). Increases in ferritin (275) NSE (276) and circulating ganglioside$_{D2}$ (277–279) are all associated with worse prognosis. Increases in LDH are nonspecific and may indicate rapid cell turn over or large tumor burden. Increased LDH levels are associated with poor outcome (280).

Genetic features that have been found to correlate with prognosis are tumor cell ploidy, MYCN amplification, chromosome 1 deletion or allelic loss, telomerase activity and expression of the TRKA-gene. Of these features a hyperdiploid karyotype and a high level of expression of the TRKA-gene, encoding for a primary component of the high affinity nerve growth factor receptor are associated with a favorable outcome (281–286). In contrast, poor survival is predicted by MYCN amplification, chromosome 1 deletion (1p36 LOH) (287), chromosome 11 deletion(unb11q-LOH) (287), or allelic loss and a high telomerase activity (288–294).

Table 5 International Neuroblastoma Staging System

Stage 1	Complete gross resection of localized tumor. Residual disease may or may not be present microscopically. Identifiable lymph node(s), ipsi- and contralateral, negative for tumor at microscopy
Stage 2A	Incomplete gross excision of localized tumor. Identifiable ipsi- and contralateral lymph nodes negative for tumor microscopically
Stage 2B	Complete or incomplete gross resection of localized tumor with ipsilateral no adherent lymph nodes positive for tumor. Enlarged contralateral lymph nodes negative for tumor microscopically
Stage 3	Unresectable unilateral tumor infiltrating across the midline, regional lymph node involvement may be either present or absent; or localized unilateral tumor with contralateral regional lymph node involvement; or midline tumor with bilateral extension by infiltration or by lymph node involvement
Stage 4	Dissemination of tumor to distant lymph nodes, bone, bone marrow, liver, or other organs except as defined for stage 4S
Stage 4S	Localized primary tumor (as defined in stage one or two) with dissemination limited to skin, liver and or bone marrow[a] in an infant $<$ 1 yr of age

[a]Less than 10% of nucleated cells are tumor cells.
Source: From Refs. 253,259,261.

Treatment

INSS stage, age and biological features are the most important determinants in the design of a specific management plan for a patient. The intensity of the treatment program depends highly on the assessment of a patient's risk for recurrent disease (295). Surgery is usually performed in all patients to establish the diagnosis, procure tumor tissue for biologic studies and staging of the tumor. If feasible, the tumor can be excised at initial surgery. Chemotherapy is the principal treatment modality (296). Multiple-agent therapy has proven to be more advantageous than single agent therapy (219,221). In many patients, however, multimodality treatment is required. Radiation therapy and autologous bone marrow transplantation are used most frequent to supplement chemotherapy. Other treatment modalities studied for their effectiveness in neuroblastomas include radionuclide therapy with ^{131}I-MIBG, retinoid therapy and immunotherapy: antibodies targeted at GD2, a disialoganglioside on the surface of neuroblastoma, neuroblastoma

vaccines, and nonmyeloablative allogeneic bone marrow transplants. Novel therapies include generalized targeting of tumor cells with drugs that induce apoptosis (e.g., fenfretinide or target angiogenesis).

In the International Neuroblastoma Response Criteria definitions are given to assess response (260). Response should be determined based on the best approximation of the volume of both primary tumor and metastatic sites. Six categories are distinguished: 'complete response,' 'very good partial response,' 'partial response,' 'mixed response,' 'no response' and 'progressive disease.' 'Complete response' is characterized by complete disappearance of tumor at primary and metastatic sites. 'Partial response' is defined by a decrease of over 50% in volume of primary tumor and metastatic sites. If the response of the primary tumor is found to be between 90% and 99% and all metastatic sites have disappeared, it is classified as 'very good partial response.' In 'mixed response' there is a response of 50% or greater at one or more sites and a reduction of 50% or less at one or more others sites. In 'no response' no new lesions occur and the volume of primary tumor and metastases changes between a reduction of less than 50% and an increase of less than 25%. 'Progressive disease' is defined by a 25% increase in any preexisting lesion or the occurrence of any new lesion. However, discussion is ongoing whether the number of categories should be decreased.

The three-year event-free survival for stages 1, 2, 4S lies between 75% and 90%. For stages 3 and 4, age is important: if a child with stage 3 is younger than one year it has an 80% to 90% chance of cure, but with an age of over one year the three years survival rates drop to 50%. For children with stage 4 disease these percentages are 60% to 75% and 15% for the three-year survival. Late effects of treatment include disturbed linear growth and gonadal function and the occurrence of second malignant neoplasms such as thyroid carcinoma or myelodysplasia/ leukemia.

Screening

Screening programs for the detection of neuroblastomas have been set up in Japan, North America and Europe. Four million children at either six to seven months or one year of age were screened by measuring urinary catecholamines. Studies results were remarkably similar and led to more neuroblastomas being diagnosed in the screened population however, the additional tumors were mainly low-stage tumors with favorable biologic features. Many of these tumors would have never been diagnosed clinically because of high likelihood of spontaneous regression.

There was no decrease in the incidence of high-risk tumors in children beyond the age of screening as well as no lower mortality in the screened populations. Therefore universal screening of infants for neuroblastoma with urine catecholmines is not recommended (297–301).

GANGLIONEUROMA

Ganglioneuroma is a rare benign tumor that originates from the sympathetic chain. It is defined by the World Health Organization as ''a benign tumor of ganglion cells in various proportions, which may be immature or dysmorphic (e.g., multinucleation and nuclear pyknosis), and Schwann cells'' (302). They either occur de novo or are a result of maturation and differentiation of neuroblastomas (216,303). According to the International Neuroblastoma Pathology Committee it is not a separate entity but should be considered a final stage of matured neuroblastomas (261). In a series of 88 patients described by the Armed Forces Institute of Pathology the majority of patients were above 10 years of age and only 16% (14 out of 88) of the patients were younger than 10 years (219). In contrast, in recent studies the median age at presentation was found to be between 5.5 and 7.6 years (304,305). Boys and girls appear to be equally affected.

The posterior mediastinum (38%) is the most frequent site of occurrence and together with localization in the retroperitoneum this accounts for most of the cases (218,304–306). For abdominal tumors a preference was observed for nonadrenal (37.5%) over adrenal ganglioneuromas (21%) (305).

Up to half of the patients are asymptomatic (306–308). If symptoms are present, they are usually unspecific and related to a mass effect of the tumor or intraspinal tumor extension. Some patients may suffer from hypertension due to excessive catecholamine secretion of the tumor. The percentage of tumors reported to secrete catecholamines or catecholamine metabolites varies in the literature between 20% to 39% (304,305,309). Seldom a ganglioneuroma is found to be the cause of watery diarrhea by secreting VIP (310,311). The tumor can be visualized with ultrasonography, CT or MRI. Furthermore, 57% of the tumors are positive at ^{123}I-MIBG scintigraphy (305). MRI is superior to CT for studying local and intraspinal extension in retroperitoneal ganglioneuromas (312). Complete excision of the tumor is curative. Usually the prognosis remains excellent even if resection of the ganglioneuroma is incomplete.

NEURAL TUMORS AND CHRONIC DIARRHEA

Chronic diarrhea caused by neuroendocrine or neural tumors often is related to the production of VIP. The most common tumors in this group are VIPomas which can occur sporadically but also in the context of MEN 1. VIPomas cause secretory, watery diarrhea, which subsequently leads to disturbances in water and electrolyte homeostasis including hypokalemia and metabolic acidosis (Vol. 2; Chap. 28). Symptomatic treatment concerns replacement of water and electrolyte losses. Definitive treatment consists of tumor removal. For VIPomas, resection of a single

and/or multiple tumors is indicated which may include a pancreatic tail resection. For neuroendocrine or neuroectodermal tumors such as VIP-producing pheochromocytoma, ganglioneuroma, ganglioneuroblastoma, and medullary thyroid carcinoma, definitive therapy also is achieved by surgical tumor removal (310,311,313–320). If the diarrhea-causing tumor is composed of chromaffin cells, it may also secrete large amounts of catecholamines. Therefore, measurements of plasma VIP and plasma/urinary catecholamines including metanephrines should be performed in children with watery diarrhea (314,321). The somatostatin analogue octreotide improves diarrhea in the majority of patients including those with carcinoid syndrome (322,323). In patients with MTC, (nonvoluminous) diarrhea may be the initial complaint. The etiology of the diarrhea in this setting is unclear (324).

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11

Puberty and Its Disorders

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NORMAL PUBERTY

Definitions and Mechanisms

Puberty is the stage of growth leading to sexual maturity and reproductive capacity. In girls, the initial physical changes are breast development; in boys, testicular growth—both consequences of increased gonadotropin and sex steroid secretion. The progressive physical changes seen in breast, genitals, and pubic hair are described using the Tanner staging system that breaks the continuous pubertal physical changes into one of five stages. Accelerated growth begins at the onset of puberty in girls and at mid-puberty in boys. Menstruation and spermatogenesis also begin mid to late puberty. Gonadotropins [luteinizing hormone (LH) and follicle-stimulating hormone (FSH)] both stimulate the synthesis of estradiol in the ovary, which in turn stimulates breast and reproductive system maturity in females. LH stimulates testosterone synthesis by the testicular Leydig cells, while FSH stimulates seminiferous tubule maturation. Androgen stimulates pubic and axillary hair development, apocrine gland maturation resulting in adult-type body odor, and skin changes related to acne. Sex steroids stimulate overall somatic growth directly and indirectly via increased growth hormone secretion. Estradiol is the key hormone stimulating skeletal maturity in both sexes.

Neural circuits in the adolescent brain are activated and remodeled, partially sex steroid driven, with prominent maturation resulting in heightened sexual motivation, responses to sensory stimuli, and sexual behaviors (1).

Feedback mechanisms of the hypothalamic-pituitary-gonadal (HPG) axis first mature during fetal life. At this stage, the pulsatile gonadotropin secretion pattern initially develops because of gonadotropin-releasing hormone (GnRH) stimulation. This fetal gonadotropin release persists into the neonatal period where it is then dampened until re-emergence during puberty. Negative-feedback mechanisms of hypothalamic-pituitary gonadotropin–gonadal control, first acquired in fetal life, are operative thereafter. During childhood, this system is down regulated with minimal GnRH secretion and consequential diminished episodic release of gonadotropins, particularly LH, during childhood. The profiles of inhibin A are detectible during infancy, with inhibin B levels rising during childhood. Both rise with puberty having positive correlations with FSH providing evidence for follicular activity during infancy and childhood (2). Mean FSH levels in childhood remain relatively higher than LH, particularly in girls. Throughout childhood, both the pituitary and gonads are capable of full function when stimulated. During the childhood years, the pituitary gland remains responsive to GnRH stimulation with the prepubertal gonadotropin response pattern having FSH release that exceeds LH release. However, the childhood gonadotropin-response pattern to GnRH stimulation may not be distinguishable from that of hypogonadotropism. While there are obvious changes in feedback sensitivity, such are inadequate to explain differences in fetal, neonatal, childhood, and pubertal physiology. Over-riding central nervous system (CNS) control remains an obligatory component.

With the onset of puberty, mean LH and FSH levels increase, with a relatively greater rise in the LH. If measurements from a single serum sample of LH level are above the intermediate range of overlap of prepubertal and pubertal individuals, ascertainment of pubertal status can be made from a single, random sample. Since the response of LH to GnRH/GnRH analog (GnRHa) stimulation becomes more pronounced than FSH with pubertal maturation, an LH level obtained 30 to 60 minutes after GnRH or GnRHa stimulation remains the gold standard for determining pubertal status. Because the incremental rise of FSH after GnRH/GnRHa stimulation during the prepubertal and pubertal periods are similar, its

measurement during such testing is often unnecessary. A marked FSH response, commonly evident in prepubertal girls, should not be mistaken to indicate a pubertal response. Because of the differences between prepubertal and pubertal LH and FSH, LH/FSH ratios may be helpful in determination of pubertal status. While it must be kept in mind that gonadotropin secretion patterns change over time, a ratio of less than one generally reflects a prepubertal pattern while a ratio greater than one suggests a pubertal release pattern.

The onset of puberty is a consequence of enhanced episodic GnRH secretion. Accentuated episodes of LH release first occur during sleep, then progressively increase in frequency and amplitude and extend throughout the 24-hours period. The mechanisms used to control or initiate episodic release of hypothalamic GnRH remain incompletely understood, but appear to reflect a balance between both stimulatory and inhibitory neurotransmitters such as: acetylcholine, catecholamines, γ-aminobutyric acid (GABA), opioid peptides, prostaglandins, and serotonin.

Genetic/Molecular Control of Pubertal Development

The role of several specific neurotransmitters, co-factors, and receptors in the development of the HPG unit and the regulation of puberty have been identified. Much of what is known about genetic control of HPG axis has come from studying patients with single gene mutations resulting in gonadotropin deficiency. Several recently described examples of this include the identification of the roles played by kisspeptins, its cognate G-protein coupled receptor (GPR54), and the fibroblast growth factor receptor 1 (FGFR-1)(KAL2) gene. Previously recognized factors include GABA, the LHBβ subunit gene, neuropeptide Y, β-endorphin, leptin, and glutamate.

The GPR54 gene encodes a G protein-coupled receptor. A mutation in the GPR54 gene has been identified in a consanguineous family with idiopathic hypogonadotropic hypogonadism (HH). Affected humans have decreased GnRH secretion and a left-shift in the dose response curve for GnRH (3). This G-protein-coupled receptor appears to be necessary in mammals for the pubertal onset of pulsatile LH and FSH secretion. In men with inactivating mutations in GPR54 there is no puberty, the reproductive organs remain immature, gonadotropin, and sex steroid levels remain low in spite of normal hypothalamic GnRH levels (4). Studies from a loss of function GPR54 mutation expressed in HEK293 cells showed a diminished responsiveness to Kisspeptin suggesting that this factor is necessary for pulsatile release but does not drive it.

In late juvenile phase primate studies, repetitive administration of the GPR54 receptor agonist, kisspeptin-10 led to a LH discharge pattern that was identical to that seen following GnRH administration. This gonadotropin response to Kisspeptin-10 was abolished following pretreatment with the GnRH receptor antagonist, Acyline. These data are consistent with the activation of GPR54 from increased expression of hypothalamic Kisspeptin (5). Data from a patient with a compound heterozygous mutation in GPR54 suggests that the GPR54 function is required for neonatal as well as pubertal gonadotropin release patterns (6).

Identification of genetic mutations resulting in Kallmann syndrome has provided new information about CNS and hypothalamic development. KAL-1 gene mutation, found in X-linked Kallmann syndrome, encodes the protein anosmin-1, a glycoprotein involved in organogenesis. The KAL-2 mutation FGFR1 is associated with deficiency of FGF signaling during embryonic olfactory bulb and gonadotropes migration. Such have been found in the autosomal dominant form of Kallmann. Transgenic mice embryos with FGFR1 mutations fail to show FGFR1 expression in the developing telencephalon (7).

NORMAL FEMALE DEVELOPMENT

Terminology

An increase in the activity of the hypothalamic-pituitary-ovarian (HPO) axis during puberty may be referred to as gonadarche. Thelarche refers to the onset of breast development; menarche is the first menstrual period and pubarche refers to the onset of sexual hair growth. Pubarche is usually the result of increased adrenal androgen secretion (adrenarche). The other source of androgen in girls is ovarian. Thelarche is the most common initial manifestation of puberty, however, in as many as 20% of girls pubarche may precede thelarche.

Hormones and Hormone-Stimulated Changes

Mean LH, FSH, and estradiol levels initially increase before the physical changes of puberty become manifest. Levels further increase during puberty, accompanied by increases of both inhibin A and inhibin B (2). An unstimulated or GnRH/GnRHa stimulated LH level and an estradiol level above the prepubertal range confirms gonadarche. Estrogen-stimulated changes include onset and progression of breast maturity, genital growth (particularly the labia minora), maturation of the vaginal mucosa, uterine/endometrial growth, and body composition changes resulting in the female pattern fat distribution. Breast growth, which may begin asymmetrically, progresses throughout puberty and is classified into one of five Tanner stages (Table 1). Pubic hair is also Tanner staged (Table 1).

Adrenarche

Adrenarche, resulting from an increased adrenal androgen production, usually begins before gonadarche. Hormonal documentation of adrenarche is done by identifying pubertal levels of the weak adrenal androgen: dehydroepiandrosterone sulfate (DHEAS).

Table 1 Staging of Pubertal Development (Tanner)

Staging	Breast	Pubic hair staging	Concomitant changes	
Girls				
1	Prepubertal, papilla elevation	No pigmented hair		
2	Budding; larger areole; palpable and visible elevated contour	Pigmented hair, mainly labial	Accelerating growth rate	
3	Enlargement of the breast and areola	Coarser, spread of pigmented hair over mons	Peak growth rate, thicker vaginal mucosa, axillary hair	
4	Secondary mound of areola and papilla	Adult type but smaller area	Menarche (stage 3 or 4) decelerating growth rate	
5	Mature	Adult distribution		
	Genital size	**Pubic hair staging**	**Concomitant changes**	**Prader orchidometer (mL)**
Boys				
1	Prepubertal	No pigmented hair	Long testis axis < 1.5 cm	1–3
2	Early testicular, penile and scrotal growth	Minimal pigmented hair at base of penis	Early voice changes; testes length 2.5–3.3 cm	3–6
3	Increased penile length and width; scrotal and testes growth	Dark, coarse, curly hair extends midline above penis	Light hair on upper lip, acne, maximal growth, testes length 3.3–4.0 cm	8–12
4	Increased penis size including breadth; pigmented scrotum	Considerable, but less than adult distribution	Early sideburns; testes 4.0–4.5 cm	>12
5	Adult size and shape	Adult distribution, spread to medial thighs or beyond	Beard growth; testes > 4.5 cm	>15

Population studies have shown that DHEAS levels begin to increase in girls around six years of age.

Growth and Progression of Puberty

Acceleration of linear growth seen at the onset of the puberty is perhaps the first manifestation of puberty in girls. Alongside this increase in stature, there is an increase in weight gain and percent body fat. The interval between pubertal onset and menarche is variable since it is a consequence of the magnitude (mean levels over time) and sensitivity to sex steroid exposure. Menarche does not necessarily indicate the onset of ovulation. The mean time between the onset of stage 2 breast development and menarche is two years. Age at menarche correlates directly with skeletal age and inversely with linear growth remaining. Those with greater estrogen exposure before menarche are closer to their adult height and may gain less than 4 cm after reaching menarche. On average, girls gain an additional 4 to 6 cm of height after menarche. In contrast, those reaching menarche early may have as much as 10 cm of additional growth remaining. Generally, younger age at menarche correlates with shorter adult height. Puberty progresses regularly with an average duration of three to four years, spending 12 to 15 months in each Tanner stage.

Age of Puberty

The best available data (8,9) indicate that the early age limit and mean age of Tanner stage 2 breast development for white girls is 8.0 and 10.4 years of age, for black girls 6.6 and 9.5 years, and for Mexican-American girls 6.8 and 9.8 years. The average age of completion of puberty Tanner stage 5 breast development is 15.2 years for White girls, 13.5 years for Blacks and 14.7 years for Mexican-American girls. Despite much discussion, data remain inconsistent on whether the secular trend toward earlier puberty noted from the mid 1800s to the 1960s is continuing.

The population data on age of menarche are meaningful only when stratified based on race (10). The median age and estimated lower age limit (2.5 percentile) for menarche from the 1988 to 1994 National Health and Nutrition Examination survey (NHANES III) survey for White girls is 12.55 and 10.65 years, Black girls 12.06 and 9.70 years, and Mexican-American 12.25 and 10.05 years (Fig. 1). There is evidence that shifts in age of menarche may occur rapidly if there is a change in socioeconomic status (11).

NORMAL MALE DEVELOPMENT

Physical Changes

While pubic hair growth (Tanner stage PH2) is usually the first identified physical change of puberty in boys, the first physical change notable in puberty is an increase in testicular volume. A prepubertal testis is 2 cc or less in volume (< 1.5 cm in length). A testis with a volume of ≥ 3 cc or a longitudinal axis of ≥ 2.2 cm indicates pubertal gonadotropin stimulation (12). Male puberty is designated according to Tanner staging of genital development and pubic hair (Fig. 2). Although Tanner staging does not include testicular volume, such correlate with stages and should be determined since this represents the key physical finding. Axillary hair begins at mid-puberty followed by hair growth in androgen sensitive areas,

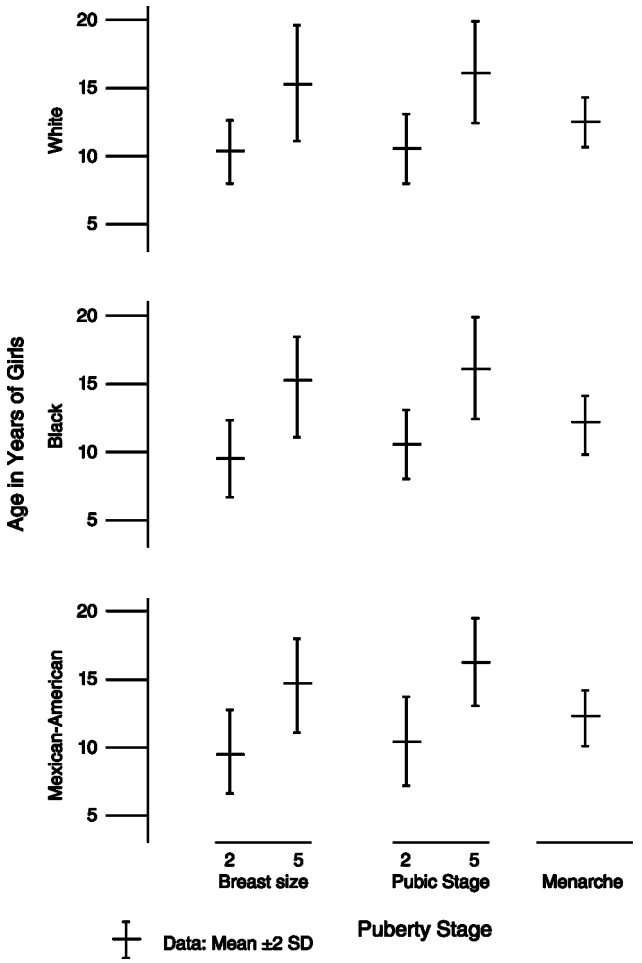

Figure 1 Estimated median ±2 SD for breast stage 2 and 5 and menarche for white, black and Mexican-American girls.

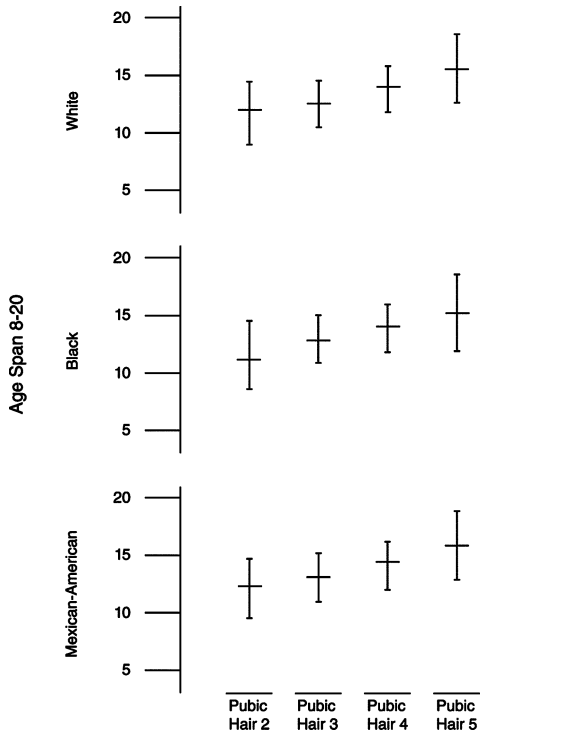

Figure 2 Estimated median ±2 SD for pubic hair stages for white, black and Mexican-American boys.

including the face, the chest, upper back, abdomen, and upper thighs progressing from mid-puberty to young adulthood. The density and distribution is related to genetic factors, as is the density and quantity in non-androgen sensitive areas such as the lower legs and forearm. What can be judged excessive lower arm hair, for example, is termed hypertrichosis and does not reflect an androgen-mediated feature. The thickness and distribution of androgen stimulated hair growth varies considerably between adult men, reflecting racial, familial, or genetic factors rather than hormonal levels.

Age of Puberty

In boys, the age of puberty has not been well documented. Evidence suggests African-American males may begin and complete puberty earlier than Whites and Mexican-Americans. The only age-appropriate large-scale data on pubertal ages in males by ethnicity come from the NHANES III from 1988 to 1992 and can be compared with earlier small studies that sampled primarily middle-class White males. There are inadequate data to determine whether the age of puberty in U.S. boys is changing. The median age of Tanner stage 2 pubic hair development (Fig. 2) (8,9) the most reliable criteria, are similar to data obtained over that last several decades in White boys at 12.0 years. In Black boys, the median age of Tanner 2 pubic hair is 11.2 years, and Hispanic boys the median age is 12.3 years. Extrapolation of these data suggest the lower age limit of pubertal onset, approximating-2 SD, is 9.0 years for White, 9.5 years for Hispanics, and about eight years for Blacks. Figure 2 also includes data extrapolated from NHANES III for the attainment of subsequent Tanner pubic hair stages. The NHANES III data for Tanner 2 genital staging differ so drastically from historical data that one can only conclude that different criteria are being applied between studies.

Hormone Changes

During puberty, there is a progressive rise of LH, FSH, and consequently testosterone that reflects an up-regulation of the hypothalamic-pituitary-testicular axis (13). Other intermediate metabolites of adrenal and testicular origin also rise (estrone, estradiol, androstenedione, 17-hydroxyprogesterone). An increase

in the levels of adrenal hormones, dehydroepiandrosterone (DHEA) and DHEAS, indicate adrenarche. Inhibin B levels, a marker of Sertoli cell mass and function, rise progressively before the onset of puberty. Both testosterone and inhibin B appear to be related to the nocturnal release of LH seen in early puberty (14). Müllerian inhibiting hormone (MIH) levels in males rise rapidly during the first year of life, peak in late infancy and gradually decline to a nadir during puberty. Outside of the neonatal period, MIH levels are inversely related to testosterone.

Growth

In boys, peak growth velocity, voice change, acne, and the onset of axillary hair occur at mid-puberty, after substantial testosterone exposure. During puberty, there is a progressive increase in total bone mineral content and lean body mass and a relative decline in body fat. These body composition changes begin with the onset of puberty, but in contrast with girls, significant composition changes do not occur until mid-puberty. In both sexes, estradiol is the primary hormone stimulating skeletal maturation and the estradiol levels attained during mid-puberty in boys is similar to those seen in early pubertal girls—a time when growth velocity peaks in females. Peak growth velocity, like puberty staging, differs based on race.

Spemarche, the onset of sperm production, has been documented by semen analyses and urinanalyses. The average male reaches spemarche while 14 years old, while he is in Tanner stage 3 genital and pubic hair development. This occurs after substantial increase in testicular volume and considerable gonadotropin and testosterone stimulation. Sperm density continues to increase to normal adult values over the next several years.

Pubertal Gynecomastia

Pubertal gynecomastia, usually involving self-limiting palpable or visible breast tissue, is a normal phenomenon that occurs in at least two-thirds of pubertal boys. It generally develops in early or mid-puberty. Findings range from isolated puffy areolar with little breast tissue to tender subareolar breast disks to a substantial degree of breast tissue. Breast growth generally plateaus after one year with some regression noted by 18 months; in most cases, total duration of pubertal gynecomastia is one to two years. The etiology of pubertal gynecomastia remains an enigma but it is felt to reflect that hormonal environment of early male puberty where the ratio of estrogen to testosterone is at its greatest. These levels of estrogen are felt to develop because of extra-glandular aromatization of adrenal/testicular androgens and the effect of high gonadotropin levels on the immature testis. In boys with significant gynecomastia, aromatization may also occur within the breast tissue. In the absence of a discrete palpable subareolar disk, it can be difficult to distinguish true breast tissue from the abundant adipose tissue (pseudo-gynecomastia) seen in overweight boys.

This common variant of normal development is not indicative of any underlying abnormality, but pathologic causes must be ruled out, if size, density, onset or duration is extreme. With pubertal gynecomastia, when quantity of breast tissue is considerable (>5 cm) or regression is not seen by two years after sexual maturity it is unlikely that satisfactory regression will occur. No medical therapy, such as those directed at blocking estrogen effect, has been shown to be producing adequate regression. Plastic surgical excision is the only therapy and should be offered. Such surgery should use only a semi-lunar incision around the areolae for removal of unwanted breast tissue to avoid trading one unwanted disfigurement for another. Other surgical techniques involving extensive chest incisions have no place in the treatment of pubertal gynecomastia.

While gynecomastia, either unilateral or bilateral, in a pubertal boy is usually a normal variant, pathologic causes should be considered if pubertal progression or development is atypical. Pathological conditions associated with gynecomastia during pubertal years include gonadal disorders, true hermaphroditism, secondary hypogonadism, Klinefelter syndrome, enzyme defects in testosterone production or partial androgen insensitivity. Rare causes include testicular tumors (Leydig cell tumors, Sertoli cell tumors, germ cell tumors), which rarely secrete estrogen and feminizing adrenal cortical tumors. Therapeutic and illicit drugs including androgens, ketoconazole, and marijuana have also been implicated in the development of gynecomastia.

If there is a persistent source of abnormal endogenous estrogen, plasma estrogen levels, or total urinary estrogen levels are elevated. Normal estrogen levels, an otherwise normal history and physical, and a negative history of contact with estrogen-containing medications or other agents is sufficient to delay further workup except for watching for regression of the gynecomastia. The presence of galactorrhea mandates that prolactin be measured.

PRECOCIOUS PUBERTY

Classification

Early physical changes of puberty are the consequence of sex steroid mediated effects, sex steroid production can stem from HPG axis activation or some other nonhypothalamic mediated increase in sex steroid production. The former category is referred to as GnRH-dependent, GnRH-driven, central or true precocious puberty (CPP); the latter is called GnRH-independent, peripheral or precocious puberty or precocious pseudopuberty (Table 2).

The diagnosis of precocious puberty has traditionally been made if physical development begins

before the lower age limit for that sex. Currently, age limits for the onset of puberty must account for racial differences in puberty development (Figs. 1 and 2). The presentation of precocious puberty includes, depending on the duration of the complaint, early onset and progression of physical development, accelerated linear growth, and advancement of skeletal age. If the onset of breast development in girls or genital growth in boys is not progressive, the diagnosis of precocious puberty may be inappropriate; at present, less than 10% of patients being referred for evaluation of early pubertal development actually meet diagnostic criteria for precocious puberty (15). In some children, particularly girls, such changes may constitute an entity described as non-progressive precocious puberty (16,17) a variant of normal development. It is important to highlight that the diagnosis of precocious puberty is based not only on early sexual development, but also on clinical, hormonal, and radiologic evidence of a progressive process. When precocious puberty has been diagnosed, the category (GnRH-dependent or GnRH-independent) and, if possible, the underlying etiology (Table 3) must be sought to determine appropriate therapy.

Table 2 Categories of Precocious and Early Puberty

Precocious Puberty
GnRH-dependent
Also termed "central" and "true"
Resulting from early onset and progression of pubertal HPG upregulation
Progression through pubertal stages with linear growth at accelerated pace
Leads to early sexual and reproductive maturity, tall stature during childhood with variably diminished adult height
GnRH-independent
Also termed "peripheral" or "precocious pseudopuberty"
Resulting from sex steroid stimulation by other than pubertal HPG activity
Early onset of physical pubertal development and growth, with varying progression
Rare, numerous etiologies
Other forms of early or inappropriate development
Premature thelarche:
Early onset of breast development without other significant pubertal changes
Independent of evidence of pubertal HPG activity
May be a consequence of normal prepubertal ovarian activity, increased sensitivity to low estrogen levels, or a metabolic consequence of obesity
May progress to precocious puberty
Premature adrenarche:
Most commonly manifest by early onset of pubic hair (premature pubarche)
Early increase of adrenal androgen production
Not clinical evidence of precocious puberty among females
May herald disorders of androgen synthesis
Intermittently progressive early puberty
Variable physical and hormonal evidence of precocious onset without morbidity
Slow or variable progression, with most pubertal milestones attained within normal pubertal age range
Growth and skeletal maturity progression without adult height compromise
Feminization of males:
Causes of childhood and adolescent gynecomastia:
Primary hypogonadal conditions
Congenital (anorchia, dysgenetic testes, enzyme biosynthetic defects, Kilnefelter syndrome, partial androgen insensitivity syndrome)
Acquired (cryptorchidism, infection, irradiation, torsion, trauma)
Drugs (amphetamines, antineoplastics, gonadotropin, isoniazid, ketoconazole, marijuana, sex steroid, tricyclic antidepressants, others)
Estrogen-secreting tumors, including Sertoli cell tumors, sex cord tumors association with Peutz-Jeghers syndrome
Systemic illness (hepatic, renal, recovery from malnutrition)
Masculinization of females:
Early onset (e.g., sexual hair, acne) or excessive androgen-mediated effects (clitoromegaly, hirsutism)
Causes include:
Hyperandrogenic disorders
Adrenal (congenital adrenal hyperplasia, Cushing disease, androgen-secreting adrenal tumors, generalized glucocorticoid resistance)
Ovarian disorders (PCOS, arrhenoblastomas, Teratoma)
Exogenous androgen
Idiopathic hirsutism

Abbreviations: GnRH, gonadotropin-releasing hormone; HPG, hypothalamic-pituitary-gonadal; PCOS, polycystic ovary syndrome.

Table 3 Differential Diagnosis of Precocious Puberty

GnRH-dependent (central)
Idiopathic (sporadic or familial):
Female:male > 10:1
More frequent among foreign adoptees
Central nervous system abnormalities (female:male~1:1):
Acquired (abscess, chemotherapy, granulomas, inflammation, radiation, surgical, trauma)
Congenital anomalies (arachnoid cysts, hydrocephalus, hypothalamic hamartomas, myelomeningocele, septooptic dysplasia, suprasellar cyst)
Tumors (LH-secreting adenoma, astrocytoma, craniopharyngiomas, ectopic pinealomas, ependymomas, glioma, including optic gliomas that may be associated with neurofibromatosis, associated with tuberous sclerosis)
Secondary to chronic exposure to sex steroids (causes of peripheral puberty; CVAH, GIP, tumors)
Reversible forms: space-occupying or pressure-associated lesions (abscess, hydrocephalus)
GnRH-independent (peripheral)
Genetic disorders (mutations):
CAH, males
Gonadotropin-independent puberty
LH receptor–activating mutations
DAXI gene mutations
McCune–Albright syndrome
Tumors:
Adrenal sex steroid secreting (adenoma, carcinoma, generalized glucocorticoid resistance)
Gonadotropin-producing (choriocarcinoma, chorioepithelioma, dysgerminoma, hepatoblastoma, hepatoma, teratoma)
Ovarian (carcinoma, cystadenoma, gonadoblastoma, granulosa cell—may be associated with Peutz–Jeghers syndrome, lipoid, sex cord, theca cell)
Testicular (Leydig cell)
Limited or reversible forms:
Chronic primary hypothyroidism
CAH
Exogenous sex steroid or gonadotropins, including iatrogenic
Ovarian cysts

Abbreviations: CAH, congenital virilizing adrenal hyperplasia; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone.

Gonadotropin-dependent Precocious Puberty

GnRH-dependent precocious puberty (GnRH-dep-PP) is the consequence of early onset of pubertal HPG activity; premature, but physiologically normal gonadotropin stimulation arising because of hypothalamic GnRH secretion. GnRH-dep-PP may result from CNS abnormalities that disrupt the balance between the inhibitory and stimulatory factors that govern pubertal onset/development or may occur in the absence of any identifiable abnormality (idiopathic). Precocious puberty occurs more frequently in girls immigrating to countries with better socioeconomic conditions (18).

Gonadotropin-Independent Precocious Puberty

GnRH-independent precocious puberty results from sex steroid stimulation, not produced because of physiological pituitary gonadotropin secretion, but from endogenous (gonadal or extragonadal) or exogenous sources. Endogenous sex steroids are autonomously produced, independent of gonadotropin stimulation or control, or because of non-pituitary gonadotropin or gonadotropin-receptor activation.

Diagnostic Evaluation

Basis

Diagnostic evaluation is based on physiology of early puberty and demonstrable pathologic underlying causes or associations. GnRH-dep-PP is diagnosed if the development of physical pubertal changes and laboratory testing are consistent with progressive changes of HPG axis activation. In patients presenting with precocious puberty, more than 90% of girls and about half of boys have GnRH-dep-PP.

Identifiable underlying neurologic abnormalities constitute less than 5% in females but are more frequent in males (20%)(19). Associated CNS abnormalities are listed in Table 3. Abnormalities may or may not be demonstrable anatomically within the CNS in areas that may disrupt the restraint typical of childhood. A unique demonstrable abnormality is the hypothalamic hamartoma (Fig. 3).

Those that cause precocious puberty are redundant CNS tissue containing GnRH neurons that function independently of CNS inhibitory influences, functioning as an ectopic hypothalamus that episodically secretes GnRH. Hamartomas may be pedunculated or sessile (20). Small, pedunculated lesions are associated with precocious puberty but not neurologic symptoms, while sessile lesions, involving the mammillary region of the hypothalamus, are commonly associated with seizures, behavior disorders and mental retardation. Seizures may be isolated or gelastic, those with gelastic seizures may present in early infancy (21). The larger sessile hamartomas are associated with precocious puberty (22). Hamartomas are identified more frequently in boys than girls, not uncommonly as young as the second year of life (Fig. 4). Abnormalities, not demonstrable using visualization techniques, include a history of encephalitis or previous CNS tumor treated with surgery, radiation, and chemotherapy. Evaluation begins by documenting history, physical findings, and assessing of hormonal status.

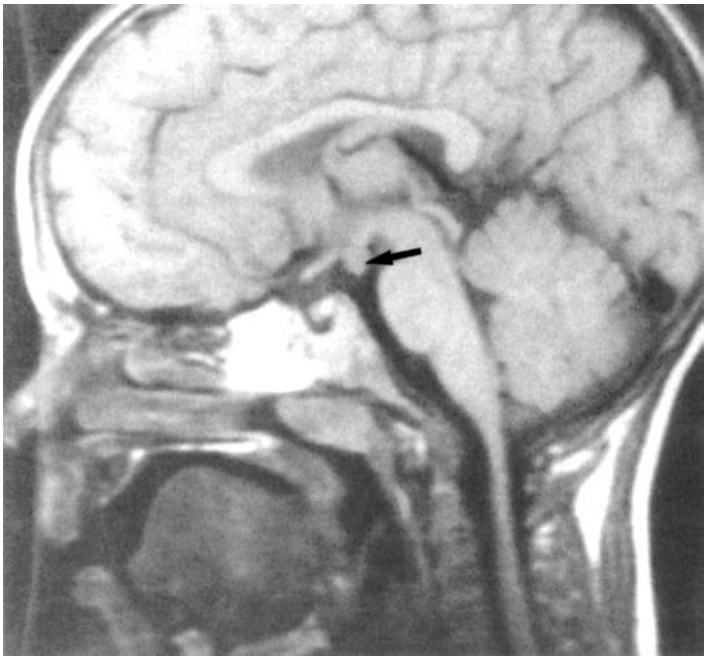

Figure 3 A hypothalamic hamartoma associated with episodic gonadotropin-releasing hormone secretion in a young boy with central precocious puberty. The arrow points to the harmartoma.

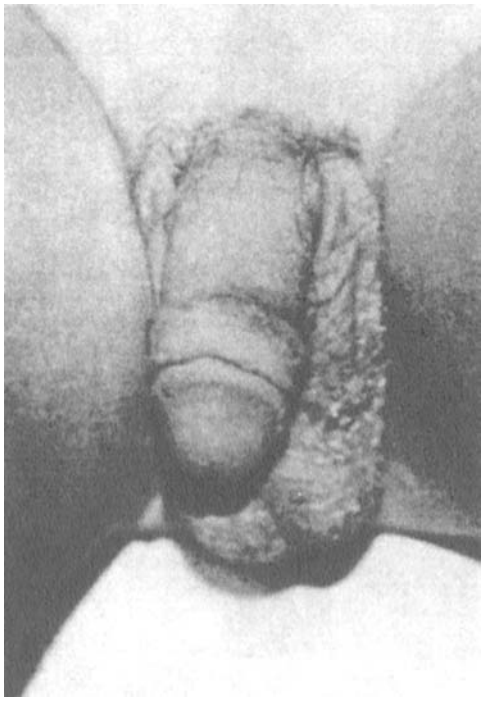

Figure 4 Genitalia of a 25-month-old boy with sexual precocity associated with a hypothalamic hamartoma. Tanner stage 2 public hair, Tanner stage 3 genetical developement, and enlarged tests for age are evident.

History

Pertinent findings on history include growth patterns since birth, age of onset and progression of physical pubertal changes, past medical, family, social, and psychological history. A complete history should be taken emphasizing careful consideration of any possible exposure to exogenous hormone, previous or current CNS abnormalities or symptoms, pubertal history of other family members, and height and growth rates. Gelastic seizures may occur in association with organic causes of CPP, including hypothalamic hamartomas.

Physical Findings

Physical examination should include careful measurement of height, weight, arm span, upper/lower body segment ratio and thyroid palpation, Tanner staging (Table 1), and general examination. For females, examination should include inspection of the genitalia for pubertal maturation, first evident by labial minora growth and increased clear mucous secretions, and visualization of the vaginal mucosa to assess the effect of estrogen, avoiding the need to obtain a potentially traumatizing vaginal smear. If the patient is positioned prone with knees drawn up and legs spread, the introitus can be visualized without touching the vulva by gently spreading the labia. A glistening red appearance is consistent with a non-estrogen stimulated mucosa, whereas a pink mucosa with a mucous covering indicates the presence of a cornified epithelia suggesting estrogen stimulation. In some circumstances, irritation or infection can result in a dull, pink appearance. An abdominal-pelvic ultrasound study (23) precludes the need for a bimanual abdominal-rectal examination.

In boys, testicular size should be carefully determined by either volume or longitudinal axis length. Testicular size and symmetry provide clues to categorization of the potential cause of precocious puberty. Prepubertal-sized testes (2 cc or less in volume or less than 1.5 cm in the longitudinal axis) consistent with staging of puberty suggest a cause other than pubertal hypothalamic-pituitary-gonadotropin function, the most common cause being excessive adrenal androgen production as in congenital adrenal hyperplasia (CAH). Pubertal-sized testes (greater than 3 cc in volume or longer than 2.2 cm) suggest gonadotropin stimulation, usually GnRH-dep-PP. As with normal puberty, bilateral growth occurs, but some asymmetry may be present. Marked asymmetry of enlarged testes or unilateral enlargement suggests a Leydig cell tumor, hyperplasic adrenal rest tissue, as occurs in inadequately treated CAH males or previous damage to the smaller testis, as following surgery, with hypertrophy of the larger testes.

History and physical findings (anthropomorphic, general and pubertal physical findings) indicate whether observation or further assessment is indicated.

Diagnostic Testing

The approach to the assessment of early pubertal development is outlined in Table 4. A diagnostic workup sufficient to discern the underlying etiology is necessary so that appropriate treatment can be given.

Initial evaluation includes assessment of hormone levels to include LH, FSH, estradiol in girls and testosterone in males, plus DHEAS if documentation of adrenarche is desired. If a girl has experienced menarche, a progesterone level may verify ovulation and the luteal phase. Basal LH and FSH levels may allow the diagnosis of CPP (24) if both are within the pubertal range for assay used, greater than the prepubertal range. Conversely, LH levels undetectable using a third generation assay, with higher FSH levels is indicative of prepuberty. With use of the same assay method, LH:FSH ratios less than one suggest prepuberty and greater than one puberty. Testosterone levels above the prepubertal range verify pubertal levels but do not differentiate the source. GnRH-dependent precocity in males is not only characterized by pubertal testosterone levels and pubertal basal and GnRH/GnRHa stimulated gonadotropin levels, but also LH levels being most useful with FSH levels often being unnecessary (Fig. 5).

A skeletal age X-ray provides information to compare skeletal maturity to age and height if lack

Table 4 Criteria to Plan Diagnostic Evaluation of Premature Pubertal Development

Clinical findings	Girls	Both sexes	Boys
A. Precocious puberty	Breast development Genital maturation Accelerated linear growth ±Sexual hair ±Menstruation		Genital development with testicular growth Sexual hair Accelerated linear growth Increased muscle mass
		Assess the following depending upon particular situation:	
		History:	
		Exposure to exogenous hormones	
		CNS trauma, anomalies or infection	
		CNS symptoms	
		Familial history of age of pubertal onset	Familial forms usually involve males
		Growth pattern and rates	
		Physical examination:	
	Tanner breast and pubic hair stage	Pubertal maturational staging (Tanner)	Tanner genital size and pubic hair stages
	Clitoral size	Body proportions (upper/lower segment ratios)	Penis size
	Rectal–abdominal bimanual examination	Body and skeletal symmetry	Stretched length
		Acne and skin pigmentation	Description of width
	Inspect for galactorrhea, estrogenized vaginal mucosa	Fundoscopic and visual field examinations	Testicular examination
		Thyroid examination	Size: long axis or volume
		Evidence of thyroid dysfunction	Symmetry
		Neurologic examination	Consistency
		Laboratory evaluation:	
		Serum or plasma assessment	Testosterone
	Estradiol	LH, FSH	
	Increased LH response	Thyroid function tests	hCG
		DHEA or DHEAS	
		GnRHa/GnRH stimulation	Increased LH rise
		Radiologic assessment:	
	Abdominal-pelvic sonography	Skeletal age	Testicular sonography
	Vaginal cytology	MRI of hypothalamic region (if "peripheral" causes excluded)	Morning void for sperm
		Other	
		History:	
B. Premature thelarche	Breast development without growth acceleration or other pubertal findings	Exposure to gonadotropins or estrogen	(See Table 2 for gynecomastia)
		Growth pattern	
		Physical examination:	
		Thorough examination (see earlier)	
		Laboratory evaluation:	
		Serum LH, FSH, estradiol	
		Skeletal age X ray	
		Pelvic sonography	
		Follow-up:	
		Reassess growth rate, pubertal progression after 2–6 mo	
		Repeat sonography for follicular cysts	
		History:	
C. Adrenarche/ pubarche	Sexual hair (pubic or axillary) without growth acceleration or other pubertal changes (Table 2)	Exposure to androgens	
		Growth pattern	
		Physical examination	
		Laboratory evaluation:	
		Plasma DHA or DHAS	
		Skeletal age X ray	
		Follow-up:	
		Reassess growth rate and pubertal progression in 3–6 mo	

Abbreviations: CNS, central nervous system; LH, luteinizing hormone; FSH, follicle-stimulating hormone; HCG, human chorionic gonadotropin; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; GnRH, gonadotropin-releasing hormone; GnRHa, GnRH analog; MRI, magnetic resonance imaging.

of precision and normal variation (± 2 SD for age) is recognized. A significantly advanced skeletal age, an accelerated growth rate and early onset and progression of physical puberty provides basal information for diagnosis, tracking progression, treatment, and subsequent growth potential.

GnRH or GnRHa stimulation testing provide the best hormonal evidence of gonadarche, whether early or not, primarily using LH responses since LH responses increase into a clearly pubertal range, while FSH levels may not change discernibly. Prepubertal children, particularly girls, have relatively greater FSH basal and stimulated levels than LH, hence are of limited or no use. Fig. 5 shows the range of responses in prepubertal girls and those with precocious puberty.

Pelvic sonography in girls determines ovarian and uterine size-enlarged for age, but appropriate for stage of puberty with GnRH-dep-PP while unilateral or asymmetric ovarian enlargement may be indicative of a tumor or cysts. Magnetic resonance imaging (MRI) of the CNS, particularly the hypothalamic region, looking for lesions are a usual part of the assessment even though neurological examination is normal. MRI's have traditionally been felt to be indicated in all boys and girls younger than six years with CPP. However, it is apparent that some girls presenting after age six years of age have abnormalities. Factors that may be useful in deciding whether this procedure should be done include ethnic background and estradiol level. A markedly elevated estradiol has been found to be a predictor of CNS abnormalities (25). Electroencephalograms is not generally indicated, although abnormalities have been reported in CPP puberty.

Causes of Gonadotropin-Dependent Precocious Puberty

Causes, outlined in Table 3, include the CNS disorders such as hypothalamic hamartomas (Figs. 3 and 4), subarachnoid cysts, glial cell tumors, and germ cell tumors (26). Association with GnRH-dep-PP is common with hypothalamic hamartomas and subarachnoid cysts, occurs in two-thirds of germ cell tumors, and unusually with glial cell tumors and rarely astrocytomas or craniopharyngioma. Pediatric oncology patients may develop GnRH-dep PP after CNS radiation therapy. This may occur in conjunction with acquired growth hormone deficiency, in which situations the characteristic growth acceleration of precocious puberty (PP) may be lacking. Trauma, surgery, or inflammation (encephalitis) to the CNS may be followed by PP, which also occurs in those with severe neurological-mental deficits of congenital or acquired origin. Precocity may be related to hydrocephalus, brain abscesses, or granulomas, apparently a consequence of excessive intracranial pressure. Such PP, in some instances, is reversible when pressure is decreased and other therapy may be unnecessary.

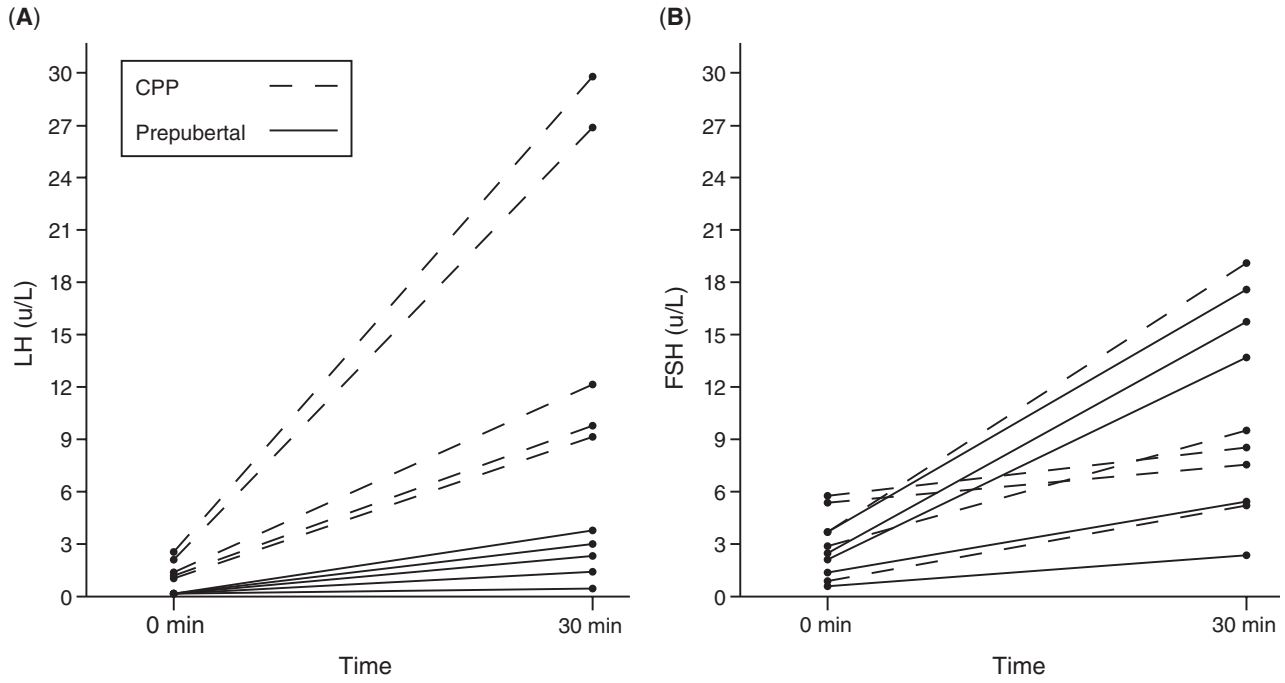

Figure 5 GnRHa stimulation test results differentiating responses between girls with GnRH-depending precocious puberty and prepuberty. Note the differences in LH responses but overlap of FSH rises. GnRH analog (leuprolide acetate) stimulus is a 30 μg/kg bolus at time 0.

In either sex, secondary GnRH-dependent precocity may develop as a consequence of prolonged sex steroid exposure associated with peripheral precocious puberty. Boys may develop CPP as a result of chronic excessive androgen impact that occurs in undiagnosed or inadequately treated congenital virilizing adrenal hyperplasia or Leydig cell tumors. Such patients, in addition to their GnRH-independent precocious puberty, develop evidence of pubertal maturation of the hypothalamic-pituitary axis including a pubertal response to GnRH or GnRHa stimulation. Advanced biologic maturation as evidenced by advanced skeletal age beyond 12 years in boys or 10 years in girls is commonly present these patients.

Causes of Gonadotropin-Independent Precocious Puberty

Precocious pseudopuberty, early puberty resulting from any other mechanism other than hypothalamic GnRH-pituitary LH and FSH stimulated gonadal activity, results from excessive sex steroid stimulation for childhood from gonadal, adrenal cortical, or exogenous source. While the presentation with early puberty is more common in girls than boys, the percentage of girls having GnRH-independent precocious puberty constitutes less than 10%. Precocious puberty is less common in males, and this form may account for up to half of patients. Etiologies are summarized in Table 3 and include the following.

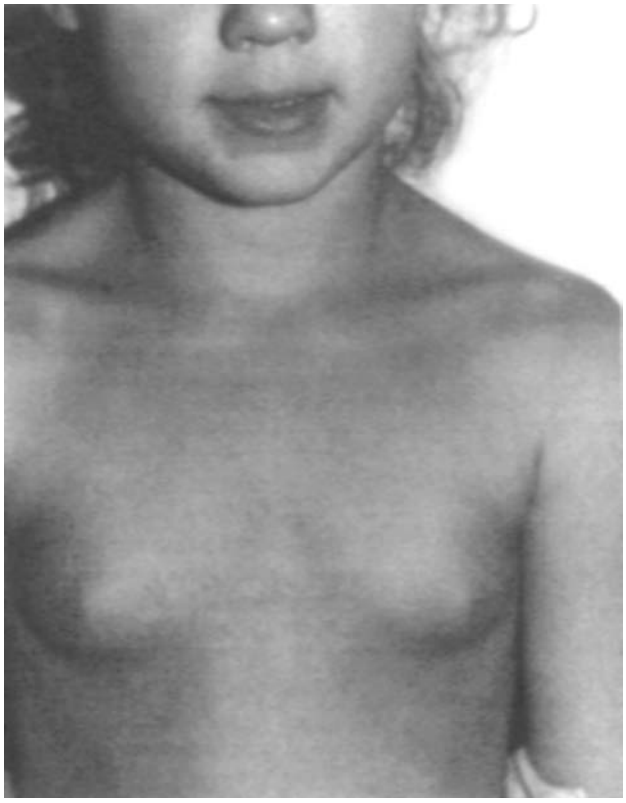

Figure 6 Premature breast development (stage 3) in a 4-year-old girl with McCune-Albright syndrome.

ETIOLOGIES OCCURRING IN BOTH SEXES

McCune–Albright Syndrome

The McCune–Albright syndrome is more frequent in females than males, includes a unique form of peripheral precocious puberty involving an activating mis-sense mutation in the gene for the alpha subunit of G_s(27). The resulting G protein stimulates cyclic adenosine monophosphate formation within the cell lines that may involve a variety of organs. Since this is a somatic cell mutation, only those organs containing such tissues exhibit the effect. This mosaic distribution of cells with this somatic mutation likely is the reason for the variable expression, exacerbations, and remissions of disease activity characteristic of this syndrome. Abnormalities consist of localized multicentered osseous lesions known as polyostotic fibrous dysplasia within bone, melanotic cutaneous macules called ''cafe au lait'' spots in skin, and endocrinopathies that may involve the gonads, adrenal cortex, thyroid, pituitary gland and parathyroid glands. Corresponding endocrinopathies include precocious puberty (Fig. 6), hyperadrenocortisolism, hyperthyroidism, GH excess (pituitary gigantism or acromegaly), and hypophosphatemia. Excessive endocrine function appears to result from autonomous excessive hormone production, a consequence of the G protein producing stimulation as if trophic hormones were present.

Females with precocious puberty present with breast maturation and vaginal bleeding, without gonadotropin stimulation, as a consequence of estrogen-secreting follicles that may enlarge into cysts. Characteristic findings include asymmetrical, enlarged ovaries containing large cysts that may spontaneously regress. Such regression generally coincides with falling estradiol levels, and regression or cessation of progression of estrogen stimulated changes of puberty. Skeletal age and biological maturity follows a similar pattern.

Most patients eventually have early pubertal HPO function (secondary GnRH-dep-PP), at which time GnRHa therapy can be used to suppress GnRH-driven gonadotropin secretion component of this condition. Before this, since ovarian estrogen secretion is a consequence of the mutation in the McCune–Albright syndrome independent of gonadotropin stimulation, GnRHa therapy is not indicated. With or without therapy, patients with the McCune–Albright syndrome usually eventually ovulate with menstrual cycling and fertility.

Other endocrinopathies associated with this syndrome result from constitutive activation other trophic hormone receptors; for example, MSH-like effects in skin, PTH-like effects in bone lesions, and ACTH-like effects on the adrenal gland.

Chronic Primary Hypothyroidism

While untreated primary hypothyroidism is typically characterized by growth, skeletal, and pubertal delay, in some circumstances pubertal changes may be associated with the presentation of children with primary thyroid gland failure and elevated TSH levels (Vol. 2; Chap. 17). The primary finding in girls is breast development, sometimes accompanied by galactorrhea, or testicular enlargement in boys. The etiology is poorly understood and it is not clear why this phenomenon is only seen in a small portion of patients with chronic primary hypothyroidism. Factors felt to contribute to the pubertal development is the excessive secretion of the alpha subunit that occurs alongside the TSH hypersecretion, both of which may occupy and activate the FSH receptor (28) and stimulation from excessive prolactin secretion from increased hypothalamic TRH secretion.

With thyroid replacement therapy, TSH and prolactin levels normalize and testes and breast size regress and galactorrhea ceases. Additional therapy is usually unnecessary.

Sex Steroid Secreting Tumors

In boys, androgen-secreting tumors (Leydig cell tumors and adrenocortical androgen-secreting tumors), and in girls, estrogen secreting tumors (Table 3) of the ovary (29), and adrenal (30–32) are rare causes. Leydig-cell tumors may be a consequence of localized, somatic activating mutations of the LH receptor (33).

Exogenous Sex Steroid

Physical changes of puberty also occur following exposure to exogenous sex steroids. Postulated examples include hormones or substances with hormone-like effects in foods, cosmetics (34), and medicines, and from repeated exposure to sex steroids used by other adults in the household (35,36).

Female-Specific Etiologies: Ovarian Cysts

Functional ovarian cysts may temporarily secrete sufficient estrogen to cause breast, genital and endometrial development; in some cases, resolution of the cyst is associated with vaginal bleeding. The progression of ovarian follicle to form cysts, with or without significant estrogen secretion, may occur as part of normal childhood (37) and GnRH-driven precocious puberty, or as a time-limited cause of GnRH-independent precocious puberty. Cysts may occur secondary to intermittent gonadotropin stimulation during childhood and in the McCune–Albright syndrome. Cysts are readily identified by ultrasonography, are usually self-limiting over a period of months, so no other therapy is usually indicated. Unless the cyst is positioned or is sufficiently large to constitute a surgical emergency, treatment should be conservative, with careful sonographic and hormonal monitoring until resolution. Recurrence of functional cysts may occur.

Male Specific Etiologies: Congenital Virilizing Adrenal Hyperplasia

Historically, undiagnosed or inadequately treated congenital virilized adrenal hyperplasia (CVAH) caused by 21-hydroxylase or 11-hydroxylase deficiency was the most frequently cause of precocious puberty in boys. With wide-spread neonatal screening for CAH, early diagnosis has made this presentation much less frequent (Vol. 2; Chap. 9). Findings include a history of excessive muscular development, accelerated growth rates, and genital growth with onset during infancy or early childhood. Suggestive findings on initial assessment are small testes, low LH and FSH and elevated androgens and advanced skeletal age for age.

Familial Male-Limited Gonadotropin-Independent Puberty

Activating mutations of the LH receptor can stimulate testicular steroidogenesis and spermatogenesis, and are a unique form of male-limited gonadotropin-independent precocious puberty (aka Testotoxicosis) (38,39). Testosterone levels may be in or above the adult male range and seminiferous tubular development is sufficient to allow spermatogenesis. These LH receptor mutations stimulate G-protein mediated increased cyclic AMP production causing autonomous Leydig cell testosterone production. Females with the activating mutations of the LH receptor do not develop precocious puberty because ovarian steroidogenesis requires both FSH as well as LH activation for steroid production.

Characteristics may include father-to-son transmission, onset at two to three years age, considerable genital growth including bilateral testicular growth. Presentation includes findings similar to GnRH-dep-PP: a mature, muscular physique, genital and testicular enlargement that may include nodular Leydig cell hyperplasia (40), accelerated growth and skeletal age advancement. Initial laboratory values include elevated testosterone alongside low LH and FSH levels. Secondary CPP typically follows. After maturation of the hypothalamic-pituitary-testicular axis, normal adult function appears to range from those with normal paternity to those with decreased testicular volume or oligospermia (41). Adult height varies from short stature to average height (42). Treatments involve several agents aimed at reducing the synthesis or action of androgens. The longest experience has been with the P450 cytochrome inhibitor, ketoconazole, which is effective in inhibiting androgen synthesis and is well tolerated (42). Combinations of anti-androgen (spironolactone) and aromatase inhibitors have also been used. The greatest experience has been with the aromatase inhibitor, testolactone, while

on-going studies are being conducted with other more highly selective anti-androgens and more potent aromatase inhibitors (43,44).

Gonadotropin-independent precocious puberty has also been reported in a boy with X-linked adrenal hypoplasia congenital due to a mutation in the DAX1 gene (45). In this case, GnRHa treatment did not suppress testosterone production but sex steroid replacement did. It has been hypothesized that the excessive ACTH attendant to adrenal hypoplasia stimulate Leydig cells.

Chorionic Gonadotropin Secreting Tumors

Excessive LH (or LH-like) stimulation in boys stimulates enough testosterone excess to cause precocious puberty. Excessive LH secretion in girls, without concomitant FSH stimulation, does not usually cause precocious puberty in girls, although rare cases have been reported (46). Human chorionic gonadotropin (hCG) secreting tumors are most commonly located in the liver, cerebrum, mediastinum and gonads (47). Chorionic gonadotropin secretion from teratomas, embryonal tumors, hepatoblastomas, and CNS germinomas stimulate Leydig cell production of testosterone with little increase in testicular volume.

TREATMENT OF UNDERLYING ABNORMALITIES OF PRECOCIOUS PUBERTY

Therapy for precocious puberty should be directed at the primary case when one can be identified. CNS tumors, as well as ectopic gonadotropin-producing, gonadal, or adrenal tumors should be treated with the appropriate surgical, radiation or chemotherapy regimen. An exception to this occurs in hypothalamic hamartomas resulting in precocious puberty where surgical treatment may cause significant morbidity depending on location. Since hamartomas are congenital malformations and rarely have neurological consequences, GnRHa therapy is used instead of surgical intervention. In some cases where the hamartomas are pedunculated and therefore easily accessible, surgical extirpation may be a viable treatment option for CPP.

Other instances where treatment of the underlying condition is indicated are adrenal suppression in CAH (Vol. 2; Chap. 9), thyroxine replacement in primary hypothyroidism (Vol. 2; Chap. 17), and cessation of inappropriate steroid or gonadotropin administration. In GnRH-independent precocious puberty, successful treatment of the underlying disease is followed by cessation and occasionally regression of pubertal development. In patients with autonomous gonadal steroid production, such as those with the McCune–Albright syndrome or male familial gonadotropin-independent puberty, therapy is aimed at reducing sex steroid production or effect. Therapies that have been used with varying success (48) include steroid synthesis inhibitors (ketoconazole), aromatase inhibitors (testolactone and anastrozole) and the estrogen-receptor antagonist (tamoxifen) (49). Since these do not completely eliminate sex steroid production/effect, secondary CPP may develop. If this occurs, GnRHa therapy, as described below, should be considered.

Natural History of Untreated GnRH-Dependent Precocious Puberty

The natural history of GnRH-dep-PP is outlined in Fig. 7.

The rate of development, as in normal-aged puberty, is determined by the magnitude of sex steroid secretion with higher levels resulting in the most accelerated physical changes. All phases of sexual development are precocious, including ovulation and spermatogenesis, leading to early attainment of reproductive capability. Depending on the inter-relationship of the accelerated linear growth rate and concomitant acceleration of skeletal maturity, adult height may be less than genetically expected height (Fig. 8).

Very early or rapidly progressive precocious puberty leads to greater compromise of adult height because of disproportionate advance of skeletal maturity beyond interval height increase. For example, a skeletal age advance of two years with a concomitant height increase of only one year suggest a loss of one year's worth of growth potential. With

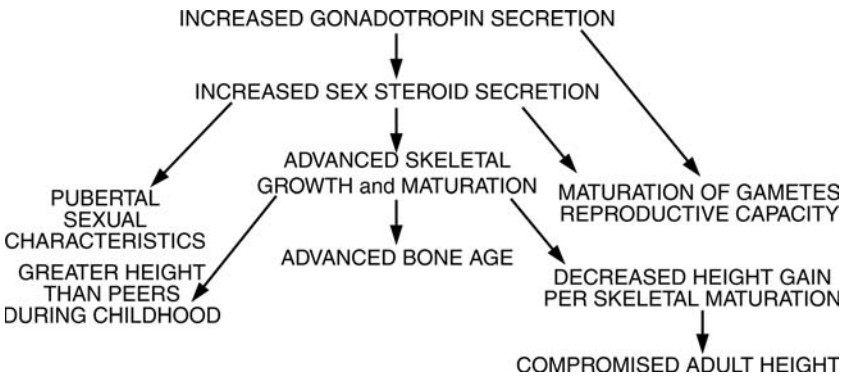

Figure 7 Schematic representation of the natural history of early pubertal development. *Source*: Used by permission of Year Book Medical Publishers.

premature growth spurt and skeletal maturation, children with precocity are tall for age during childhood. However, their projected adult height diminishes proportional to the rate of skeletal maturity. The typical outcome of untreated rapidly progressive precocious puberty is completion of growth when epiphyses fused at a younger age and shorter height than if puberty and the pubertal growth spurt had occurred during the usual years. However, it appears that many patients do not have rapid advance of skeletal age exceeding concomitant growth rates (Fig. 8) so that adult heights reach the normal range (50).

Therefore, indications for treatment of progressive CPP include an attempt to preclude or reclaim loss of height potential, halt pubertal progression, and alleviate or prevent psychological stress. Tall stature, advanced pubertal development, erections, menses and misunderstood urges during childhood may cause social and psychological adjustment problems. Psychosocial concerns are an indication for counseling and consideration of medical treatment to suppress excessive growth rate and pubertal advancement.

Thus, whether or not GnRHa therapy is considered, the patient and parents need to understand what is happening. If puberty is GnRH-dependent, they should understand that normal things are happening, but at an early age. An explanation, geared to the child's ability to understand, that it is normal for the body of a child to change into the body of an adult but sometimes it starts too soon and happens too fast is usually satisfactory. Simply an explanation to a girl that her body has started changing from a girl to a woman, something that is supposed to happen, is starting too soon. Depending on the sense from the parents, it may be appropriate to suggest that she, the girl, deserves to be a girl longer. Similar discussion can be tailored for boys, together with some references to male sexual issues, if felt to be appropriate, to include recognition of universal male phenomenon such as erections. During such discussions with the patient, often with the parent present, consideration should be given to the inclusion of other age-appropriate sex education topics.

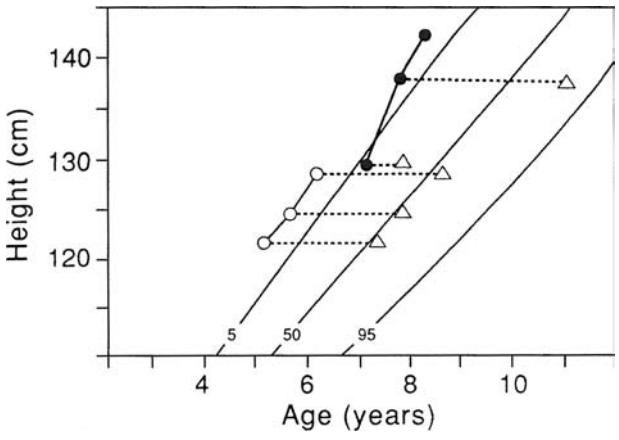

Figure 8 Growth chart for girls shows percentile of height for age. The solid circles represent height for age for a girl presenting with central precocious puberty; the connected triangles depict height for bone age. Note that although growth rate is accelerated, bone age has accelerated considerably more in the same time period, resulting in decrease in projected adult height. In contrast, the patient whose heights are depicted (open circles) with Tanner stage 2 breast and pubic hair development did not have an acceleration in growth rate or bone maturity. Although she was tall for age with early pubertal development, she did not have a decreased adult height potential.

Psychosexual development is generally commensurate with age not physical maturity, although there is evidence of withdrawal behavior, anxiety, depression and somatic complaints (51). While adolescent-type masturbation occurs in males with precocious puberty, inappropriate sexual behavior is seldom a problem and if it is, an external cause should be excluded. In contrast with being sexually aggressive, patients tend to be naive and thus are likely, as other children, to misinterpret sexual advances by older individuals. Girls and, to a lesser extent, boys are potential victims from sexual encounters with older persons who would take advantage of their childlike concept of, and vulnerability to, intimacy. It must be remembered that both boys and girls may become potentially fertile. Generally, once children with precocious puberty recognize that they are more physically mature than their age mates, they tend to avoid incidents of childhood sex play. Generally, children with early puberty share interests with age peers.

Therapy for GnRH-Dependent Precocious Puberty

Criteria for Treatment with GnRHa

Patients with GnRH-dep-PP, idiopathic or otherwise, are diagnosed based on pubertal gonadotropin secretion based on basal LH and FSH levels or a pubertal gonadotropin response to GnRH stimulation, data that can be used as a basal reference to evaluate effectiveness of therapy. GnRHa is the treatment of choice (52) and in fact the only effective therapy, acting by removing the GnRH stimulus for gonadotropin synthesis and release.

The decision for or against treatment should involve the parents and consider all aspects of the child's physical and mental maturity. General criteria for therapy include: (i) a pubertal response to GnRH or GnRHa stimulation testing or some other documentation of pubertal gonadotropin secretion (basal plasma or urinary levels clearly above the prepubertal range); (ii) a sustained accelerated linear growth rate; (iii) an advanced or accelerating skeletal age, and (iv) physical changes consistent with progressive pubertal development (assignment of Tanner stage 2 without progression may not merit therapy). Additional criteria include: (i) physical development at a rate which is clearly well outside the range for age and race, (ii) skeletal age that has exceeded the growth rate (height age) with compromised adult height potential, and (iii) a perceived

need by the parents or child to delay further pubertal growth and development. If the primary reason for treatment is to preserve or regain height potential in the child who has not had progression of skeletal age in excess of height gain, predicted adult height may be a useful criterion in deciding whether to treat. There is evidence that if height prediction does not fall below 155 cm in girls, adult height is preserved in more slowly progressing puberty (53).

The pharmacologic basis for GnRHa therapy is the suppression of the episodic secretion of gonadotropins by overriding the obligatory episodic release of GnRH with continual occupation of the GnRH receptors on the pituitary gonadotropes in the pituitary with high levels of GnRHa.

Institution of Therapy

GnRHa are available for subcutaneous implantation, as depot injections, short-acting injection and nasal spray. GnRHa initially causes an initial LH and FSH release, followed, with adequate dosage, by down-regulation of responsiveness including decrease in GnRH receptor numbers. Circulating gonadotropins levels decrease, with concomitant return of sex steroids to prepubertal levels. With adequate dosing, suppression occurs within weeks of the onset of therapy. If a period does not occur before institution of GnRHa therapy, withdrawal bleeding would be expected with suppression.

Monitoring GnRHa Therapy

Dosing adequacy and effectiveness can be monitored by lack of progression of clinical indices and hormonal testing, including GnRH or GnRHa stimulation of LH release. In boys, a plasma testosterone in the prepubertal range is indicative of adequate dosage, making LH stimulation testing un-necessary. In girls, estradiol levels are less helpful, as for diagnosis because levels fluctuate and may be low at times during puberty. Recommended dosages are adequate in the vast majority of patients to achieve suppression. Once suppression is verified, patients can be monitored at four to six month intervals to verify ongoing suppression. Monitoring can involve GnRH or GnRHa stimulation testing or involve a single sample at 30 to 60 minutes for LH after the injection of the therapeutic depot medication (54,55). Such treatment results in a deceleration of the puberty growth rate and a cessation of pubertal development. Obviously, when suppression is not achieved, the dosage should be increased and testing repeated until suppression is documented.

Impact of GnRHa on Pubertal Development and Growth Rates

In female patients, breasts may regress, vaginal mucosa becomes nonestrogenized, and, if menarche had occurred, menses cease. During the institution of suppression, an episode of withdrawal bleeding may occur as a consequence of the deceased estrogen level. If an endometrial stripe is discernible by ultrasonography before therapy, this is to be expected.

In boys, both genital and pubic hair development ceases and may regress. Testicular volume decreases. Skeletal age maturation rate slows, notable after the first six months of suppression. Growth slows to prepubertal rates, and with prolonged therapy or in those with markedly advanced skeletal age, growth rates may decelerate markedly. Projected adult heights, which are based on skeletal age, age, and height at any given point in time, can be expected to increase and should not further diminish. Such change is a consequence of point of onset of therapy. Children begun before markedly advanced skeletal age, and hence, before loss of height potential are expected to preserve, rather than regain, growth potential on therapy. Those begun on therapy with very advanced skeletal ages can not be expected to gain much growth potential.

Children with precocious puberty have increased weight for age, related to sex steroid effects causing an increase in lean body mass. As many as one-quarter of girls have increased body mass index (BMI) at presentation with early puberty, although it is unclear how this is related to the current increased incidence of childhood obesity. During and after therapy, BMI does not increase further, and in most BMI decreases into the normal range (56,57).

GnRHa does not influence adrenal androgen secretion. Hence, if adrenarche has occurred, it is expected to be maintained or progress during treatment. If there is significant secretion of adrenal androgens, this may be adequate to maintain or cause progression of sexual hair. Thus, maintenance or advance of Tanner pubic hair staging on therapy should not be interpreted as evidence of lack of GnRHa suppression of gonadal activity. Documentation of pubertal levels of adrenal androgens (DHEA or DHEAS) can be used to verify this.

Adequacy of suppression can be monitored by low random LH levels or, if needed, demonstration of the lack of gonadotropin response to exogenous GnRH stimulation. With adequate dosing, suppression is demonstrable after the first eight weeks of therapy.

Skeletal Maturity

The stimulus for accelerated skeletal maturity is removed once sex steroid levels are suppressed. However, since the steroid effect on bone maturity is delayed, it may be more than six months before growth deceleration is documented. Thereafter, skeletal maturity slow to normal until the skeletal age reaches the average age of puberty for that gender—approximately 10.5 years in girls and 12.5 years in boys. Beyond this pubertal skeletal age, bone age maturation may progress very slowly in the absence of sex steroids.

Adult height forecasts are based on the relationship between skeletal maturation and standing height. During therapy, at the time of periodic skeletal age

determinations, height predictions can be estimated to judge impact of therapy. This information can be used to judge the impact of treatment on growth potential. Changes in height prediction during therapy are directly related to skeletal age at onset of therapy.

Ancillary Tests

Monitoring of ovarian ultrasound and bone density is not typically indicated. Ovarian and uterine volume both diminish. Bone mineral density can be expected to remain relatively constant. Since density is greater than expected for age, lack of accrual of greater bone density during the hiatus of therapy is not considered detrimental.

Addition of Growth Hormone Therapy

Among children with CPP with advanced skeletal age in relation to height for age, GnRHa therapy may be inadequate to reclaim lost height potential. In these patients, the addition of growth hormone therapy is often considered. The rationale for such combined therapy is that GnRHa therapy, as a consequence of lowering sex steroid levels, is associated with decreased growth hormone and insulin-like growth factor1 (IGF1) secretion (58). Hence, growth hormone is given in an attempt to stimulate linear growth while GnRHa is used to preclude the advance of skeletal age allowing for height gain. Outcome studies indicate that height gained with combined therapy is statistically greater than with GnRHa therapy alone, although this gain is only a few centimeters (59). Thus, the cost: benefit ratio should be carefully considered when combined therapy is being considered.

Discontinuation of Therapy

This decision must be individualized. Characteristically, therapy is discontinued when the child reaches the age when puberty typically is occurring, assuming growth potential is adequate and the child is psychologically prepared. When predicted height is still less than target height, therapy may continue in an attempt to gain greater height. However, since the gain diminishes with advancing age and skeletal age, because of waning growth rates, the benefit of continuing therapy must be reassessed at intervals of not more than six months.

Recovery of Hormonal Secretion after Discontinuation

Resumption of pubertal HPG activity begins promptly, becoming complete within weeks or months. LH and FSH responses to GnRH or GnRHa stimulation are pubertal before six months. There is variability in the resumption of physical development, with attainment of Tanner stages and testicular growth, and ovarian volume based on ultrasound, proceeding at a rate similar to that during normal puberty. Menses in females who had experienced menarche before therapy occurs within months. Most other girls experience menarche within 18 months, although some have required considerably more time (60). Ovulatory cycling begins and menstrual regularity has been documented, following at a pace similar to that in similarly-aged females.

Long-Term Outcome Data

Psychological Adjustment

The incidence of neurotic accentuation concerning physical appearance and increased insecurity has been reported in girls with precocious puberty while receiving therapy (61). No long-term psychological detrimental consequences are recognized for females with a history of precocious puberty or GnRHa therapy.

Fertility

While there are no large series documenting fertility outcome after GnRHa therapy, there is no evidence of problems with sexual function or fertility. Pregnancy with birth of normal infants has occurred numerous times. In instances where sampled, semen analyses have indicated normal spermatogenesis.

Growth after Discontinuation of Therapy and Adult Height

Data concerning the benefit of therapy in preservation or reclaiming growth potential leading to normal adult heights are difficult to ascertain since patients are begun on therapy at different stages of maturity. While adult height is greater than predicted at onset of therapy, whether or not adult height is within the target height range depends on height potential at onset of therapy (62–64). Growth rates and total height gained after discontinuation of GnRHa therapy is less than projected based on height and skeletal age at discontinuation. Adult heights are usually within the normal range for sex (65), are greater and closer to target height in those with early onset of precocity, younger, less advanced skeletal age, without delay in treatment, and longer duration of treatment (66).

Bone Mineral Density

Because GnRHa, by lowering sex steroid levels, decreases the rate of accrual of bone density in children with precocious puberty and is accompanied by loss of bone mineral density (BMD) in adults after chronic use, the impact on the attainment of normal BMD at the end of adolescent growth in those treated with GnRHa is pertinent. Available data indicate that at diagnosis and onset of therapy, children with precocious puberty may have greater than average BMD for age, within the range for skeletal age, have little of no change during therapy resulting in lower BMD for age at end of therapy, but by

mid-adolescence after puberty physiology resumes, BMD is normal for age (67,68). For children thus treated, as during normal growth and adolescence, better calcium intake is associated with greater BMD.

Hyperandrogenism

Early androgen stimulation in girls has been associated with subsequent ovarian hyperandrogenism. A minority percentage of girls with GnRHa-dependent precocious puberty have as the initial sign androgen effects, usually sexual hair. It is unclear in what portion of such patients this finding is the first evidence of a hyperandrogenic state. There is no evidence that ovarian hyperandrogenism, a common diagnosis, is diagnosed more frequent in girls with precocious puberty, nor after treatment with GnRHa. The withdrawal of gonadotropin and estrogen during GnRHa therapy may lead to the development of ovarian cysts which develop secondary to interruption of the stimuli for follicle growth. However, an increased incidence has not been reported during or after such therapy.

OTHER FORMS OF EARLY OR GENDER-INAPPROPRIATE DEVELOPMENT

The partial forms of early development should be considered as possible variants of normal that should be carefully followed to determine whether further inappropriate changes occur or whether there is any evidence of an underlying developing abnormality. A further category involves feminization in males and masculinization in females, changes that have been referred to as contrasexual or heterosexual precocity since such are inappropriate for sex.

Partial Pubertal Development

Premature Thelarche

Traditionally, premature thelarche is the onset of premature breast development without other associated pubertal changes. In cases of isolated premature thelarche, breast tissue is most commonly noted during the first two years of life. In most instances, the history suggests that this is a persistence or increase of the palpable breast tissue that is present at birth and the neonatal period as a consequence greater ovarian hormone production during infancy. Breast development is usually limited and often regresses by 24 months of age. Thelarche persisting or occurring beyond this age is consistent with enhanced follicular development and is typically associated with increased FSH and inhibin B secretion (69). Breast development may be a consequence of fluctuations of the female childhood HPO axis, with temporary FSH-stimulated increases of ovarian steroid secretion.

As occurs in the onset of puberty, initial breast growth may be unilateral or asymmetrical. When a girl presents with Tanner stage 2 breast development without other evidence of puberty, careful monitoring for progression over subsequent months is mandatory but only a limited assessment is indicated (Table 4). If growth is normal and breast development is minimal, additional studies may not be necessary. If so determined, estradiol levels and skeletal age should be obtained and are normal or slightly advanced for age; pelvic ultrasound should demonstrate normal size and configuration of the uterus and ovaries, including ovarian follicular microcysts (70). As long as this condition does not progress, treatment can be limited to education and counseling. However, since childhood in girls may be characterized by episodes of greater gonadotropin secretion, it may be difficult to ascertain where along the continuum from premature thelarche, non-progressive precocious puberty and GnRH-dep-PP a given patient lies. A small portion of girls presenting with premature thelarche progress to CPP (71). However, more commonly there is minimal progression until the usual age of onset of puberty. Without hormonal evidence of pubertal onset, the later diagnosis should not be made.

Premature Adrenarche and Premature Pubarche

Premature pubarche, the early development of sexual hair, is usually a consequence of premature adrenarche, an early onset of the increase of adrenal androgen production. Premature adrenarche precedes pubertal maturation of the gonad, is independent of gonadarche (72), is not accompanied by a mature GnRH stimulation response and is not suppressed with GnRHa therapy. Prepubertal growth velocity is greater than other children, whereas the pubertal growth component is reduced so that adult height is not impacted. Height and skeletal maturity in the child with adrenarche ranges from normal, to borderline to minimally, but significantly, advanced.

In girls, premature adrenarche may be a early manifestation of androgen excess (73) of adrenal or ovarian origin and is a known risk factor for the development of polycystic ovarian disease [polycystic ovary syndrome (PCOS), ovarian hyperandrogenism] (74). (Vol. 2; Chap. 13) The hyperandrogenism that results from increased androgen synthesis by the adrenal cortex or ovary may exaggerate adrenarche and predispose for the development of PCOS. The phosphorylation defect involved in this may also affect insulin metabolism. The hyperinsulinism may influence LH secretion and amplify the hyperandrogenism (Vol. 1; Chap. 11). Androgen excess states of childhood may present similarly, but are progressive (75) and make up a small minority of patients presenting during late childhood with sexual hair.

Premature pubarche occurs more commonly in girls than boys and is rare before six years of age. The evidence of androgen stimulation may include

mild acne, oily skin, onset of adult-type body odor, and axillary hair with or preceding early pubic hair. If there are no other associated findings or pathological conditions, a minimal assessment including androgen levels to verify that levels are not greater than those consistent with early adrenarche is required (Table 4). Documentation of adrenarche is made by demonstrating circulating levels of DHEAS, DHEA, or androstenedione within the range of early adrenarche and above the prepubertal range. Because of the incremental rise of DHEAS associated with adrenarche, this hormone alone is usually adequate as a screen.

Height and skeletal age are normal or transiently advanced. While subsequent growth and pubertal development is expected to occur normally, children should be monitored if they are overweight or have other risk factors for the development of the metabolic syndrome. Abnormal progression or excessive virilization mandates further evaluation.

Inappropriate Sexual Development for Gender

Feminization in boys and virilization in girls has been called contrasexual pubertal development. This may occur before during or after normal puberty and causes sexual characteristics inappropriate for sex of the individual (androgen excess in girls and estrogen excess in boys). Pathologic gynecomastia is discussed above in the section concerning pubertal gynecomastia.

Masculinization in Girls

Although premature development of sexual hair (premature pubarche) in girls is not uncommon, excessive virilization is rare and presents with clitoromegaly, deepening of the voice, hirsutism, acne and oily skin. Etiologies, include adrenocortical neoplasms (76), are listed in Table 2. Severity of virilization is generally related to the severity of the abnormality, being greatest with adrenal and ovarian tumors, less with adrenal hyperplasia and ovarian hyperandrogenism. The mildest forms of adrenal hyperplasia may present only with sexual hair requiring ACTH stimulation testing or DNA analysis for diagnosis (Vol. 2; Chap. 9). Amount of virilization, skeletal age advancement, and acceleration of growth rate indicate assessment. Initial laboratory evaluation should include 17-hydroxyprogesterone, androstenedione, testosterone free testosterone, cortisol, LH, and FSH levels. Measurement of adrenal steroid intermediate metabolites may be useful in identifying mild forms of adrenal hyperplasia. Adrenal suppression may be necessary to differentiate adrenal hyperplasia from adrenal tumors or ovarian sources. As discussed above, abnormal adrenarche may be indicative of or contribute to the development of PCOS. PCOS, ovulatory dysfunction and functional hyperandrogenism often manifest by mild virilization, amenorrhea and insulin resistance.

DELAYED PUBERTY AND HYPOGONADISM

Definition and Classification of Delayed Puberty

Evaluation for causes of delay or lack of pubertal development should be considered if the initial physical changes of puberty are not present by age 13 years in girls or age 14 in boys. Further, an abnormality may be present if there is lack of appropriate progression of puberty, more than four years between the first signs of puberty and menarche in girls or the onset and completion of genital, including testicular, growth in boys.

Delayed puberty results from a lack of pubertal maturation of the neuroendocrine axis or to gonadal dysfunction. This may develop due to primary dysfunction of the HPG axis or may occur as a secondary phenomenon to systemic illness (e.g., occult Crohn disease) or undernutrition (Vol. 2; Chap. 1). Accordingly, a commonly employed classification system has been developed that sorts patients with delayed puberty into one of two main categories based on the levels of basal gonadotropins and sex steroids: those with primary hypothalamic-pituitary dysfunction and those with a primary gonadal disorder.

General Approach

Delay may be evidence of permanent hypogonadism requiring a lifetime of therapy, or may be only a transient delay with potential for normal gonadal function. Assessment is done to determine whether the delay or lack of development is consistent with a lag in normal pubertal maturation of the HPG axis or to underlying abnormalities associated with permanent hypogonadism (Tables 5–7). Permanent hypogonadism is a consequence of a defect of GnRH or gonadotropin secretion or gonadal inability to secrete sex steroids.

Hence, the approach to delayed puberty is to categorize based on gonadotropin status. However, at initial assessment it is often impossible to differentiate with certainty the non-pathologic and pathologic causes, and the transient from the permanent causes within the pathologic grouping. Causes of hypogonadotropism include the non-pathologic, i.e., constitutional delay, and the pathologic. Pathologic causes may be permanent or transient. Hypergonadotropic causes are always pathologic.

The initial approach to patients of both sexes with delayed or lack of progression of puberty is to determine gonadotropin status and skeletal age. Elevated gonadotropins indicate gonadal failure. If bone age, an index of biologic age, is at or beyond the age of puberty, gonadotropin (LH and FSH) levels should be elevated if gonadal failure is present because of maturation of the HPG axis but absence of negative feedback by sex steroids. If gonadotropin levels are mildly elevated, GnRH stimulation may unmask an excessive response of LH and FSH documenting primary gonadal failure. However, if levels are clearly elevated such GnRH stimulation is unnecessary.

Table 5 Causes of Pubertal Delay Associated with Hypergonadotropic Hypogonadism

Chromosomal, gene, syndromic and congenital disorders:
Adrenal hypoplasia congenital[a]
Androgen receptor mutations/androgen insensitivity syndrome (may present as primary amenorrhea)
Anorchia (vanishing testis syndrome)
Aromatase deficiency
Ataxia-telangiectasia syndrome
FSHβ gene mutations
Galactosemia
Gonadal dysgenesis (46,XX;46XY-Swyer syndrome)
Gonadotropin receptor inactivation mutations
Klinefelter syndrome (47,XXY and variants)
Leopard syndrome
Leydig cell agenesis/LH receptor mutation
LH subunit gene mutations
Mixed gonadal dysgenesis (45,X/46,XY)
Myotonic dystrophy
Noonan syndrome
Nephropathic cystinosis
Partial androgen insensitivity syndrome
Testicular regression syndrome
Testicular synthesis abnormalities
Turner syndrome (45,X, and variants)
Acquired disorders:
Autoimmune
Chemotherapy
Galactosemia
Infectious (coxsackie, mumps)
Infiltration (sickle-cell disease, hemochromatosis)[a]
Irradiation
Orchitis
Secondary to cryptorchidism
Surgical
Torsion (bilateral)
Trauma

[a]May be associated with hypogonadotropic hypogonadism as well.

Table 6 Pubertal Delay with Potential for Normal Puberty

Constitutional delay of growth and puberty
Chronic systemic illness
Cardiac
Diabetes mellitus
Gastrointestinal (Crohn's disease)
Hematologic(Sickle-cell disease)
Malignancy
Pulmonary (asthma/cystic fibrosis)
Renal
Sickle cell
Drug abuse
Excessive energy/exercise expenditure (female athletic triad-secondary amennorhea, dietary dysfunction, osteoporosis; wrestlers syndrome)
Exogenous obesity (premature early changes, slow progression)
Endocrinopathies
Growth hormone deficiency
Glucocorticoid excess
Hypothyroidism
Hyperprolactemia
Uncontrolled diabetes mellitus
Malnutrition/malabsorption (chronic)
Psychiatric illness
Anorexia nervosa
Psychogenic stress
Psychosocial dwarfism

Low gonadotropin levels include those with constitutional delay, temporary delay associated with chronic disease and permanent gonadotropin deficiency including identified genetic disorders. There are no known tests that definitely differentiate physiologic delay from gonadotropin deficiency until age is well into adolescence (77), since childhood is a relatively hypogonadotropic state. If biologic maturity, as indicated by skeletal age is less than biological age of the onset of puberty (10–11 years for girls and 12–13 for boys), parameters, including random or stimulated gonadotropin levels, overlap between with hypogonadotropism and an immature hypothalamus. In the older adolescent, minimal response to GnRH or GnRHa stimulation suggests gonadotropin deficiency at the hypothalamic or pituitary level, while over time, the pattern in the child with delayed puberty but a potential for normal function shows a pubertal rise.

In Girls

Definition and Classification for Females

The current age at which puberty is considered delayed in American girls is 13 years of age for breast development and 15 years for the onset of menarche (78). The mean duration from the onset of puberty to onset of menarche is 2.4 ± 1.1 years. Puberty may also be considered abnormal if it develops at an unusually slow pace after beginning at a normal age, failure to reach Tanner stage 5 in four years is excessive. When there is normal breast and pubic hair development but a lack of menses in a girl more than 15 years of age, the more appropriate term of primary amenorrhea should be used.

In girls, it is important to determine whether the physical signs of puberty are absent or incomplete and whether there is marked discordance between the development of pubic hair and breast. In individuals with androgen insensitivity syndrome, for example, there may be well-developed breasts with scant to no pubic hair.

In girls, as well as boys, delayed puberty, the lack of pubertal maturation of the neuroendocrine axis, results from primary dysfunction of the HPG axis, may occur as a secondary phenomenon to systemic illness or undernutrition, or as a variant of normal. A useful classification system involves two main categories based on the levels of basal gonadotropins and sex steroids: a temporary or permanent primary hypothalamic-pituitary dysfunction and a primary gonadal disorder. A third diagnostic category occasionally used in girls is eugonadotropic eugonadism, a category to describe girls with an appropriate constellation of secondary sexual characteristics and normal HPG function who have delayed menarche.

Although less frequent than in boys, the most common cause of pubertal delay in girls is an excessive variation of normal development called constitutional delay of growth and development, in which the pace

Table 7 Hypogonadotropic Hypogonadism (Compromised Gonadotropin-Releasing Hormone or Gonadotropin Synthesis Release or Action)

Idiopathic HH
Lack of GnRH synthesis
Kallmann syndrome
Mutations of KAL gene (KAL-1) which encodes anosmin-1, associated with visual defects, nystagmus, ataxia, synkinesia, midfacial defects and renal agenesis
Mutations of FGFR1 (KAL-2) associated with cleft lip/palate, dental agenesis, synkineses
Defective GnRH release or action
Leptin and LeptinR—HH associated with severe obesity
DAX-1-hypogonadotropism associated with x-linked adrenal hypoplasia
Congenital[a], and contiguous gene defect leading to Duchenne muscular dystrophy
GNRHR-mutations on the GnRHR gene
Prohormone convertase deficiency
Isolated LH deficiency
LHβ-subunit mutation
Associated with multiple pituitary hormone deficiencies
HESX1 (Septooptic dysplasia, panhypopituitarism)
LHX3 (associated with hypothyroidism, hypoprolactinemia)
PROP1 (associated with growth hormone deficiency, hypothyroidism, hypoprolactinemia)
Absence of corpus callosum
Hypopituitarism (idiopathic, empty sella, pituitary agenesis, Rathke pouch cyst, acquired (granulomas, inflammation, infiltration (sickle cell diseasea[a], hemosiderosisa, thalassemia, histiocytosis), hypophysitis, irradiation, surgery, trauma, tumors (craniphyaryngioma, hypothalamic glioma, optic nerve glioma, pituitary adenomas, prolactinomas)
Syndromes
Alstrom
Borjenson–Forssman–Lehmann
Carpenter
CHARGE
Gordon–Holmes spinocerebellar ataxia
Laurence–Moon–Bardet–Biedl
Multiple lentigines
Noonan
Prader–Willi
Prosencephalon defects (associated with central incisor, cleft—lip and palate, midfacial cleft)

[a]May also be associated with hypogonadotropic hypogonadism.
Abbreviations: HH, hypogonadotropic hypogonadism; FGFR1, fibroblast growth factor receptor 1; GnRH, gonadotropin-releasing hormone; GnsRHR, gonadotropin-releasing hormone receptor.

of physical developmental is slowed. A review of 74 patients presenting with pubertal delay at an academic center, 30% had benign constitutional delay, 19% had functional hypothalamic hypogonadism related to another systemic disease, 20% had permanent HH, 26% had permanent hypergonadotropic hypogonadism and 5% had another cause of pubertal delay (79). This report substantiates those of previous investigators (80) demonstrating that in most circumstances pubertal delay in girls, in contrast to boys, is not a benign entity and most likely represents disease.

Hypergonadotropic Hypogonadism (Ovarian Failure)

The hypergonadotropic state implies: (i) the hypothalamic component of puberty has been activated, and (ii) the negative effects of sex steroid feedback on the hypothalamus are not present. Accordingly, this biochemical state suggests ovarian dysfunction of various etiologies (Table 5). Hypergonadotropic hypogonadism is always a pathological state. Assessment should include history of surgery, irradiation and chemotherapy, examination for features of the Turner syndrome and consideration of a karyotype.

Turner Syndrome, Gonadal Dysgenesis/Agenesis

The Turner syndrome results from a sex chromosome aneuploidy where critical segments of the X chromosome is missing, occurring in approximately 1/3000 live born female infants (Vol. 2; Chap. 12). This syndrome is associated with other abnormalities such as: right-sided cardiac abnormalities (bicuspid aortic valve, coarctation of the aorta), fetal lymphedema (resulting in webbing of the neck, low posterior hairline and persistent hand/foot edema) and facial dysmorphism (mid-face hypoplasia, high arched palate, frequent ear infections). After birth, progressive short stature and gonadal failure are the most common features of Turner syndrome that bring affected girls to medical attention. Although most girls with Turners syndrome have primary gonadal failure requiring exogenous sex steroid replacement, a great deal of phenotypic variability exists and as many as 15% to 30% of affected girls show spontaneous breast development; 5% to 15% reach menarche and 1% to 3% achieve unassisted pregnancy (81).

Because the default development of sexual differentiation is female. pure gonadal dysgenesis, with 46,XY karyotype, results in female genital differentiation because of the complete lack of testicular development or function. Rarely, pure ovarian agenesis with a 46,XX karyotype also occurs.

Autoimmune Ovarian Failure

Ovarian failure has been reported in both type I (Addison's disease, hypoparathyroidism, mucocutaneous candidiasis) and type II (Addison's, autoimmune thyroid disease, type 1 diabetes mellitus) Autoimmune Polyglandular Syndromes (APS) (Vol. 2; Chap. 26). As many as 60% of girls with type I APS and as many as 10% of girls with APS II develop ovarian failure. In women with type 1 APS, the presence of side chain cleavage enzyme auto-antibodies appears to be a strong predictor of ovarian failure (81,82).

Mutations in Gonadotropin and Gonadotropin Receptor Genes

Homozygous mutations in the FSH β gene have been identified and both partial and complete FSH function defects have been described (83,84). In females, isolated FSH deficiency presents with pubertal delay and/or impaired follicular development. In contrast, females with LH receptor defects often develop secondary sexual characteristics but present with primary amenorrhea. In cases of gonadotropin receptor

dysfunction, gonadotropins are elevated and sex steroids (estradiol, progesterone) are low (85).

Galactosemia

As many as 50% of girls with classic galactosemia develop ovarian failure (86). The classic form of galactosemia, which results from a homozygous mutation in the GALT enzyme gene (9p13), is associated with primary ovarian failure. Although the exposure to persistently elevated values of galactose-1-phosphate are felt to be involved in the pathogenesis of ovarian failure, the high rate of ovarian failure in appropriately treated girls who maintained normal gal-1-phosphate levels suggests that ovarian damage results from both intrauterine and extrauterine exposures.

Irradiation

Roughly 50% of females exposed to pelvic irradiation develop primary ovarian failure. The severity and likelihood of ovarian dysfunction is dependent on the dose of radiation delivered and the age when it is received (Vol. 2; Chap. 30). The actively proliferating cells of the mature ovary are more susceptible to the effects of irradiation than those of the quiescent pre-pubertal ovary. The LD50 for oocytes appears to be around 2 Gy and exposure to more than 6 Gy generally result in permanent ovarian failure (87).

Chemotherapy

Cytotoxic chemotherapy may cause premature ovarian failure in as many as 60% of treated females. Implicated agents include: cyclophosphamide, busulfan, procarbazine, and etoposide (Vol. 2; Chap. 30). Like irradiation, the actively proliferating cells of the mature ovary appear to be especially sensitive to the effects of cytotoxic agents. GnRH agonist therapy has been used to preserve ovarian function during chemotherapy with alkalating agents and have successfully resulted in the return of spontaneous menses and ovulation in most survivors. Accordingly, gonadal suppression should be considered in all pubertal and post-pubertal females undergoing chemotherapy (88).

Infectious Disease

Ovarian failure has been associated with Mumps, Shigella, Malaria, and Varicella infections.

Enzyme Deficiency

Most steroidogenic enzyme deficiencies present with findings at birth or during childhood, milder forms that block estrogen synthesis include 17-hydroxylase-17,20-desmolase deficiency (elevated progesterone, pregnenolone 17-hydroxyprogesterone and 17-hydroxypregnenolone levels) and desmolase deficiency (all steroids low), may present with pubertal delay.

Hypogonadotropic Hypogonadism

If gonadotropin levels are low, the search for the cause is also an attempt to determine if the hypogonadotropism represents a temporary or permanent hypothalamic-pituitary defect. In an attempt to differentiate patients with a permanent defect from those with delayed or temporary hypogonadotropism, GnRH- or GnRHa-stimulated gonadotropin levels can be measured although such testing is often not helpful except in the older adolescent patient. Documenting and tracking skeletal maturation provides an index of biologic maturity.

Causes of HH range from benign, self-limited causes such as constitutional growth and development delay and treatable underlying conditions (Table 6) to genetic defects in gonadotropin production (Table 7). to secondary causes of central hypogonadism such as chronic illness or infiltrative disease. Constitutional delay in growth and development is the most common in this group but distinguishing this etiology from other more serious causes can be difficult and therefore should be a diagnosis of exclusion that may require waiting until the patient becomes older to verify.

Constitutional Delay

Constitutional delay represents an exaggerated delay of puberty in an otherwise healthy girl in which the physical and biochemical markers of growth resemble those of a younger child. In these children, puberty will occur spontaneously and progress normally at a later than average age. There is often a family history of constitutional and pubertal delay (89). At presentation, these children are also conspicuous because of their relative short stature (2–3 SD below the mean) when compared to their same aged peers. In these children, pubertal staging and stature are commensurate with the bone age. Among such children, adult height is generally consistent with the genetic potential. A diagnosis of constitutional growth delay requires that other causes of pubertal delay be eliminated. Constitutional growth delay can be especially difficult to distinguish from isolated gonadotropin deficiency, a process that often requires an extended period of observation.

Permanent Hypogonadotropic Hypogonadism

Persistence of low basal or GnRH-stimulated LH and FSH levels and lack of demonstration of episodic secretion in a patient without an underlying cause, or the finding of such levels in a patient in late-teenage years with a bone age over 11 to 12 years is indicative of a defect of gonadotropin secretion.

Isolated Gonadotropin Deficiency

Idiopathic HH (IHH) and Kallman syndrome are isolated defects in pituitary GnRH secretion (90). By

definition, individuals with this diagnosis have no other structural or hormonal deficits to explain the hypothalamic-pituitary deficiency. These conditions can occur in a partial or complete state. Patients with partial HH may also present with arrested or stalled pubertal development. IHH can be sporadic (66%) or familial (33%). Inheritance can be X-linked, autosomal dominant and autosomal recessive. Because the defect occurs at the hypothalamic level, reproductive function may be restored with pulsatile GnRH administration.

Kallmann Syndrome

Kallmann syndrome is a form of isolated idiopathic hypogonadotropism associated with anosmia (absence of smell) or hyposmia and is the most common form of isolated gonadotropin deficiency, rarer in females than males (1/50000 females; 1/10000 males). Most cases are sporadic; however, 5% of patients harbor a mutation in the Kal1 gene (Xp22.3). The product of this gene, Anosmin-1, is a neural cell adhesion protein involved in FGF signaling. Carrier females may have hyposmia, delayed menarche or irregular menarche but are usually fertile. The Kal2 gene (8p12) codes for FGFR1; an inactivating mutation in this gene has been found to be responsible for an autosomal dominant form of Kallmann syndrome. Midline facial defects (cleft lip, cleft palate, and more extensive midline facial defects) are associated with Kallmann syndrome.

DAX-1 Gene Mutation

DAX-1 is an orphan nuclear receptor essential for adrenal cortex development and regulation of gonadotropin secretion. DAX-1 also plays a role in ovarian formation and duplication of this gene in males can lead to sex-reversal. Inactivating mutations in the DAX-1 gene, located in the dosage sensitive sex reversal locus at Xp21 may result in HH and adrenal insufficiency (X-linked adrenal hypoplasia congenita). Carrier females have been reported to show delayed puberty (91). Homozygous states are unusual in females but have been described. Genotype phenotype relationships have been inconsistent.

Gene Mutations

GnRH binds to a G-protein-coupled receptor on 4p13.1 that signals through the phosphoinositol pathway to increase intracellular calcium and stimulate gonadotropin synthesis. Mutations in the GnRH receptor have been identified in 30% of families with isolated gonadotropin deficiency not associated with anosmia and in approximately 5% of patients with IHH. Identified mutations interfere with ligand binding; reduce phospholipase C activation and phosphoinositol production. However, currently, no mutations in the GnRH gene have been identified in patients with HH. Females exhibit poor breast development and amenorrhea. Curiously, 75% of affected patients will exhibit a rise in LH and FSH when challenged with a single intravenous dose of GnRH (100 μg). Therefore, GnRH mutation does not preclude therapy with pulsatile GnRH (92).

Loss of function mutations of GPR54 have been identified as a cause of HH. As discussed above, both a functional GRF54 and the KiSS protein-derived peptide play a key role in puberty and the normal function of the gonadotropic axis (5,93).

Structural CNS Lesions and Tumors

Craniopharyngioma, the most common tumor resulting in childhood hypopituitarism, may present with delay of puberty (Vol. 2; Chap. 3). One series of patients with craniopharyngioma of pubertal age, all had pubertal delay and in this group this was the most common reason for referral (94). Associated symptoms are usually neurological (headaches 13%, visual disturbance 35%), endocrine disturbance is present at diagnosis in up to 80% of patients. CNS lesions as a consequence of head trauma, surgery, infections and infiltrative diseases can also disrupt hypothalamic/pituitary function resulting in HH. Midline defects such as septo-optic dysplasia (SOD) or absent septum pellucidum may also be associated with pituitary gland hypoplasia and gonadotropin deficiency.

Genetic Syndromes

The Prader–Willi Syndrome (PWS) results from a loss of function of the paternal allele for the imprinted genes such as SNRPN, a spliceosome protein, and SNURF, a gene regulating imprinting, because of deletions or maternal uniparental disomy of chromosome 15q11-q13. PWS is commonly associated with HH. Other features include global hypotonia, mental retardation, and small hands/feet. Noonan syndrome (1/2500) is associated with hypogonadotropism and is characterized by delayed puberty, short stature, valvular pulmonic stenosis, cardiomyopathy, nuchal webbing of the neck, a low hairline and chest deformity. Girls with the Laurence–Moon–Bardet–Biedl syndrome show mental retardation, pigmentary retinopathy, postaxial polydactyly, obesity and both central hypogonadism as well as ovarian failure.

Single Gene Defects

Leptin or leptin receptor gene mutations can cause obesity, hyperinsulinemia and HH. PROP-1 (5q) gene mutations can cause an autosomal recessive deficiency of GH, prolactin, TSH and FSH/LH (Chapter 1 of Vol. 1).

Temporary Hypogonadotropism (with Potential for Normal Function)

If there is an underlying chronic illness, if there has been prolonged pharmacologic glucocorticoid

therapy, if there is excessive emotional stress, unusual physical activity, or an inadequate nutritional state, the hypogonadotropism is likely a secondary and potentially reversible condition. If the underlying problem can be adequately treated, normal gonadotropin secretion is expected to follow. Response to estrogen treatment while the underlying problem persists is variable.

Body Composition/Undernutrition

Intense exercise can result in primary amenorrhea, a condition depleting energy needed for growth. Similarly, anorexia nervosa and other causes of under/malnutrition are other well-known causes of menstrual disturbance and HH (Vol. 2; Chap. 1) (95).

Chronic Disease/Systemic Illness

Any major illness, particularly inflammatory illnesses, can cause pubertal delay and poor growth. Poor nutrition, chronic pain and psychosocial factors can all disrupt the HPG axis. Hemochromatosis may result in pituitary damage and HH (96). Human immunodeficiency virus may cause both primary and secondary hypogonadism (97). Elevated lead levels have been shown to be associated with pubertal delays (98). Prolactinomas are also associated with hypogonadotropism.

Eugonadotropic Eugonadism

Eugonadal patients with normal development of secondary sexual characteristics who have delay in menarche may have an anatomical defect, a chronic anovulatory state or rarely a disorder of intersex. In girls with partial or incomplete canalization of the vagina or imperforate hymen, cyclical endometrial shedding accumulates and results in chronic lower abdominal pain. In all of these cases, a vaginal exam or ultrasound is diagnostic.

Evaluation

Evaluation of pubertal delay includes a careful review of general health and growth, questioning about exposures to irradiation or chemotherapy, history of intense exercise as well as the family pattern of pubertal timing and attainment of menarche. A physical examination including anthropometric measurements (standing height, upper to lower segment ratio, and assessment of subcutaneous fat), pubertal staging, testing of sense of smell and neurological assessment is required.

Laboratory assessment may include measurement of a CBC, electrolytes, LFT's, ESR, prolactin, cortisol, IGF-1, TSH, Free T4, sex steroids including DHEAS, FSH, LH, karyotype and bone age determination when indicated. In some cases of HH, brain imaging may be indicated to rule out intracranial mass. A pelvic ultrasound may be helpful to evaluate endometrial stripe and Müllerian anatomy. GnRH/GnRHa stimulation testing can be especially helpful in equivocal cases. If there is clearly a pubertal response or essentially no response, these suggest respectively the potential for or lack of normal gonadotropin secretion.

Management

When pubertal delay results from systemic disease or chronic illness, treatment should be directed at the primary illness. When hyperprolactinemia has been identified, head MRI should be obtained and therapy can be started with a dopamine agonist, bromocriptine mesylate or carbergoline.

Puberty can be induced using low dose estrogen therapy started at 10 to 12 years of age, an age that allows the induction of breast development without risking undue bone age advance. In girls with Turners syndrome, sex steroid replacement may be delayed to optimize the effects of supplemental growth hormone therapy (Vol. 2; Chap. 5). The starting dose of estrogen can be 0.3 mg of conjugated estrogens every other day, 5 μg of ethinyl estradiol daily or transdermal estrogen preparations (0.025 mg) twice weekly. Transdermal estrogen replacement may be preferred in those girls with a history of poor compliance or a family history of thromboembolism. Low dosage patches have been used only at night in an effort to mimic spontaneous puberty (99). The dose of estrogen can be increased every 6 to 12 months in order to reach full replacement doses after two to three years of therapy. Daily doses of 0.625 mg of conjugated estrogen or 20 μg ethinyl estradiol are accepted as full replacement doses. Replacement therapy, with the possible exception of those without internal reproductive structures, eventually involves cyclic estrogen-progesterone therapy. Once full estrogen replacement has been reached cyclical progesterone 5 to 10 mg of medroxyprogesterone acetate or 200 to 400 mg of micronized progesterone daily for 12 days can be added every month to induce monthly menstrual bleeding. Cyclic estrogen-progesterone therapy can involve low-dosage estrogen birth control pills, a daily oral estrogen regimen or the transdermal form for 21 days with the addition of progesterone added for 12 days (day 10–21) followed by a week of no hormones. Once full pubertal development has been reached, the estrogen dosage should be the minimum that will maintain normal menstrual flow and prevent calcium bone loss, equivalent to 0.625 conjugated estrogen. After maturity and growth are complete, the replacement regimen may be altered to allow less frequent withdrawal bleeding.

Androgen replacement using small doses of methyltestosterone or DHEA may be helpful to stimulate androgen dependent pubic hair growth, especially in those girls with hypopituitarism. This therapy should be balanced with the risk of hirsutism, male pattern baldness, clitoral enlargement and deepening of the voice that occurs with higher doses.

In cases of permanent gonadotropin deficiency, induction of fertility may be accomplished with pulsatile GnRH pulse therapy. When hypogonadism is due to pituitary dysfunction, hCG and/or (human menopausal gonadotropin/recombinant FSH) can also be used.

It should be recognized that gonadotropin secretion may resume in patients with a temporary cause. In addition, a small minority of patients with the Turner syndrome may have spontaneous pubertal development with ovulation, menstruation and fertility. In these situations, replacement therapy may not be needed in all patients. However, these groups of patients are at risk of developing hypogonadotropism and a need for therapy. While ovulation does not occur with permanent gonadal failure as in Turner syndrome, those with an adequately developed uterus may be candidates for pregnancy via ova donation and in vitro fertilization.

In Boys

Definition and Classification

While most boys presenting with the complaint of delayed puberty have constitutional delay, care should be taken in the initial assessment to consider pathologic causes. The initial workup should involve an evaluation aimed at the differential diagnosis listed in Tables 5–7 and discussed below.

It is useful to classify patients based upon gonadotropin levels: hypergonadotropic hypogonadism (testicular failure) or hypogonadotropic states. Low gonadotropin levels may be a consequence of an immature hypothalamic-pituitary axis, a down-regulated axis, or a permanent defect.

Hypergonadotropic Hypogonadism (Testicular Failure)

Elevated gonadotropins after initial laboratory testing will identify testicular failure. Categories of primary testicular failure listed in Table 5 provide a guide to further evaluation.

Klinefelter and Multiple X Syndromes

The most common cause of hypergonadotropic hypogonadism is the Klinefelter syndrome, occurring in about 1 in 600 males). The majority have a 47,XXY karyotype, with variations including 47,XXY, 48,XXXY, 48XXYY, and 49,XXXYY. This syndrome is currently more commonly diagnosed during fetal or neonatal life or during childhood. Presentation is seldom with delay of onset of puberty, with slow progression or lack of completion being more common. Testosterone may be normal during puberty, with testosterone levels reaching adult levels, subsequently gradually diminishing, with concomitantly increasing estrogen-testosterone ratios. Diagnosis may be delayed until infertility becomes manifest. Small testes, low inhibin B levels and elevated LH and FSH are essentially ubiquitous findings. Azoospermia is expected, except in some with a mosaic karyotype. Typical physical features include tall stature, disproportionate long limbs, poor muscular development, and gynecomastia. Relatively long limbs and smaller penis size may be noted during childhood. Degree of virilization and body habitus often varies depending on genetic factors and relative androgen deficiency. Findings include decreased sexual hair, muscle mass and a feminine fat distribution. Neurological, psychological and behavioral problems often manifest as difficulties in school. Those with three or four X chromosomes are more severely affected.

Other Partial and Complete Forms

Primary hypogonadism results from prenatal or acquired diminished or lacking testicular function. Examples include by the neonate with anorchia (Vanishing testis syndrome), bilateral cryptorchidism with dysgenetic testes, atrophic testes, bilateral torsion at any age, damaged parenchyma (sickle cell disease), trauma, infection (mumps and coxsackie virus), chemotoxicity, irradiation and as a consequence of surgery (attempted correction of cryptorchidism). Elevated gonadotropins are associated with hepatic and renal failure. Genetic associations include myotonic dystrophy, Noonan syndrome, and linked with a mutations resulting in congenital adrenal hypoplasia. Inactivating mutations of the LHβ and the LH receptor are associated with pubertal delay. The LHβ mutations are associated with elevated LH levels, low FSH and testosterone, male genitalia and small testes. Partial LH receptor mutations may be associated with pubertal delay and normal to undervirilized genitalia.

Hypogonadotropic Hypogonadism

Low gonadotropin levels and pubertal delay may result from a physiologic delay or a permanent defect. Delay of puberty in a boy with the potential for normal puberty and reproductive function may be a variant of normal ''physiologic delay'' or a consequence of a chronic systemic condition including malnutrition. Permanent defects result from genetic mutations, demonstrable defects or destruction of the hypothalamus or pituitary, the later usually being associated with other pituitary dysfunction. When gonadotropin levels are low, when there are no organic or associated conditions of pituitary malfunction, and when there is no other cause to account for delayed maturation, it usually is not possible to determine whether gonadotropin deficiency or delayed maturation of a potentially functional HPT axis exists until well into pubertal years. Gonadotropin responses to GnRH/GnRHa stimulation are helpful only if there is a clear pubertal response or if

the response is so minimal that it is below the childhood response range.

Low Gonadotropins without Permanent HPT Defect

In boys, constitutional delay of growth and puberty is most likely if the medical history is otherwise normal and abnormal findings on physical examination relate only to delay of puberty. Such patients are healthy, have a history of short stature with growth rate at the lower limit of normal. Those with delayed puberty associated with systemic disease can be expected to have the potential for normal gonadal function unless the underlying disease cannot be fully treated or the therapy damages the HPG axis (Table 6).

Constitutional Delay of Growth and Development

This variant of normal development is characterized by growth, height, and hormone levels characteristic of a younger boy, with a history of progressive maturity, albeit delayed. Commonly, boys decelerate in growth velocity by three years of age, have a similar delay in height and skeletal maturation, progress throughout childhood at a low normal growth and skeletal maturity rate. Short stature and physical immaturity become more noticeable as the usual age of puberty approaches with somatic and sexual pubertal growth occurring spontaneously, at an older age than typical. Such delay may be associated with negative psychological effects, impaired academic performance and limited social involvement. The outcome of this condition, generally considered benign, is normal physical development, sexual and reproductive function. It is unclear whether there is compromised adult height and post-adolescent BMD. Androgen therapy during pubertal years has not been shown to improve BMD but men with a history of constitutional pubertal delay having been reported to have BMD below and within the normal range (100). Males presenting with delay who are relatively tall and have better growth but more immature body ratio segments attained better adult heights regardless of their skeletal age (101).

Pathologic Causes of Pubertal Delay with Normal HPG Axis Potential

Systemic illness, e.g., Crohn disease, other chronic inflammatory conditions, malignancies, cystic fibrosis (102), severe cardiac disease, hypothyroidism, poorly controlled diabetes mellitus, systemic therapy for chronic conditions, especially Cushing disease or long-term pharmacologic dosages of glucocorticoids. Hemochromatosis, as may occur in sickle cell disease, may result in both pituitary and testicular dysfunction. Nutritional and psychosocial deprivation may be associated with pubertal delay while the boy has the potential for normal adult HPG axis function (Table 6).

Permanent Gonadotropin Deficiency

Isolated

Categories of HH include forms of the Kallmann syndrome, known gene and anatomic deficits and idiopathic. Isolated defects in GnRH secretion involve a growing list of defined gene mutations and IHH, are not associated with anatomic defects and are not associated with other hormone deficits (Table 7). A positive family history may be found. Defined genes include: KAL1 which encodes anosmin-1, a neural adhesion protein present during organogenesis, necessary for normal embryonic migration and appropriate neural spatial relationships, associated defects including visual deficits, nystagmus, ataxia, synkinesia, midfacial defects and renal agenesis. FGFR1 (KAL 2 or FGFR1) is associated with deficiency of FGF signaling during olfactory bulb morphogenesis and an autosomal dominant form of Kallmann syndrome. Both Leptin and LeptinR gene mutations are associated with HH and severe obesity.

DAX-1-hypogonadotropism is associated with x-linked adrenal hypoplasia congenital and may be associated with a contiguous gene defect leading to Duchenne muscular dystrophy. Mutations of the GnRH receptor gene, FSH or subunit genes may result in HH.

Multiple Pituitary Hormone Deficiencies

Multiple pituitary hormone deficiencies may be congenital or acquired. Defects associated with panhypopituitarism include tumors, trauma, irradiation, surgery, infections, infiltrative disorders and specific gene defects. Tumors include craniopharyngioma, and anatomic abnormalities (Table 7). There are also poorly understood syndromes that are associated with hypogonadotropism.

Evaluation

History includes a review of rates of weight and height gain, testicular descent, any other evidence suggestive of gonadal endocrinopathy, including associated hypopituitarism, hypothyroidism, adrenal insufficiency, diabetes insipidus or midline facial malformations; current or prior illnesses and their treatment, including irradiation, surgery, chemotherapy, or glucocorticoid therapy; family history of pubertal delay, hypogonadism, or infertility; evidence of craniofacial-CNS midline defects, including anosmia or hyposmia. Anosmia and midline facial defects suggest hypogonadotropism while those with constitutional delay often have a positive family history for delayed puberty and short stature. Cryptorchidism, a small penis or low gonadotropins noted in the neonatal period, a positive family history, or anosmia suggest permanent deficiency. A growth curve of heights-for-age that parallels

standards below the normal range suggests constitutional delay, while normal stature, with disproportionately long limbs, suggests hypogonadism.

Physical examination is normal, typical of a younger, immature boy. This includes careful documentation of height, weight, pubertal stage (Table 1), and upper/lower (U/L) segment ratios. This ratio is determined by subtracting, from standing height (U), the vertical distance from the pubic symphysis to the floor (L) or by subtracting the sitting height (U) from standing height (L). Body proportions in the boy with constitutional delay may be juvenile, a greater upper/lower body segment ratio (relatively short legs and arms for height) than usual for age while those with a pathologic cause have normal or low ratios. A low ratio indicative of excessive leg (long bone) growth is suggestive of a defect or prolonged delay. Such ratios may be pronounced in primary hypogonadism, such as the Klinefelter syndrome. Normal ratios differ by race, mature ratios approximating 1.0 in Whites and 0.9 in Blacks. A careful neurological examination should include assessment of fundi, visual fields, and sense of smell. Testicular location (scrotal, inguinal, or nonpalpable), size, and consistency are important. A testis less than 2.0 cc or longitudinal axis less than 1.5 cm is prepubertal in size. A volume larger than 3.0 cc or longer than 2.2 cm is evidence of pubertal growth. A testis in a pubertal-aged boy of 1.0 cm or less, particularly if unusually firm or soft, is suggestive of a hypogonadal state.

Laboratory evaluation after measurements of LH, FSH levels and skeletal age are based on history and physical findings. If growth rate is subnormal, assessment of growth hormone secretion using IGF1 assay and thyroid function may be indicated, although it is important to recognize that growth rates are slowest just before the onset of puberty in normal boys. Studies to rule out CNS, renal, or gastrointestinal disease may include blood and urinary pH, urinary specific gravity, sedimentation rates, blood urea nitrogen, creatinine, and CNS MRI. A karyotype is indicated if evidence such as small, firm testes, gynecomastia, body segment ratios, and gonadotropin levels suggest Klinefelter syndrome. If history is consistent with known genetic forms of hypogonadotropism, specific gene tests should be considered. If gonadotropin levels are not elevated, testes are nonpalpable or a testicular defect is suspected, MIH levels (103), as an indication of testicular presence and integrity, and hCG stimulation testing (104) may be indicated. Numerous hCG regimens are used depending on the status of the patient, one to three injections daily or on alternate days of stimulation may be adequate to demonstrate a pubertal response. A rise in circulating testosterone levels to greater than 170 ng/dl after a single injection, greater than 200 on day 3 or greater than 300 on day 5, obtained within 24 hours of the last injection indicates normal testicular potential. A dosage of 3000 units/m^2 per injection is adequate, limiting maximum stimulation to 3000 units per injection.

Treatment

Patients with primary hypogonadism and their parents should be informed about therapeutic possibilities, with counseling appropriately for the patient's level of understanding. Counseling should be based on an understanding of the two basic functions of the testes: male hormone and sperm production. With appropriate hormone replacement, patients can be assured that they will develop physically normally and will be able to function sexually, as any other man. They need to understand that they, as other men who have a problem producing sperm, may chose to have children by adoption or using currently available methods and procedures, including sperm donation. If testes are absent, the choice of placement of prosthetic testes into the scrotum should be presented, clearly indicating that such testes are placed only for appearance purposes, have no function but feel and appear normal. While prostheses are available in graded sizes, they generally are not placed until the scrotum is large enough to accommodate an adult-sized testis. There are increased operative risks in subsequently replacing prostheses. Deferring surgery until after pubertal hormonal stimulation to enlarge the scrotal capacity is appropriate. There is no evidence that having an empty scrotum during boyhood has detrimental psychological effects. The knowledge of the option of testicular implantation after early puberty should be psychologically satisfying.

Hormonal replacement can begin at the usual age of the onset of puberty, 12.5 to 13 years, as soon thereafter as the need is recognized, or earlier if desired for reassurance. Occasionally, there are reasons to delay treatment, including allowing catch-up growth for short stature, emotional or psychological immaturity. The initial dosage, particularly in the completely immature boy, should be low to avoid the risk of priapism and overly rapid pubertal development, then adjusted based on response, age, social and intellectual maturation, and psychological needs of the patients. When skeletal age is immature, there is a risk of disproportionately accelerating bone age in relation to concomitant growth ultimately diminishing adult height. However, when replacement therapy is started at a pubertal bone age, there should be no detrimental effect on adult height.

Even though this differentiation cannot be made when pubertal delay occurs with low gonadotropin levels, sex steroid therapy is often begun before it is possible to differentiate delay from deficiency. Such therapy, if desired, is appropriate when age and psychosocial development are ready for physical pubertal maturation. Treatment for three to six months with testosterone (Fig. 9) leads to somatic and genital growth.

Such therapy has been used without detriment if begun at a biological age (bone age) of 12 to 13 years

or older for either constitutional delay or HH. Subsequent puberty occurs in those with adequate potential. Among gonadotrophin deficient men there is no evidence of any deteimental effect of previous testosterone treatment upon the potential for spermatogenesis in response to exogenous gonadotropin stimulation.

Before a diagnosis is possible, a short-term therapy of four to six months of testosterone therapy may be given using depot intramuscular or topical forms of testosterone. Titering the dosage is difficult using the currently available patches while dosing selection is possible with gel and depot preparations. The greatest experience involves depot testosterone (such as enanthate or cypionate) given at a dosage ranging from 50 to 100 mg IM every four weeks. In the completely prepubertal boys, low dosages are used since higher dosages may result in frequent, prolonged erections, and stimulation of rapid changes, with marked reddening and sensitivity of genital tissues. To differentiate temporary from permanent deficiency, treatment should be stopped for several weeks after the initial months of stimulation and testosterone levels measured to determine endogenous androgen production. Testosterone levels clearly within the pubertal range (>50 ng/dl) suggest constitutional delay. Whether further treatment is given or not, such patients should be followed to assess progression of puberty and endogenous testosterone levels. Once normal adult male levels (>275 ng/dl) have been documented, if physical including testicular examination is normal, hypothalamic-pituitary-testicular function can be assumed.

If testosterone levels remain low, this is evidence of gonadotropin deficiency. Therapy with androgen should be continued after this diagnosis is made, based on persistently low LH, FSH, and testosterone levels and available pertinent gene studies. Gonadotropins can be used to induce puberty (105), although practically biosynthetic LH and FSH administration is utilized, when paternity is desired, to stimulate spermatogenesis and fertility. A portion of patients treated this way respond with adequate spermatogenesis, response being better with larger initial testis size. This response is not adversely affected by prior exogenous androgens. Because of expense and injection schedules, such treatment is not indicated until fertility is desired. Testosterone replacement is the treatment of choice until that time.

The initiation of pubertal changes are described under treatment leading to differentiation of causes of pubertal delay with low gonadotropin levels. Dosages can be gradually titering upward subsequently to full-replacement dosages after three to four years. Males with constitutional delay are commonly given a course of three to six months of testosterone to stimulate more rapid catch-up in growth and maturity. Such therapy has been shown not to reduce adult height in the boys

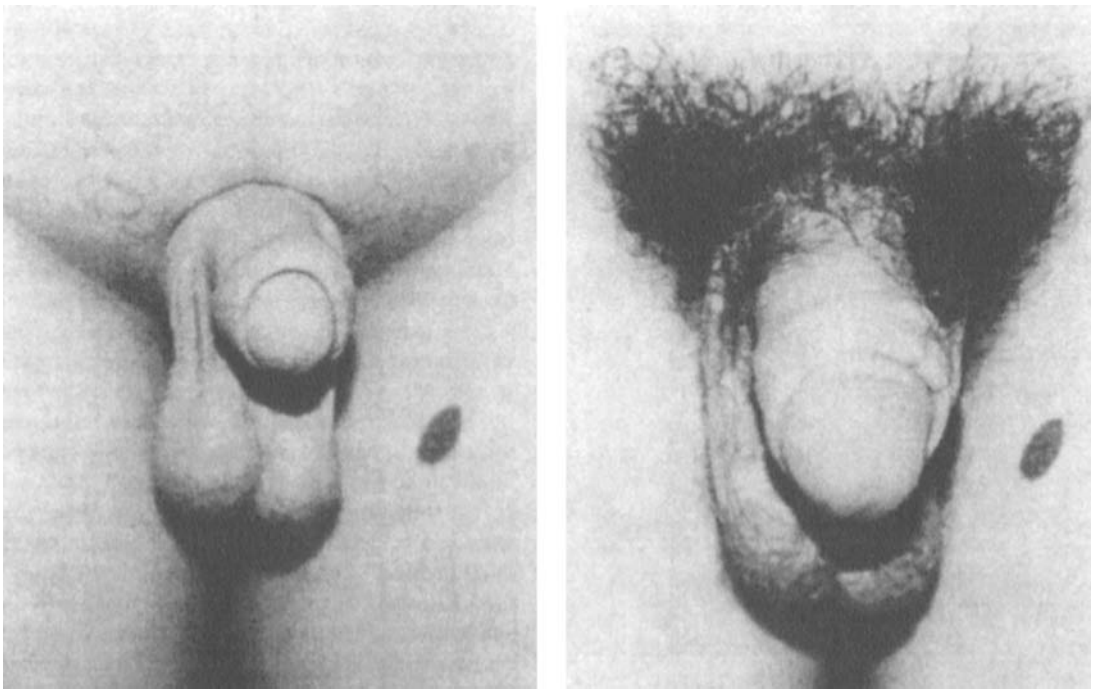

Figure 9 Genitalia of a boy with delayed puberty before (*left*) and after (*right*) five monthly injections of 200 mg testosterone enanthate IM. Pubrectal development was Tanner 2 before the injections and skeletal age was 12 years 6 months at 14 years 8 months age. After the injections at 15 years 2 months pubertal development progressed to Tanner 4. A subsequent endogenous testosterone level of 192 ng/dl verified a diagnosis of constitutional delay of puberty.

with a skeletal age above 12 years (106). This may be initiated even after physical and hormonal changes verify that puberty is beginning (Fig. 9). Patients with permanent hypogonadism learn that their physical development (except testicular, of course) and sexual function become normal. If accessory sexual glands are appropriately formed, ejaculation, semen volume, and appearance are normal.

Full replacement of androgen leads to attainment of physical and sexual maturity while continuation of therapy is required to maintain this adult male state. Therapy may be transdermal via gel or patch or by injection of depot testosterone. The full replacement depot dosage is not more than 100 mg/wk, commonly given at two (200 mg) or three (300 mg) week intervals. Longer intervals are not recommended since supraphysiologic levels are reached early and subnormal levels late in the cycle. The skin gel preparation involves the daily administration of 50, 75, or 100 mg., with absorption over a 24-hours period, with the goal of maintaining a steady-state serum testosterone concentration. Dosing can be used to maintain the desired circulating level. Recommended sites of application are the shoulders, upper arms and abdomen. Gel preparations are available in multi-dosage pumps, thus can be used in smaller dosages if desired during stimulation of pubertal development. Skin contact with gel should be avoided by other than the patient. The testosterone patch is available in strengths to deliver 2.5 and 5.0 mg of testosterone. Evening applications produce levels with circadian variation over 24 hours and maintain circulating levels within the normal range.

Older forms of androgen therapy replacement are usually inadequate to produce full androgen stimulation and oral forms have hepatic toxicity. Preparations of oxandrolone, fluoxymesterone, and methyltestosterone buccal or oral tablets do not provide full replacement and have limited usefulness.

As noted above, some hypogonadotropic hypogonadal males have the potential for spermatogenesis and testosterone production if appropriately stimulated with gonadotropin or GnRH. The expense and frequency of administration usually limits such therapy for adult men attempting paternity.

Since delayed puberty alone may be associated with short stature (107) or may coexist with idiopathic short stature, treatment to promote a greater adult height is being tried using aromatase inhibitors, since estrogens play a crucial role in epiphyseal closure. Initial results find a delay in skeletal maturation and height SDS for skeletal age (108–110).

Outcome

Outcome information depends upon the underlying cause of the pubertal delay. It can be expected that men with hypogonadism will respond to androgen therapy with full physical development and sexual responsiveness. The potential for success with assisted fertility techniques depend upon the presence and accessibility of spermatogonia or mature sperm. The adult height attained also depends upon co-existing factors, even for those with constitutional delay. Delay of treatment for pubertal delay is related to both failure to attain target height and diminished BMD (100,111). Timely therapy and current medical therapies result in a good quality of life for most individuals (112).

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12

Turner Syndrome

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INTRODUCTION

Humans need an intact X chromosome to survive. When all (or part) of the second sex chromosome—the X or the Y chromosome—is absent, a characteristic prenatal and postnatal phenotype results. Of the small percentage that survives to term, affected individuals are phenotypic females with dysgenetic ovaries, short stature, and other typical but highly variable dysmorphic features (Fig. 1).

Otto Ullrich first described the syndrome in a case report in the German literature in 1930 and later recognized the neonatal presentation with lymphedema (1). University of Oklahoma Professor Henry Turner, whose name is inextricably linked with the syndrome, described seven patients with "infantilism, congenital webbed neck, and cubitus valgus" in 1938 (2). Turner became the first physician to use medical therapies in a patient with Turner syndrome (TS), administering injections of various pituitary extracts and estrogen-containing preparations to several of his patients. Rudimentary ovaries ("streak gonads") were identified as the cause of TS's sexual infantilism in 1944 by Wilkins and Fleishman (3), and the term 45,X gonadal dysgenesis is often used to identify TS (4). Other manifestations of the syndrome were catalogued during this period and collectively termed "Turner stigmata" (5).

TS was associated with X chromosome monosomy in 1959 by Ford et al. (6) in a prepubertal 14 year old with short stature and typical features. The development of routine cytogenetic analysis in the 1960s led to recognition of the diversity of chromosomal complements associated with TS (7,8). Most patient series have reported that approximately half of TS patients have a 45,X karyotype, while others have a mosaicism of 45,X cells with normal cells or with structural anomalies of the second X, the most common being isochromosome (duplication) of the long arm (9). Mosaicism is more common, however, when stringent cytogenetic methods are utilized. The relative proportions of karyotypes found in representative patient series are presented in Table 1 (10,11).

Investigators have recognized that haploinsufficiency of X chromosome genes normally expressed by both copies should be responsible for features of TS. One such gene, short stature homeobox (SHOX), was discovered in 1997 and found relevant in particular to the characteristic short stature and bone anomalies of TS (Vol. 2; Chap. 1).

FEATURES OF TURNER SYNDROME

Ascertainment Patterns

TS may be detected incidentally during prenatal diagnostic procedures for advanced maternal age, and it appears that the majority of these pregnancies are electively terminated. Unlike trisomies or Klinefelter syndrome, the incidence of TS does not increase with maternal age (13), therefore a relatively small proportion of affected fetuses are ascertained. TS is also suspected, like other aneuploidies, by increased nuchal thickness or other ultrasonographic anomaly, such as aortic coarctation, or by elevated maternal α-fetoprotein, estriol, or βHCG, leading to confirmation by karyotyping.

Perhaps a third of TS patients are identified at birth by Pterygium coli (webbed neck), lymphedema of the hands and feet, or coarctation of the aorta. Most of these infants have 45,X karyotypes (Fig. 2) (14), because these physical findings, in contrast with other stigmata of TS, are more common in patients with complete X monosomy than in patients with mosaicism or structural abnormality of the X.

A report derived from baseline data of a U.S. growth hormone trial (initiating therapy prior to four years of age) demonstrated fewer phenotypic features in the subjects found incidentally by prenatal diagnosis compared with those ascertained postnatally in infancy or early childhood (15). Furthermore, 56% of the patients discovered prenatally were 45,X/46,XX,

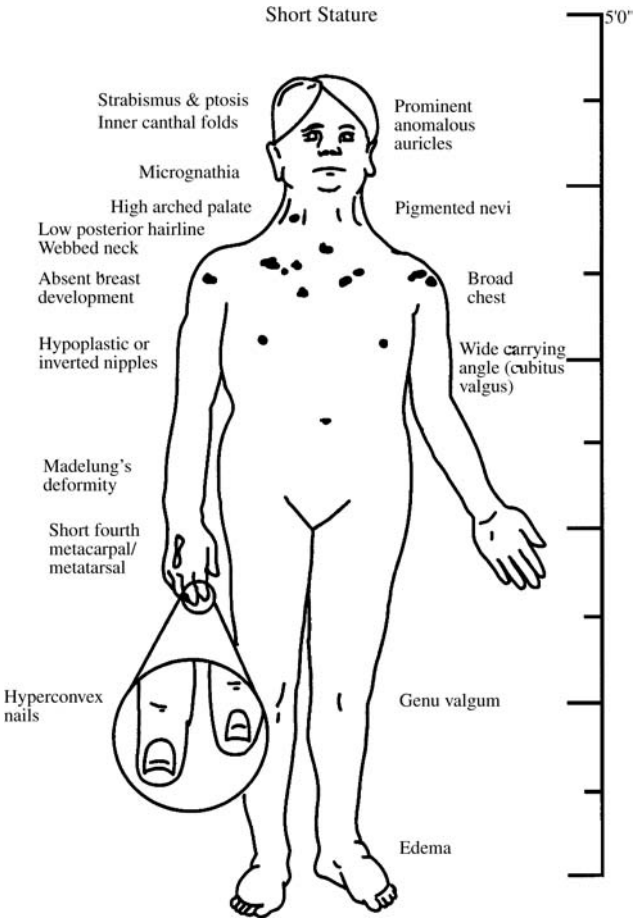

Figure 1 Schematic drawing of features of Turner syndrome commonly visible on physical examination. *Source*: From Ref. 179.

implying an ongoing strong bias toward diagnosis of monosomic rather than mosaic TS. As a consequence, depending upon the feature, the phenotype reported in patient series will be skewed toward an artifactually higher percentage of affected individuals.

TS is additionally suspected in many girls in early to mid-childhood when growth failure results

Table 1 Percentages of Karyotypes in Four Series of Patients with TS

Karyotype	Palmer and Reichman (8)	Hall et al. (10)	Park et al. (11)	Held et al. (12)
45,X	58.2	55.0	61.2	20.7
46, XisoXq	7.3	5.5	6.0	5.7
Mosaicism (with 45,X)				
/46,XX	8.2	13.4	11.2	17.2
/46,XisoXq	11.8	4.7	7.8	12.6
/46,XringX	5.5	3.9	1.7	3.5
/46,XY	5.4	3.1	2.6	2.3
/46XXp-	0.9	0.8	2.6	2.3
/46,XXq-	0	1.6	1.7	3.5
/47,XXX and / 46,XX/47,XXX	1.8	0.8	0.9	6.9
/46, X+marker	0	0.8	0	18.4

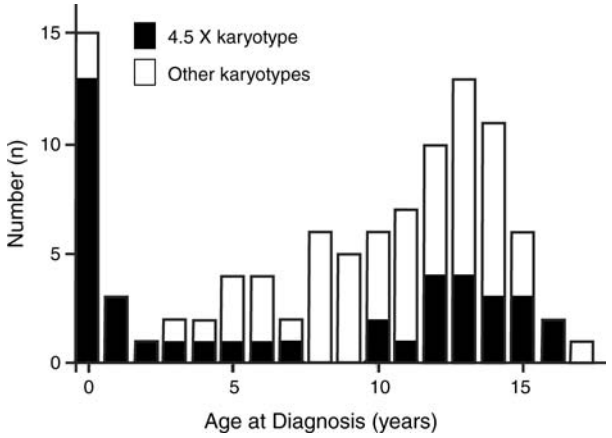

Figure 2 Age of diagnosis in 100 patients with TS. Note the greater percentage of patients with 45, X karyotype diagnosed in infacny. *Source*: From Ref. 14.

in a height noticeably below the normal range. Nevertheless, many patients will not be recognized until pubertal failure or short stature in the teenage years leads to medical evaluation. A recent update (16) of the Belgian ascertainment experience revealed that only a few patients with TS were diagnosed prenatally, 30% were ascertained prior to one year of age, 48% in childhood, and 22% after 12 years old. The diagnosis may even be delayed until referral in adulthood for primary or secondary amenorrhea, infertility, or recurrent miscarriage. Patients also are occasionally detected on the basis of one of the less evident features of TS, such as facial or bony abnormalities, learning problems, strabismus, or otitis or hearing loss. It is highly likely that many patients with mosaicism have manifestations that are mild enough to escape detection altogether, but the magnitude of this phenomenon is not known.

Fetal Demise

X monosomy is the most commonly occurring sex chromosome anomaly, but 90% or more of Turner conceptions are spontaneously aborted. It is probable that more than 1% of conceptions involve X chromosome loss. As many as 10% of spontaneously aborted fetuses have 45,X karyotypes (17). As a result, TS is common prenatally but occurs with an incidence of only about one in 2000 to 2500 live female births, making the syndrome less common postnatally than either 47,XXY (Klinefelter syndrome) or 47,XXX. Because the peak of fetal deaths occurs at about 12 weeks of gestation, most 45,X fetuses are spontaneously aborted prior to amniocentesis. A smaller proportion of fetuses are aborted in the second trimester, with autopsy findings of massive lymphedema and cystic nuchal hygroma (18). Cardiac hypoplasia has been noted as a major cause of second trimester fetal demise in TS (19).

It has been contended that all fetuses with 45,X karyotypes are spontaneously aborted and that all live born individuals with TS, due to early mitotic errors, are X chromosome mosaics, whether cytogenetically detectable or occult (18,20). Aborted fetuses with X chromosome loss are, indeed, overwhelmingly 45,X, implying a relative fetoprotective effect of mosaicism. By employing techniques such as polymerase chain reaction (PCR) and fluorescent in-situ hybridization (FISH), Held et al. (12) were able to document approximately 80% mosaicism in live born TS, largely by the detection of small marker chromosomes in some cell populations (Table 1). Mosaicism may be present in multiple tissues without detectability in blood cells, and presence of mosaicism in the trophoblast or in placental tissue might be all that is required to avoid lethality. A methodical survey (21) uncovered a previously unreported sex chromosome mosaicism in 90% of TS patients, primarily by FISH for X and Y markers. It seems clear that mosaicism occurs more commonly that usually reported, but obligate mosaicism in live born TS has not been unassailably proven.

Cardiac Anomalies

Echocardiography should be performed as soon as the diagnosis of TS is confirmed. Long known to be associated with TS, coarctation of the aorta occurs in approximately 10% to 20% of TS patients (22–25). Echocardiography has revealed other common left-sided anomalies in TS, including aortic stenosis, aortic insufficiency, and anomalous pulmonary venous return (Table 2).

The most frequent anomaly is probably bicuspid aortic valve, either alone or associated with an accompanying lesion such as coarctation (26). Both aortic coarctation and bicuspid aortic valve occur more commonly in association with a 45,X karyotype than with mosaicism or structural abnormality of the X. The spectrum of left-sided cardiac lesions in TS includes the hypoplastic left heart syndrome (27,28), which, like aortic coarctation, is an indication for chromosomal analysis in a female fetus or infant.

Table 2 Prevalence of Cardiac Malformations in TS by Echocardiography in Three Large Series

	Gøtzsche et al. (22)	Sybert (23)	Mazzanti et al. (24)
Total number of subjects	179	244	594
Number of subjects with structural abnormalities (%)	46 (26)	96 (40)	136 (23)
Coarctation	18 (10)	34 (14)	41 (7)
Bicuspid valve	25 (14)	28 (11)	74 (13)
Aortic stenosis/ insufficiency	5 (3)	11 (5)	19 (3)
Anomalous pulmonary venous drainage	NA	2 (1)	17 (3)
Other (ASD, VSD, hypoplastic left, etc.)	0 (0)	25 (10)	17 (3)

In studies of second trimester fetal pathology, a high frequency of these types of heart vessel defects has been noted in association with edema, nuchal cystic hygromas, and lymphatic aberrations at the base of the major vessels, suggesting a causal link between lymphedema and cardiac anomalies (19,29–31). There is a clear postnatal association between neck webbing and bicuspid aortic valve or coarctation. However, cardiac anatomy has been normal in many fetuses with severe edema, and the etiology of cardiac defects remains conjectural.

Various arterial abnormalities have been observed in TS (32,33). Clinically, TS patients are at risk for catastrophic aortic dissection and aneurysmal rupture, as more than 50 cases have been reported to date (34,35). Most aortic ruptures in TS are thoracic. Most of the fatalities have occurred in young women with preexisting cardiac anomaly or hypertension (23,36), but a significant percentage of deaths has occurred in individuals without known risk factors. Cardiac evaluation is mandatory in any TS patient with chest pain or shortness of breath, and cardiac status must be followed carefully during assisted reproduction and pregnancy (37). Mild aortic root dilation (about +1 SD) by echocardiography or magnetic resonance imaging (MRI) is observed in TS girls matched with controls for body size. It is not known whether routine follow-up echocardiography is helpful in anticipating aortic dissection. We currently recommend regular cardiologic and echocardiography follow-up for those with a cardiac lesion or hypertension, and repeat echocardiography in all other TS patients at cessation of growth therapy.

Renal Anomalies and Hypertension

Renal ultrasound is recommended for all patients at the time of diagnosis. The etiology of renal abnormalities in TS is unknown, and cardiac and renal anomalies usually occur independently. Most renal anomalies are silent and do not become clinically significant. Renal dysmorphology was documented by ultrasound in one-third of 141 patients with various karyotypes in the early study by Lippe et al. (38), still the largest published series, although antedating technical improvements in ultrasonography. The most frequent abnormalities were double collecting systems, horseshoe kidney, and rotational abnormalities, each occurring in 5% to 10% of patients. Abnormalities requiring urologic referral included ureteropelvic and ureterovesicle junction obstruction (6%) and absent kidney (3%). In their study, the incidence of renal dysmorphology in girls with 45,X karyotype was 45%, compared with 18% in other karyotypes combined. The greater prevalence of some renal anomalies in 45,X has been confirmed by other patient series. A Turkish study of 82 TS patients found the prevalence of horseshoe kidney to be 11%, with eight out of nine associated with a 45,X karyotype, whereas collecting duct abnormalities (overall 21%)

were as likely with mosaicism or X structural abnormality (39). Multicystic kidney dysplasia has also been reported (40).

Hypertension is common in adolescents and adults with TS. Although elevated pressures secondary to renal etiologies can occur, hypertension in TS is usually essential (Vol. 1; Chap. 13). Studies have uniformly demonstrated systolic pressures above the 95th percentile in over 20% of adolescents and adults with TS (41–44), although there is some disagreement regarding elevation in diastolic pressures. Because hypertension is a strong risk factor for aortic dissection (44) or other long-term cardiovascular complications, intervention is mandatory.

Craniofacial and Skeletal Anomalies

The facial appearance of girls with TS may include epicanthal folds, ptosis, down slanting palpebral fissures, maxillary and mandibular hypoplasia and retrognathia, prominent or malformed ears, neck webbing, low hairline, and short neck. Many of these features may represent consequences of fetal edema. However, patients may have few of these phenotypic characteristics, and the features most classically associated with TS, neck webbing and low hairline, are only present in a minority of girls. Reconstructive surgery of the ears or neck is controversial, due to the reported frequency of keloid formation.

Girls with TS may have a high arched palate, which can contribute to the feeding difficulties commonly encountered in infancy or to speech problems. Anatomic anomalies at the cranial base probably alter the angle of the Eustachian tube, leading to the high incidence of otitis media. Sinusitis and mastoiditis are also common complications, and sleep apnea has been reported. Incidence of hearing loss in TS, which can be conductive but is predominantly sensorineural, may be as high as 30% in children and 90% in adults (45–48). Hearing impairment in adulthood correlates with lower psychological well-being. The small jaw and high palate are associated with orthodontic problems, such as crowding and malocclusion (49).

Diverse bone abnormalities are found in TS, but they are usually documented only after the diagnosis has been made (50). The most common anomalies are short fourth metacarpal and cubitus valgus. Infrequent skeletal abnormalities include Madelung deformity of the wrist, sternal deformities, and genu valgum. These bone abnormalities appear to be seen equally in patients with 45,X monosomy, structural abnormality, or mosaicism, but are variably expressed, even in X chromosome monosomy. These features are likely attributable in some degree to SHOX haploinsufficiency, whether directly or mediated by fetal lymphedema. Cubitus valgus and Madelung's wrist deformity are easily seen on physical examination, and short fourth metacarpal is found on examination or on bone age films.

Patients should be routinely evaluated for scoliosis and kyphosis, which may worsen with age, particularly as growth therapies are initiated. Congenital dislocation of the hip may be seen in infants with TS. Many investigators have documented short-leggedness and abnormal body segment ratios in TS, and patients tend to exhibit a short, "square" body habitus (51). The appearance of widely spaced nipples (shield chest) may be attributable to this phenomenon.

Osteopenia

Osteopenia has been widely reported in adults with TS, but the relative contributions of an underlying bone dysplasia versus insufficient acquisition and maintenance of bone mass, due to a presumably avoidable hypoestrogenism, are still unclear. Typical modern assessment of bone mineral is by dual X-ray absorptiometry (DXA) determination of bone mineral content (BMC) and bone mineral density (BMD). Because both are dependent upon body and bone size, DXA underestimates bone density in TS, unless adjusted volumetrically or interpreted using appropriately matched controls. This premise has been overlooked in many studies of bone density in TS.

In a survey of Swedish TS patients, half of those over 45 years old had osteopenia by DXA (52), and even patients receiving long term estrogen replacement therapy had deficits in spine and radial bone density. In a report by Stepan (53) mean lumbar BMD was −4.5 SD in untreated subjects and −2.3 SD in those who had received estrogen replacement. Sylven et al. (54), comparing 47 TS women (mean age 47.9 years) with controls matched for age and weight, found a mean whole body BMD of −1.23 SD in the TS subjects; length of hormonal replacement therapy was found to be the significant variable. In another study evaluating BMD and fracture rate in 40 TS patients and 40 subjects with other forms of primary amenorrhea (PA), BMD was low in both TS and PA compared with controls (55). Corrected for height and weight, the TS group had a better bone density measure than the PA group, but fractures were increased in both groups.

Taken together, these studies argue that osteopenia can be a problem in TS, potentially leading to fractures. Although an intrinsic skeletal factor may predispose women to low bone density, the treatable hypoestrogenism is the most important element leading to the generally low BMC in adult TS patients (56,57). Increased fracture risk has been confirmed in many surveys, but it is generally confined to wrist fractures (58,59). Some studies of bone density in TS support the notion that cortical bone, as is present in the forearm, is selectively affected in TS (60). Indeed, the wrist may be specifically susceptible as part of the constellation of bone anomalies secondary to SHOX haploinsufficiency. There are no convincing reports of increased rates of spinal compression or hip fractures in Turner women. This is surprising given that for each

standard deviation that BMD falls below the mean in the normal population, the risk of pathologic fracture rises two to three fold.

Reports of osteopenia in children and adolescents with TS are unconvincing and have not always been careful in controlling for body size or delay in estrogenization (61). Our group has reported that adolescents with TS receiving human growth hormone (hGH) therapy exhibit normal bone mineral properties (62), a finding confirmed by other groups. In another study of TS adolescents receiving hGH and ethinyl estradiol (EE), phalangeal DXA BMD was normal both at baseline and three years after discontinuation of GH (63). There is only slight evidence that hGH contributes to bone mineral other than indirectly by increase in body size, whereas estrogen augments bone density in adolescents and adults independently of growth (64–66). Adult bone density may plausibly be affected by the timing of initiation of estrogen replacement in early adolescence, but it most certainly is adversely affected by insufficient estrogen during adulthood.

Gonadal Dysgenesis

Gonadal dysgenesis is one of the hallmarks of partial or complete X chromosome loss. In most 45,X individuals during childhood, the ovaries are already gonadal streaks consisting of fibrous stroma with diminished numbers of primordial follicles. Histological studies of the gonadal ridge have documented normal densities of primordial germ cells at 12 weeks of gestation, but in the early second trimester oocyte formation and folliculogenesis fail to occur normally and connective tissue proliferates. Oocytes undergo premature atresia in a highly variable manner that may be completed before birth or may continue into adolescence or adulthood (67,68). The phenotypic manifestations of the declining numbers of functioning ovarian follicles constitute a spectrum from absence of any pubertal development in most girls, to mid-pubertal failure without progression to menarche, secondary amenorrhea, or early menopause. In a retrospective Italian review of 522 adolescents and women, spontaneous puberty was documented in 14% of 45,X and 32% of mosaic patients (74). Most studies state that spontaneous menarche occurs in fewer than 10%, primarily in those with mosaicism and usually in 45,X/46,XX.

Ovarian failure is reflected in reduced steroid feedback on the hypothalamic-pituitary axis and marked elevations in serum follicle stimulating hormone (FSH) and leutinizing hormone. Most infants and adolescents with TS have markedly elevated FSH values, indicative of ovarian failure. The degree of FSH elevation is dependent on karyotype. A longitudinal study (75) of FSH values in 56 girls with 45,X, 14 girls with 45,X/46,XX, and 18 girls with another mosaic karyotype revealed that 45,X TS girls have significantly elevated, but gradually declining, FSH values from nine months to six years old. Although not clearly established, it is likely that FSH is elevated throughout the prepubertal period; thus, an FSH level may be predictive at any point. In contrast, the 45,X/46,XX girls had minimally elevated FSH values, even at one to two years, when FSH is very elevated in monosomic females (Fig. 3).

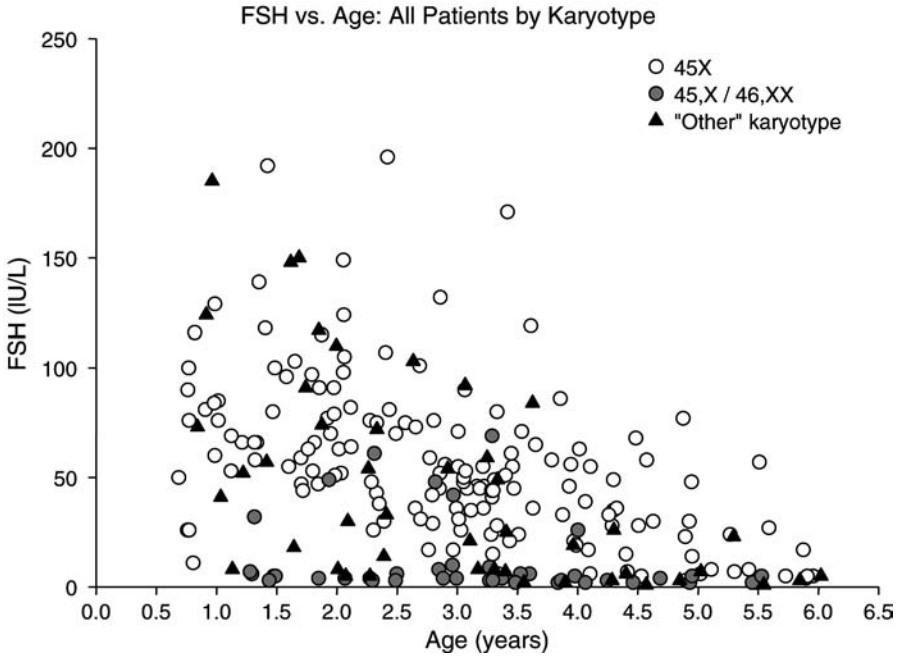

Figure 3 Scatter plot of mixed cross-sectional and longitudinal data of serum follicle stimulating hormone by age in Turner syndrome, grouped by karyotype. *Source*: From Ref. 70.

Massarano et al. reported the ultrasound appearance of ovaries in 104 TS girls of varying ages (76). The ovaries of two-thirds of the subjects were classified as streak gonads, with ovarian volumes in the remaining one-third of subjects in the lower range of normal. Because the incidence of spontaneous puberty correlates with ovarian volume (77), it is not unreasonable to obtain an ovarian ultrasound concurrently with the initial renal ultrasound, taking care to warn families of the predictive imprecision of the information. It is not known whether ovarian volume or FSH is the better predictor of spontaneous puberty. Ovaries that are near normal in size can be most easily detected in infants and toddlers or in girls of pubertal age, matching the temporal pattern of gonadotropin stimulation.

Prepubertal uterine volumes are in the normal range. Recent studies from Germany (78) examined uterine length in 50 women with 45,X who had puberty induced and were receiving estrogen and progestin replacement. A uterine length of less than -2SD was present in 26% of the 45,X TS women, whereas all four of the 45,X/46,XX TS women had normal uterine length. The presence of a smaller uterus may lead to decreased success of a donor oocyte pregnancy and thus needs to be evaluated carefully. Age of initiation of estrogen therapy or type of estrogen used to induce puberty may play a role in uterine development. Although the vagina and external genitalia are usually normal in TS, vaginal atresia has been reported, and genital ambiguity can occur in 45,X/46,XY individuals.

From a management point of view, it is important to remember that progressive follicular atresia results in two functional deficits, failure to secrete estrogen and progesterone on the one hand, and loss of germ cells and infertility on the other. For the pediatric endocrinologist, only the former requires medical intervention, as will be discussed in a later section. Nevertheless, families appreciate a frank discussion of the probability of infertility and potential options, including adoption and assisted reproductive technologies. Spontaneous pregnancy occurs in an occasional TS patient, predominantly but not exclusively in those with mosaic karyotype (74). In these cases, miscarriages are common and prenatal diagnosis is mandatory, due to the increased incidence of chromosomal anomalies and malformations in the offspring. Freezing ovarian wedges is theoretically an option; if fertilization and implantation of these oocytes later in life is successful, the issue of chromosomal defects arises. Assisted reproduction utilizing an oocyte donor may be preferable. Pregnancy in TS following oocyte donation has approximately the same success rate as in the general population (75). The American Society for Reproductive Medicine has published guidelines regarding careful cardiac monitoring of women with TS contemplating pregnancy, given the 2% risk of aortic dissection and 100-fold increase in mortality associated with pregnancy (37).

Stature and Body Composition

Manifestation of growth failure in TS begins with mild intrauterine growth retardation. Mean birth weight is about 2800 g, a relatively mild deficit that is ordinarily overlooked. Although growth velocity in the first few years of life had been previously considered relatively normal, Davenport and others have demonstrated from recent longitudinal studies that growth velocity is indeed subnormal in the first 18 months (76). Nevertheless, the 95th percentile of the TS curve does not diverge from the fifth percentile of the normal female curve until nine years of age (Fig. 4), so that in many girls TS is not diagnosed until late in childhood. Growth failure becomes even more obvious in adolescence, due to the absence of a pubertal growth spurt, and the height nadir in TS relative to normal females occurs at about 14 years of age.

In the absence of growth therapies, most TS patients continue to exhibit slow, consistent growth through late adolescence, due to delayed bone maturation and epiphyseal fusion, with typical closure after 17 years old in the absence of hormonal interventions. Historically, this delay has afforded some measure of catch-up growth before complete cessation.

Lyon et al. (77) combined their growth data with those from other European studies to create growth standards for TS in the absence of hormonal therapy. The Lyon curve, as well as other growth standards (78,79), provides an important means of evaluating growth therapies. These standard curves permit an accurate projection of adult height on the basis of the current height, and a high degree of correlation has been demonstrated between first measured height in childhood and final adult height (80). Adult heights projected on the basis of standardized curves are as accurate as heights predicted from bone age, which may be difficult to interpret in TS (81). The mean final height of the Lyon curve is 142.9 cm, a figure close to the height reported for the combined European–American experience over the last 20 years. Mean heights of approximately 147 cm are reported in untreated TS populations of Northern European descent (82,83). Final height in TS is clearly affected by parental height and ethnicity; as a rule of thumb, untreated adults with TS are approximately 20 cm shorter than expected from mid-parental target height.

The effect of karyotype on the height deficit should generally be minimized. Subjects who are mosaic for ring X and isoXq are just as short as 45,X subjects, whereas those with 46,XX and 46,XY mosaicisms may be on average slightly taller. The influence of spontaneous puberty on adult height also has not been conclusively determined (84,85). In the minority of patients in whom it occurs, it is generally associated with only a modest pubertal growth spurt, especially if hGH therapy has already maximized growth velocity, and the pubertal estrogen levels may truncate the duration of growth.

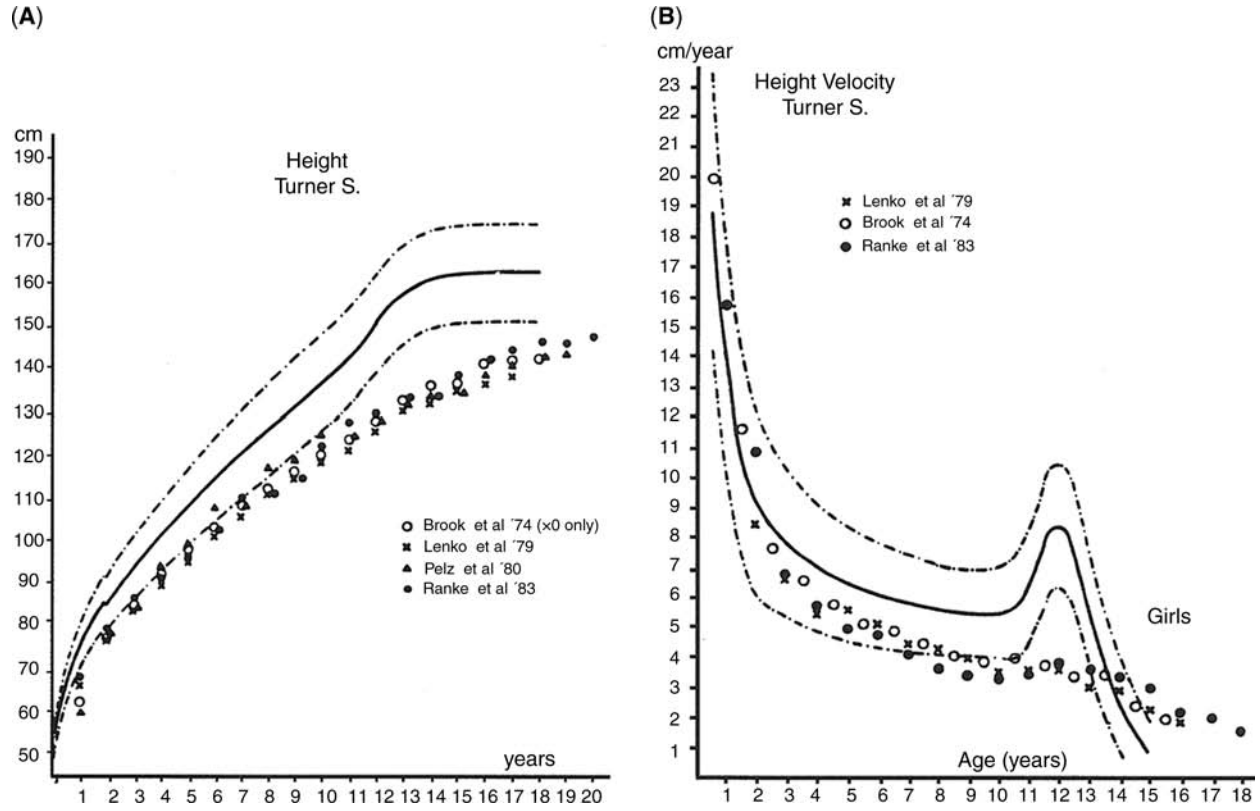

Figure 4 Mean height (**A**) and height velocity (**B**) in untreated European girls with Turner syndrome from four European studies, plotted on the growth curve for normal females. Note the steady decline in height velocity beginning in early childhood and the absence of a pubertal growth spurt. *Source*: From Ref. 77.

The mean body mass index (BMI) in TS is above age-specific norms except in early childhood, and BMI increases with age (82,86). Girls with TS have relatively large trunk, hands, and feet in relation to their height, as well as broad shoulders and pelvis (87), contributing to the short, square appearance. As a group, adults have a higher than expected prevalence of obesity, increased fat mass, and lower lean body mass (51). Consequently, TS adults are vulnerable to comorbidities, such as type 2 diabetes, hypertension, and cardiovascular risk, especially if estrogen deficiency is not adequately treated (88) (Vol. 1; Chaps. 1 and 11).

Etiology of Growth Failure

The mechanism of growth failure in TS is poorly understood, but it may be due to the combined effects of aneuploidy (89), a primary skeletal dysplasia, mild GH secretory dysfunction, and estrogen deficiency. Pathological bone development may represent either an intrinsic defect in ossification secondary to loss of critical genes on the X chromosome, such as SHOX, or alternatively might be another manifestation of intrauterine edema. However, no consistent histological abnormality has been found in bone tissue of patients with TS. Nevertheless, growth failure is obvious in most girls with TS during childhood, before apparent deficiencies in the GH/insulin-like growth factor (IGF) axis or estrogen production, implying that an intrinsic bone defect, not an endocrine abnormality, is primarily responsible for the short stature of TS.

A subset of patients with TS is GH deficient by provocative GH testing or by nocturnal GH secretion rates. An Italian study reported that 32% failed to have GH >1 mcg/l on two provocative tests and 62% had reduced spontaneous GH secretion (90). Diminished GH levels are strongly correlated with high BMI (91), accounting for much of the variation in GH testing. Furthermore, serum IGF-I levels are generally normal, even in those with diminished GH levels. IGF-I levels do fall below age-related norms in adolescence, probably reflecting estrogen deficiency, a thesis supported by an increase in GH concentrations in response to low-dose estrogen therapy (92–94). GH testing also fails to predict either pretreatment growth rate in TS or response to exogenous hGH. Accordingly, GH testing and IGF-I (or IGFBP-3) levels are not needed in the initial evaluation of TS, although intermittent IGF-I levels may be useful in determining appropriate hGH dosing throughout the course of therapy (Vol. 2; Chap. 5). GH deficiency,

as well as hypothyroidism and chronic disorder, should be considered in any girl whose height or growth velocity is clearly below normal for TS.

Cognition and Behavior

It is essential to remember that individuals with TS are not mentally retarded, as was erroneously reported in early studies. Later studies have reported an increased prevalence of retardation (full scale IQ less than 80), in addition to aggressiveness and seizure disorder, only in patients carrying a small ring X (95,96). Paradoxically, this effect is likely not due to loss of X alleles, but rather to overexpression of unidentified X genes because of either loss of XIST (the X inactivating center) or interference with normal expression of XIST in these small rings (97–99). Autism in 45,X patients has also been reported (100).

Modern neuropsychological investigations have revealed specific impairments in cognitive functioning in TS. The most commonly reported deficits relate to visual–spatial processing and visual memory, often reported as difficulties on tasks assessing spatial and numerical abilities (101–103). These deficits result in measurable reductions in performance IQ, in contrast with a normal verbal IQ, in TS children and adults. Tests of memory, executive function, and affect recognition may be impacted. These deficits are detectable in childhood and into young adulthood. TS subjects also have reduced scores on motor tests. Ross and others have observed improvement in both motor and nonverbal processing skills following the initiation of estrogen or androgen therapy (104), but not with hGH therapy. Numerous imaging studies, including functional MRI, have documented anomalies in brain morphology and function in TS subjects (105–109), establishing a sophisticated profile of the neurological correlates of cognitive and psychological dysfunction in TS. Ross has pointed out that classic TS cognitive findings are present even with small deletions of the X chromosome, for example the Xp22.3 region alone (109), showing that cognitive genotype/phenotype correlations are still poorly established.

Few studies have examined academic outcome in large numbers of subjects, but school-age academic achievement is generally within the mainstream (110), with the exception of mathematics (111). In fact, the percentage of women with TS who attend college is actually higher than in the general population. Children with TS are not disruptive in the classroom setting and so may not be referred by the schools for evaluation even if they have learning problems. Nevertheless, it is crucial that girls with TS receive ongoing educational and psychological evaluations throughout their school careers. Hearing evaluation is important as an adjunct measure to improve social and academic skills of girls with TS.

Numerous behavioral and psychosocial studies in TS children and young adults demonstrate problems in social competence (112–115). The ability to assimilate and interpret social cues is diminished. TS girls have reduced self-esteem, more internalizing behavioral problems, and fewer social interactions, and these problems may worsen at the transition to early adolescence. Physical self-image may be poor, possibly related to peer teasing. Anorexia and clinical depression have been reported. Girls may benefit from psychological counseling during childhood and adolescence. Adults with TS clearly have a high prevalence of mood disorders (116). Quality of life surveys note hearing loss and delayed initiation of estrogen, rather than short stature, as the most aggravating factors for TS adults (117,118).

Skuse et al. (119) compared neuropsychological test results in 80 45,X females aged 6 to 25 years according to parental origin of the X chromosome. In an initial Achenbach screen, the TS subjects with maternal X ($n = 55$) had much higher rates of academic failure and social difficulties than those with paternal X ($n = 25$). On further testing, subjects with an X of paternal origin had superior executive function, better social adjustment, higher verbal IQ, and better verbal memory (120). The authors attributed these findings to maternal imprinting of X chromosome loci, but these putative social and cognitive effects of parental origin of the X still need to be confirmed (121).

Autoimmunity

Several types of autoimmunity are found with increased prevalence in TS, including thyroid autoimmunity, inflammatory bowel disease, and rheumatoid arthritis. Although type I diabetes has been reported in TS, it has not been conclusively established that the incidence is greater than in controls.

Prevalence in TS of antibodies against thyroid peroxidase and thyroglobulin is 20% to 30% in several large patient cohorts (122–124) (Vol. 2; Chap. 26). As in a control population, thyroid autoimmunity increases with age from the first to third decade of life. Although occurring in patients with any karyotype, those with long arm isochromosomes are at highest risk of thyroid autoimmunity (125). Interestingly, both mothers and fathers of patients with TS have a higher than expected incidence of thyroid autoimmunity (126). This has led to the speculation that familial thyroid autoimmunity might be associated with nondisjunction or related chromosomal defects. Alternatively, maternal thyroid autoimmunity might be associated with a protection against the lethality of X chromosome monosomy in utero (127).

Hypothyroidism (or hyperthyroidism) develops in approximately 10% to 20% of TS patients. Individuals should be screened on an annual or biannual basis for thyroid disorder by measurement of TSH, and more frequently if thyroid antibodies have been detected (Vol. 2; Chap. 17). There does not appear to be an increase in polyglandular autoimmunity

associated with thyroid autoimmunity, as islet cell and adrenal antibodies are not elevated. Both ulcerative colitis and Crohn's disease have a greater than expected prevalence in TS. In a series of 135 TS adults, four had inflammatory bowel disease (128). As with autoimmune thyroid disorders, a disproportionate number of TS patients with inflammatory bowel disease have an X isochromosome (129,130). Intestinal telangiectasia and celiac disease have also been observed in TS (131). The risk of juvenile rheumatoid arthritis in TS has been estimated to be six fold increased from a survey of pediatric rheumatology centers (132). The increased risk of these autoimmune disorders in TS mandates pediatric endocrinologists to be attentive to the rheumatologic and gastrointestinal complaints of their TS patients. Although not due to autoimmunity, elevated liver enzymes and other hepatic abnormalities have been documented in TS (133,134).

Metabolic Abnormalities

An increased prevalence of diabetes mellitus in TS has been repeatedly cited, but the supportive data are weak regarding the type of diabetes. An early paper by Forbes and Engel (135) reported from a cohort of 41 TS patients that six (15%) had onset of an unspecified type of diabetes after the age of 30. The supposition has been that TS patients are prone to developing type 2 diabetes, but of interest, all of those with karyotypes reported in that 1963 paper had an X isochromosome, which suggests type 1 diabetes, because of the known propensity for other autoimmune disorders. There was no additional mention of a meaningful increase in diabetes in subsequent TS series, until a hospital records review in all Danish TS patients over a 10 year period (88) reported a higher than expected number of admissions with a diagnosis of both type 1 or type 2 diabetes. It now seems likely that TS patients have an increased risk of both disorders.

Fasting glucose levels have been consistently normal in numerous series of TS subjects. Fasting insulin levels have been reported normal or elevated, study differences plausibly attributable to the wide normal range. In contrast, an increased frequency of abnormal oral glucose tolerance tests (OGTT) has been compellingly documented in children and adults with TS, including the nonobese (136–141). A review of the literature through 1991 cited a prevalence of abnormal tests of 32.5% in 326 patients, but the reported prevalence in later studies is somewhat lower, usually in the range of 15% to 20%. The preponderance of evidence points to insulin resistance as the mechanism of carbohydrate intolerance, but some papers remark upon delayed insulin response to glucose challenge in TS (142–146).

Studying 71 TS subjects before and during hGH and/or oxandrolone therapy, Wilson et al. (137) reported that all girls had normal fasting glucose and insulin levels, both at baseline and during growth therapy. Fifteen percent had an abnormal OGTT at baseline. Integrated glucose and insulin concentrations following oral glucose challenge did not change during hGH treatment, but did rise with the addition of oxandrolone, a finding confirmed in other studies. However, other investigators have since demonstrated a rise in both fasting and glucose-stimulated insulin levels during hGH therapy, which may be dose-dependent. The Dutch hGH studies found only a 6% rate of abnormal OGTT that was not increased by hGH, but insulin levels were elevated throughout hGH therapy, albeit reversible at the cessation of treatment (139,140). Accordingly, it is prudent to survey patients for carbohydrate intolerance during hormonal therapies in TS.

Mild lipid abnormalities have been reported in TS. Total cholesterol is elevated in adolescents, but not children, and, like insulin resistance, correlates with body mass (147). hGH therapy results in a reduction in low-density lipoprotein and an increase in high-density lipoprotein (141). There is no literature to date on possible physiologic benefits of hGH therapy in adults with TS. The reported effects of therapeutic estrogen on lipid profiles have been mixed (148,149).

THE GENETIC BASIS OF TURNER SYNDROME

Phenotypic Correlations with Karyotype

The availability of chromosomal analysis in the 1960s allowed for initial attempts to correlate phenotypic manifestations of TS with the variations of X chromosomal loss (150). In his 1965 review, Ferguson–Smith hypothesized that 45,X subjects demonstrated the ''complete TS'' and that the short stature and stigmata of TS were attributable to monosomy of loci on the short arm (7). According to his review of available data, 45,X patients exhibited a higher prevalence of neck webbing, congenital lymphedema, and cardiac malformations when compared with patients with mosaicism or X structural abnormalities. Short stature was a universal feature when the short arm was missing, as in 45,X and karyotypes with long arm isochromosomes. In contrast, normal stature occurred in 20% of 45,X/46,XX, 50% of 45,X/47,XXX, and 63% of long arm deletions. Spontaneous pubertal development and menses, which occurred in only 8% of 45,X patients, were more likely in girls with 45,X/46,XX mosaicism (21%) and deletions of the short arm (25%).

Large patient series since the 1960s have corroborated Ferguson–Smith's summary. Various studies have confirmed the lower incidence of common TS features in certain mosaicisms, although many minor features occur equally in X monosomy and mosaicism. Later improvements in banding techniques and use of X chromosome probes provided a more detailed localization of X chromosome breakpoints, allowing Simpson to provide an updated review of phenotype/karyotype correlations in the late 1970s (151). Location of the breakpoint correlates poorly with the

Table 3 Frequency (%) of Phenotypic Features in Turner Adults with Complete X-Monosomy (45, X), Short Arm Deletion (46, XXp-), or Long Arm Deletion (46, XXq-)

Feature	45, X ($n = 332$)	46, XXp- ($n = 52$)	46, XXq- ($n = 67$)
Short stature	100	88	43
Gonadal dysgenesis	91	65	93
Short neck	77	38	21
Cubitus valgus	77	25	16
"Shield chest"	74	35	13
Low hairline	72	19	9
Delayed bone age	64	17	10
Pigmented nevi	64	27	19
Nail anomaly	57	8	7
Short metacarpal	55	29	12
Renal anomaly	44	8	6
Webbed neck	42	2	1
Hypertension	37	8	7
Cardiac anomaly	23	2	0
Thyroid disease	18	6	3

Source: Adapted from Ref. 152.

resulting phenotype, and any feature of TS can be seen with major deletion of either Xp or Xq. Nonetheless, there is a crude association of short arm deletions with short stature and of long arm deletions with ovarian dysgenesis. Therman and Susman reviewed the literature on phenotypes of nonmosaic adults with X long or short arm terminal deletions (152). Short stature occurred in 43% and 88% of the Xq- and Xp- cases, respectively (Table 3).

Ovarian failure, including both primary and secondary amenorrhea, occurred in 93% and 65% of Xq- and Xp- karyotypes, respectively. Essentially the entire pericentromeric region and long arm are involved in ovarian development and maintenance, as deletion breakpoints with apparently identical degrees of ovarian failure are scattered throughout. Lymphedema and cardiovascular anomalies were rare in both short and long arm deletions, compared with 45,X, although lymphedema has resulted from loss of the distal short arm of the Y in XY females.

Identification of parental origin of the remaining X chromosome in TS has been undertaken to look for clues to the etiology of X monosomy and to evaluate whether genomic imprinting might explain the phenotypic variation seen with the 45,X karyotype. Different methods have verified that the intact X in 45,X monosomy is maternal (Xm) 70% to 80% of the time (153). However, there is no difference in the prevalence of maternally or paternally imprinted X in spontaneous abortuses (154), and studies have not discerned any postnatal phenotypic distinctions, other than in the neuropsychological study described above, in 45,X subjects on the basis of parental chromosomal origin.

A model to explain the TS phenotype invokes halved expression of a gene or genes on the X chromosome; such a gene must have homologs on the X and the Y and must escape X inactivation. Although many loci on the second X are inactivated throughout all developmental phases and tissues, except in the oocyte and during initial zygotic cell divisions, many other genes which escape X inactivation have been identified in recent years, including the entire pseudoautosomal regions at both termini. Despite sophisticated comprehension of the X chromosome and the rapidly expanding list of genes that escape inactivation (155,156), there has been relatively slow progress in elucidating the genetic basis of TS since phenotype–karyotype correlations were first drawn 30 years ago.

Short Stature Homeobox

The only X chromosome locus to have been convincingly associated with TS is SHOX, for short stature homebox-containing gene, located in the pseudoautosomal region of the short arm. Isolated and sequenced independently by two groups of investigators (157,158), SHOX is highly conserved across species and has two transcripts from alternate splicing: SHOXa is widely expressed, whereas expression of SHOXb is highest in bone marrow fibroblasts. Rao et al. (157) demonstrated deletion of the locus in 36/36 short individuals with Xp22 or Yp11.3 breakpoints, and absence of a deletion in normal controls or in subjects with X or Y rearrangements and normal stature. A SHOX mutation was found in 1 out of 91 subjects with otherwise unexplained short stature, and other individuals have since been identified.

Belin et al. (159) and Shears et al. (160) reported SHOX deletions in many family members with an autosomal dominant bone dysplasia called Leri–Weill dyschondrosteosis (LWD). Thought to be more severe in females, the syndrome is characterized by a short forearm with Madelung deformity and limited mobility, tibiofibular shortening, variable metacarpal and metatarsal involvement, and mild short stature (161). In one LWD family, a fetus with Langer mesomelic dysplasia (severe shortening of distal extremities—long considered the homozygous form of LWD) had loss of both SHOX alleles. Thus, loss of one SHOX allele results in LWD, and loss of both results in Langer mesomelic dysplasia.

It was immediately recognized that the SHOX locus meets gene dosage requirements for a Turner gene, being a pseudoautosomal locus, thus not X-inactivated, with a homolog on Yp. Postnatal expression is largely confined to osteogenic cells, such as trabecular cells and bone marrow fibroblasts, correlating with a putative role for the gene in bone physiology. It is also expressed in the pharyngeal arch, suggesting a role in some head and neck anomalies of TS. Although the Turner phenotype is broader and more variable than that of LWD, it seems likely that many of the craniofacial and skeletal features of TS, including cubitus valgus and Madelung deformity, and some degree of the short stature, are due to SHOX haploinsufficiency (162). A comparison of heights of girls with LWD (-2.3 to -2.7 SDS) and TS (-2.4 to -2.7 SDS) suggests

that SHOX haploinsufficiency may account for the majority of the height deficit seen in TS (163). Ross et al. also suggest that the low prevalence of the Madelung deformity in TS may not be due to estrogen deficiency but rather loss of other nonpseudoautosomal X genes which are required for the development of the Madelung deformity. While other craniofacial and cardiac features may be a consequence of the putative lymphedema locus, SHOX is the first legitimate Turner gene to be identified.

Y Chromosome Mosaicism

X chromosomal monosomy occurs in mosaicism with 46,XY cells in 5% or more of patients with TS. The phenotype associated with the 45,X/46,XY karyotype, ranging from female to male, provides an interesting story and a cautionary tale about ascertainment bias. The phenotype has historically been described predominantly as ambiguous genitalia and mixed gonadal dysgenesis (streak gonad with dysgenetic testicular elements and possible asymmetry of Wolffian and Mullerian structures). In an early review, 60% of cases exhibited ambiguous genitalia and mixed gonadal dysgenesis, 25% were phenotypic females with bilateral streak gonads and other features of TS, and the remaining 15% had the appearance of undervirilized males (164). Approximately two-thirds of 45,X/46,XY individuals diagnosed at birth are raised as females. In contrast, 90% to 95% of the cases diagnosed prenatally have been normal phenotypic males at birth, and features of TS have been rare (165,166). These data suggest that characteristics of TS are seen less commonly in 45,X/46,XY individuals than in comparable mosaicisms, such as 45,X/46,XX. However, adequate longitudinal studies have not been performed and the incidence of TS features and gonadal dysgenesis may be higher than expected from first reports of normal male external genitalia.

The incidence of gonadal malignancy in 45,X/46,XY mixed gonadal dysgenesis engenders considerable clinical discussion about its translation to the TS patient. In Scully's series of 30 cases of gonadoblastoma, 10 were associated with 45,X/46,XY mosaicism, but most of those individuals had ambiguous genitalia (167). The risk of a patient with this karyotype developing gonadoblastoma/dysgerminoma has been estimated at 15% to 20%. However, this risk assessment was derived prior to recognition that the postnatal phenotype is usually male, and it undoubtedly overestimates the risk in 45,X/46,XY overall. Nevertheless, gonadoblastomas do occur in unvirilized phenotypic females with this karyotype, and the risk of malignancy, albeit unclear, is greater than in the general population. Assuming an increased risk of malignancy and a low likelihood of functional ovarian tissue in the presence of XY mosaicism, gonadectomy should be recommended in these TS patients, at least by adolescence and preferably in early childhood.

This recommendation has consequently raised the question whether techniques other than routine cytogenetics should be utilized to detect occult Y material in TS. Southern blot, FISH, or PCR analysis of cells from series of TS patients with prior 45,X karyotyping has revealed an incidence of previously undetected Y material ranging from 0% to 15%. Gravholt et al. (168) reported Y material in more than 10% of their TS patients, half of them not previously detected by routine cytogenetics. In a recent Italian study (169), 8% of 171 subjects had Y material detected. Of these, 4 out of 12 had a gonadoblastoma—all found at gonadectomy prior to 16 years of age, and in two of these individuals the Y material had not been detected by routine cytogenetics. FISH utilizing X- and Y-specific probes can now be performed by most cytogenetics labs and certainly should be utilized to determine the origin of marker chromosomes or small rings (170), either to warn of potential malignancy from Y genetic material or to explain unusual features of TS due to X chromosome fragments. The potential benefit of adjunct FISH or PCR studies in all TS patients is not established but warrants further evaluation.

MEDICAL THERAPIES (TABLE 4)

Androgens for Growth

Androgens were utilized for growth promotion in clinical trials in TS prior to the time that hGH became widely available. Most studies demonstrated short-term efficacy in stimulating growth, and a few reported modest increases in final adult height (171–175). Lenko et al. (172) analyzed growth and final height data in 76 girls treated with fluoxymesterone and/or conjugated estrogens and found that initial growth velocity was greatest when both were used, but mean final heights were not distinguishable. In a retrospective analysis of 66 TS patients, Sybert (83) reported that the mean adult height of patients given either oxandrolone or fluoxymesterone (148 cm) did not differ significantly from the height of untreated patients (146.3 cm). In contrast, a 3 to 5 cm increase in final height relative to predicted height was documented in three oxandrolone trials (173–175). As described below, several trials of hGH used with and without oxandrolone have shown an improvement in growth and final height from the androgens. Despite this apparent growth benefit and a relatively low cost, androgens are not commonly utilized in TS in the United States and only as an adjunct to hGH therapy. Oxandrolone has limited androgenic side effects at a dose of 0.0625 mg/kg/day and, as a rule, can be added to hGH if growth rate is unsatisfactory or if growth therapy has been delayed to a relatively advanced age (>12 years old). In support of the use of androgens in TS is the fact that serum androgens are low in TS adults because of ovarian failure (176). The potential

utility of low-dose androgen therapy to improve bone density, body composition, and sexual function in adults with TS has not been adequately evaluated.

Growth Hormone

Turner himself undertook the first documented administration of GH in TS. His treatment was unsuccessful, presumably utilizing injections of bovine pituitary extract, since human GH from cadavers was not available until the 1950s. In 1960, Escamilla et al. (177) administered pituitary-derived hGH to a patient with TS, resulting in an increase in growth velocity from 3.8 to 7.5 cm/yr over several months, a typical degree of first-year growth augmentation from either pituitary-derived or recombinant hGH, which in turn was introduced in the mid-1980s. TS patients constituted the first group after GH deficiency in which clinical trials of recombinant hGH were initiated, principally because of the straightforward identification and homogeneity of the population and also the lengthy potential treatment period, due to the absence of endogenous puberty.

hGH trials in TS have universally demonstrated an increase in growth velocity. In almost every study, hGH has augmented pretreatment growth velocity by 50% to 150% in the first year (Vol. 2; Chap. 5). Many of the early trials combined hGH with estrogens or androgens, both of which had been established as short-term growth stimulants, complicating interpretation of the results. Most early trials used three times a week dosing regimens, and until recently none of the published trials utilized a randomized control group past the first year. As in hGH treatment of GH deficiency or idiopathic short stature, growth acceleration in girls with TS is sustainable but is most pronounced in the first years of therapy (178).

An early and influential hGH trial in the United States (180) treated TS girls at a mean age of 9.3 years with hGH (0.05 mg/kg/day) with or without oxandrolone (0.0625 mg/kg/day, due to virilization in 30% of subjects at 0.125 mg in the first year of study). In subjects receiving hGH alone, growth velocity increased from 4.5 cm/yr in the pretreatment period to 6.6 and 5.4 cm/yr in the first and second year, respectively, whereas growth rates on hGH plus oxandrolone were greater at 9.8 and 6.7 cm/yr. Compared with a mean adult height of 144.2 cm in historical controls, final heights after hGH and hGH plus oxandrolone were 150.4 and 152.1 cm, respectively, for gains over pretreatment projected height of 8.4 and 10.3 cm, respectively. A Swedish study (181), also using combination daily hGH and oxandrolone, began at an older mean age of 12.2 years and had no hGH-alone arm. Growth velocity increased from 3.9 to 9.4 and 6.8 cm/yr in the first two years of therapy. Final height after combined hGH and oxandrolone therapy was 154.2, 8.5 cm greater than the original projected final height.

Results of the Swedish, United States, and other early studies convinced most investigators that hGH augmented final height in TS, but some doubts persisted because of the lack of long-term randomized controls. This last concern was resolved with results of a randomized Canadian study (182) using a concurrent untreated control group for the duration of the study. Preliminary results were initially presented at the Food and Drug Administration hearings prior to approval of hGH for use in TS in 1996. In recent long-term results from this study, the mean difference in final height between the treatment and the control groups was 7.2 cm, convincing the remaining doubters that hGH is efficacious in TS.

A significant gain in ultimate height is now beyond controversy, but the expected magnitude of the increase is still being revised as further studies are reported. Key questions of optimal age of initiation and optimal dose have not been completely resolved. However, it is likely not a coincidence that the greatest height gains have been reported from studies using relatively early starting ages. The TS subjects in the U.S. study (180) gained about 8 cm from hGH alone after beginning therapy at nine years old. A recent Italian tabulation of 60 TS girls, with a mean hGH starting age of 10.8 years and not adding estrogen until 15 years, reported a mean final response of 8.2 cm over pretreatment predicted height (183). In general, the height gain when age at initiation of hGH is 11 to 13 years has been 6 cm or less, depending upon the use of androgen and estrogen. In the large Genentech hGH database, 622 TS patients treated for a mean 3.7 years beginning at 12.9 years of age had a mean final height of 148.3 cm, for an increase of 6.4 cm over pretreatment projected height (184). Mean height gain was 3.7 to 4.7 cm in 136 girls with a similar late start in a European Lilly study (185). Thus, age of diagnosis and initiation of hGH are important for final outcome.

In a provocative Dutch study (186,187), 68 girls were begun on 0.045 mg/kg/day, then randomized to stay on that dose or to be increased in subsequent years to 0.0675 or 0.090 mg/kg/day. No estrogens were given until four years of hGH therapy had elapsed and the subject reached 12 years of age. Final adult height in 60 of the 68 subjects showed that 85% had achieved a height within the normal range for Dutch girls. After a mean of 8.6 years of hGH therapy, final adult height was obtained at a mean age of 15.8 years. The mean heights for the three dose groups were 157.6, 162.9, and 163.6 cm, respectively, with height gains of 11.9, 15.7, and 16.9 cm, respectively. The height gain achieved with hGH alone at the standard dose, far superior to that of any other hGH study in TS, is plausibly attributable to earlier initiation of therapy, averaging 6.6 years old, plus the standardized delay of estrogen. Girls with TS clearly respond to hGH started even earlier in childhood: in a randomized, controlled study of hGH in 88 TS girls between the ages of nine months and four years at entry, the between-group height difference was 1.6 SDS after two years of therapy (188). However, it is unknown

whether this early start contributes significantly to final height.

Additional height achieved in the Dutch study can be attributed to the higher doses of hGH in later years of therapy. In a smaller, nonrandomized study, Carel et al. (189) also progressively increased the hGH dose, to a maximum of 0.1 mg/kg/day when growth velocity declined to less than 200% of the pretreatment level. Height gain was 10.6 cm, compared to 5.2 cm in a matched group of 17 patients who had received a fixed dose of approximately 0.045 mg/kg/day. Thus, it is possible that TS patients could benefit from hGH doses in later years of therapy in excess of the current standard of 0.05 mg/kg/day.

In a long-term Japanese study (190), a standard dose of hGH, as compared with a low dose, stimulated a greater growth velocity for two years, but reported final heights in the two groups were not different. Thus, initial growth rate is likely dose-dependent, but it is unclear whether final height can be significantly improved using higher doses of hGH from the start. In approaching that question, a large multicenter, randomized dose response study in the U.S. randomized 232 girls with TS to either 0.27 or 0.36 mg/kg/wk hGH in conjunction with either low-dose ethinyl estradiol (EE) or placebo (191). Mean near final height has been reported for 99 girls whose age at baseline was 10.9 years and at endpoint was 16.4 years. Mean near final height for those receiving the lower hGH dose with estrogen was 145.1 cm (149.9 cm without estrogen), whereas mean near final height for those receiving the higher hGH dose with estrogen was 149.1 cm (150.4 cm without estrogen). The higher hGH dose resulted in significantly greater near final height (SD 1.0 ± 0.1 vs. 0.6 ± 0.1).

Many studies have attempted to predict individual response to hGH. In an Australian study, the best response over two years occurred in younger, heavier girls with the most delayed bone age and tallest parents (192). In a British study of 52 girls who were prepubertal at start of therapy, final height gain after 5.8 years averaged 5.2 cm and correlated best with total dose, duration of therapy, and first year response (193). In a model developed by Ranke et al. from the Pharmacia database (KIGS) (194), hGH dose was the best predictor of height velocity in year one of therapy, with younger age and oxandrolone use associated with higher growth velocities in subsequent years. However, some of these factors may influence only short-term benefit and not result in gain in final height (195). Factors that were found to influence the final height outcome in the Lilly U.S. trial included the higher hGH dose, younger age at initiation of hGH, lower bone age/chronological age ratio, lower body weight, and greater height SDS at initiation of therapy (191). The French StaTur Study followed 704 patients to final height. The mean adult height gain was 8.5 cm, and factors which accounted for final height variance included: age at initiation of treatment, duration of treatment, age at onset of puberty, and hGH dose and number of injections per week (196). The common theme in these results is the need for early initiation of hGH and substantial duration of therapy at a reasonably aggressive dose.

Estrogen

Estrogen replacement was also tried historically as a growth therapy in TS. While estrogen does result in short-term growth acceleration, most studies have failed to document any improvement in final or even predicted adult height, in contrast with androgens or hGH. Indeed, because estrogen is the primary mediator of skeletal maturation, it accelerates short-term growth while abbreviating the duration of growth, as in normal puberty. Not surprisingly, several early TS studies, therefore, demonstrated that full estrogen replacement during early adolescence resulted in final heights below the expected height in TS. Investigators then attempted to identify a reduced dose of estrogen that might be an effective long-term growth stimulant without causing accelerated skeletal maturation. Significant short-term growth stimulation from low-dose EE was demonstrated, but unfortunately it does not occur without accompanying effects on bone maturation, particularly in younger patients with bone age less than 11 years (197,198). As a result, there is no perfect dose of estrogen adequate to achieve a normally timed feminization without inducing an acceleration of bone age and potential reduction in adult height.

Although low- or full-dose estrogen replacement given alone provides a notable growth spurt, little additive growth is observed when estrogen is started during hGH therapy (199,200). The estrogen-induced advance in skeletal maturation without benefit of substantially greater growth velocity means that early introduction of estrogen therapy can negate gains achieved by hGH therapy. This has now been confirmed in several studies. In the Swedish hGH study mentioned earlier (181), height gain from the combination of hGH and oxandrolone was 8.5 cm; addition of a relatively low-dose of EE (100 ng/kg/day) to this regimen at 12 years old resulted in a mean height gain of only 3.2 cm. A second Genentech study (201) randomized 60 girls on hGH from a mean of 9.5 years old to begin estrogen at either 12 or 15 years old (Fig. 5). Gains in final height were 5.1 and 8.4 cm in the two groups, respectively.

The U.S. multicenter trial mentioned above showed that the concomitant use of low-dose oral EE with hGH resulted in a 4.8 cm decrease in final adult height when hGH dose was 0.27 mg/kg/wk and a 1.3 cm decrease in final adult height when hGH dose was 0.36 mg/kg/wk (191). Thus, an effective long-term growth regimen requires reasonably early initiation of hGH, or alternatively a lengthy delay in estrogen initiation. Interestingly, the French StaTur Study found that use of transdermal estrogen

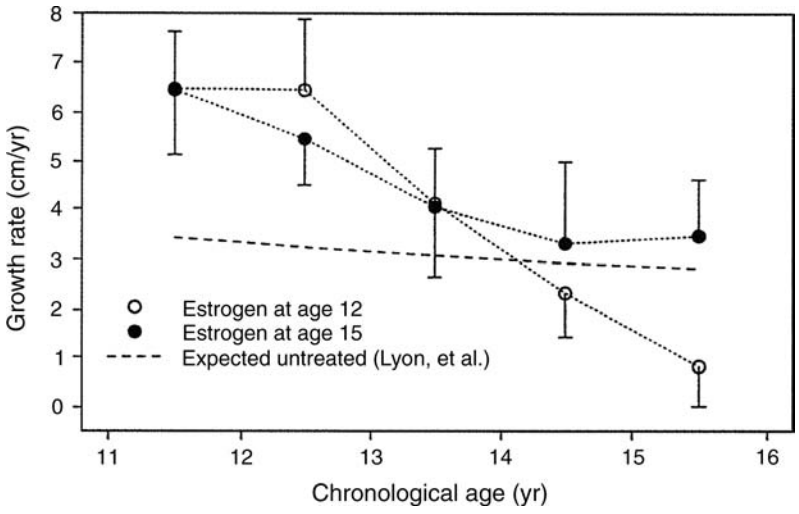

Figure 5 Differences in annual growth velocity after TS girls on hGH begin estrogen at either 12 or 15 years old. *Source*: From Ref. 201.

to induce puberty was associated with a 2.1 cm increase in final height (196). Although they were not able to show that the association was causal, the use of transdermal estrogens for induction of puberty in TS does bear further investigation.

Although there are theoretical concerns that absence of the normal low prepubertal levels of estrogen might contribute to the growth failure seen in TS, estrogen therapy is contraindicated in preadolescent patients, plays no legitimate role for the moment in growth therapy in TS, and, as a corollary, must be delayed for feminization until certain growth thresholds are achieved. The apparent estrogen-induced acceleration in skeletal age jeopardizes the primary objective of hGH therapy. However, the practice of delaying estrogen replacement until 15 years or older in an effort to maximize growth, which was the clinical practice prior to introduction of hGH, guarantees significant delays in pubertal development, risks a lifelong diminution of BMD, and potentially accentuates the social isolation and stigmatization of girls with TS. A prudent and sympathetic approach is to customize therapy according to the current height, hGH history, and psychological needs of each patient. At a minimum, estrogen should be delayed until 12 years old, but only if a girl has received hGH for three years or more. In those with a brief duration of hGH therapy, estrogen may need to be delayed until 13 or even 14 years old, and oxandrolone could be considered as an adjunct therapy.

Balancing the legitimate psychological and physiological need to begin pubertal development against concerns about final height, our typical practice is to begin conjugated estrogens (Premarin) at about 13 years old at an initial dose of 0.3 mg/day, increasing to 0.625 mg/day 6 to 12 months later. Approximately two-thirds of girls will achieve Tanner 3 breast stage after 12 months. After one to two years of therapy, estrogens are modified to day 1 to 26 only and medroxyprogesterone acetate 5 to 10 mg (Provera) is added on days 17 to 26, in order to induce menses and diminish the risk of endometrial hyperplasia or carcinoma. Other estrogen preparations, such as oral EE (starting at 100 ng/kg/day or less) or 17β-estradiol, as well as dermal patches or percutaneous estrogen gel, are also effective in feminization (202,203). Some practitioners believe oral estrogens have more deleterious effects upon hepatic metabolism than transdermal preparations, but the relative effects are still unclear (204).

Few direct comparisons of different estrogens for feminization have been made. Ironically, most studies of estrogen in TS have concentrated upon the objective of growth stimulation and have failed to provide detailed analysis of the feminizing effects. Once progesterone has been added, many TS adolescents and adults prefer oral contraceptive

Table 4 Management of TS in Childhood

Medical surveillance
Karyotype: rule out Y material
Renal ultrasound
Echocardiography (repeated in adolescence)
Regular thyroid screening
Hypertension monitoring
Ophthalmology (strabismus, etc.)
ENT (recurrent otitis) and craniofacial
Possible orthopedic referral (scoliosis)
Audiologic evaluation
Cognitive evaluation
Psychological counseling
Dietary advice
Support groups
Medical therapy
Begin hGH therapy (0.05 mg/kg per day) by 7-9 years old; earlier treatment may be indicated
Add oxandrolone (0.0625 mg/kg per day), if hGH is started late
Initiate estrogen at ± 13 years old
Cycle with progesterone within 2 years

preparations for convenience. This issue should be discussed as one of the many topics in maintaining adequate medical care as TS patients move into adulthood (Table 4) (205–208).

Summary of Medical Therapy in Turner Syndrome

The height deficit in TS is approximately 20 cm. It is tempting to conclude from informal meta-analysis that the portion of the height deficit remediated by hGH depends upon the age therapy is initiated. The expected height gain when hGH is begun at 12 may be only 5 cm, whereas hGH therapy begun at seven years of age could add in excess of 12 cm. It is therefore prudent to initiate hGH by seven years old, or earlier if a girl is in the lowest portion of the TS height curve. Better height gains may be achieved by even earlier therapy. Although no data are currently available to prove greater efficacy from an earlier starting age, growth failure is apparent in the first years of life in TS. The standard hGH dose is 0.05 mg/kg/day, but higher doses may be justified in later years of therapy or as growth velocity or IGF-I levels subside. One approach to dosing is to maintain IGF-I levels in the high normal range (Vol. 2; Chap. 5). We do not routinely attempt to diagnose GH deficiency in TS, unless height is well below that expected from parental height. The optimal time to discontinue hGH has not been established, but convention calls for continued therapy until growth is largely completed, as evidenced by a velocity below 2.5 cm/yr. Estrogens are not beneficial for long-term growth and may be deleterious for final height if started too early; accordingly, estrogen for feminization should be delayed until 13 years old, preferably after several years of hGH therapy. In tall girls or in those with lengthy duration of hGH treatment, earlier initiation of estrogen may be possible.

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13

Hirsutism and Polycystic Ovary Syndrome

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INTRODUCTION

Hirsutism, menstrual disorders, acne, and chronic anovulation are symptoms typically associated with disorders affecting androgen secretion and metabolism in adolescent girls (Table 1). In prepubertal children, androgen excess is associated with premature pubarche, which is defined as the development of pubic hair, axillary hair, acne, and/or adult-type apocrine odor earlier than eight years in girls and nine years in boys. Hyperandrogenemia is generally due to excessive androgen secretion by the adrenal cortex, ovaries, or testes. Additional manifestations of hyperandrogenemia include menstrual disorders, clitoral enlargement, masculine body habitus, male-pattern baldness, voice changes, and breast atrophy. The magnitude of the clinical features of androgen excess does not always correlate with circulating androgen concentrations.

The adrenal cortex secretes mineralocorticoids, glucocorticoids, and the adrenal androgens (Vol. 2; Chap. 8). The adrenal cortex is composed of three zones. The outer zona glomerulosa synthesizes aldosterone and is principally regulated by renin and angiotensin. The middle zona fasciculata synthesizes cortisol. The inner zona reticularis secretes the adrenal androgens, dehydroepiandrosterone (DHEA), its sulfated form (DHEAS), and androstenedione. The ovary and testes, respectively, synthesize and secrete the sex steroids, estrogens and androgens. The theca cell of the ovary synthesizes androstenedione, which serves as the substrate for estradiol biosynthesis in the granulosa cell. The Leydig cells of the testes synthesize testosterone. This chapter reviews the functional genomics of androgen metabolism and disorders of androgen metabolism in young women.

STEROIDOGENESIS

Androgens

Androgens are C-19 steroids secreted by the adrenal glands, ovaries, and testes. Androgenic potency depends on the presence of a ketone group (=O) or a hydroxyl group (–OH) at position 17. DHEA and Δ^4-androstenedione possess a keto group whereas testosterone and dihydrotestosterone (DHT) possess a hydroxyl group at C17. Only testosterone and DHT bind directly to the androgen receptor (AR). DHEA, DHEAS, and Δ^4-androstenedione are inactive precursor steroids capable of conversion to more potent androgens and estrogens in peripheral tissues such as liver, adipose tissue, and other target organs. Tissue-specific enzyme expression modulates steroid biosynthesis and intracellular steroid concentrations (1).

The human fetal adrenal cortex synthesizes large quantities of DHEAS which serve as the precursor for placental estrogen biosynthesis. During childhood, DHEA and DHEAS concentrations are low. Beginning between six and eight years of age, adrenarche characterized by increased DHEA and DHEAS secretion occurs. The physical manifestations of adrenarche include the development of pubic and axillary hair, apocrine body odor, and acne. Peak DHEAS concentrations occur between 20 and 30 years of age; subsequently DHEAS concentrations decline (2). In young men and women, plasma DHEAS concentrations are significantly greater than testosterone and estradiol. Adrenarche occurs independently from puberty; puberty being defined as reactivation of the GnRH pulse generator leading to increased pituitary gonadotropin secretion and ultimately to reproductive competence (Vol. 2; Chap. 11).

Regulation of Steroidogenesis

Steroidogenesis is governed by the activity of the hypothalamic–pituitary–target organ axis. For gonadal steroidogenesis, the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and their specific receptors play crucial roles. The ovary consists of two compartments surrounding each follicle: theca cells and granulosa cells. LH stimulates the theca cells to synthesize androstenedione, which subsequently diffuses into the granulosa cells. Within the granulosa cells, FSH induces expression of aromatase, which catalyzes the conversion of androstenedione to estrogens.

Table 1 Causes of Hyperandrogenism in Peripubertal and Postpubertal Females

Adrenal
Congenital adrenal hyperplasia
Cushing's syndrome
Adrenal tumor
Inherited glucocorticoid resistance
Bilateral adrenal dysfunction
Ovarian
Polycystic ovary syndrome
Ovarian tumor
Gonadal enzyme deficiencies
Aromatase deficiency
Aberrant gonadal differentiation

The carefully orchestrated reciprocal relationships between the hypothalamus, pituitary, and ovary are crucial for regulation of the menstrual cycle and reproductive success. Through negative feedback inhibition, the hypothalamic–pituitary–adrenal axis governs cortisol secretion by the adrenal cortex. A negative feedback regulatory pathway governing adrenal androgen production has not been identified.

The first step in steroidogenesis is the binding of trophic hormone ligand to its cognate cell surface G-protein-coupled receptors. Following ligand binding and conformational changes in the transmembrane domain, intracellular cAMP levels rise and protein kinase A is activated leading to phosphorylation of specific proteins such as cholesteryl ester transferase. Trophic hormone stimulation maintains transcription and translation of the steroidogenic enzymes in the adrenals and gonads. Adrenocorticotropin (ACTH) provides trophic stimulation to the adrenal cortex, LH provides trophic stimulation to ovarian theca cells and testicular Leydig cells, and FSH provides trophic stimulation to ovarian granulosa cells. For the LH receptor, ligand binding also activates protein kinase C (PKC) leading to activation of the downstream PKC pathway.

Steroidogenesis

Starting from the cholesterol molecule, steroid biosynthesis involves a series of sequential modifications occurring in multiple subcellular compartments (Fig. 1). Cholesterol can be acquired from circulating lipoproteins or synthesized de novo. From the cytosol, steroidogenic acute regulatory protein (StAR) promotes transport of cholesterol across the outer mitochondrial membrane. During this rate-limiting step in steroidogenesis, the 37-kDa StAR precursor protein is converted to a 30-kDa mature form after uptake and processing by mitochondria (3). In the mitochondria, the cholesterol side chain cleavage (P450scc) enzyme encoded by CYP11A1 converts cholesterol to pregnenolone.

Through the actions of P450–17α-hydroxylase/17,20-lyase (P450c17) encoded by CYP17, pregnenolone is sequentially converted to 17-hydroxypregnenolone and DHEA. The 17,20-lyase activity is influenced by the presence of cytochrome b_5 and post-translational modifications. Cytochrome b_5 is a membrane-bound electron transfer protein. By forming a complex with P450c17 and oxidoreductase, cytochrome b_5 appears to act as a "switch" to promote 17,20-lyase activity (4). In vitro, cAMP-mediated serine phosphorylation of P450c17 increases the lyase activity illustrating the possible role of serine phosphorylation to modulate lyase activity (5). In the adrenal, DHEA is converted to DHEAS through the actions of DHEA sulfotransferase encoded by the SULT2A1 gene.

The 3β-hydroxysteroid dehydrogenase (3β-HSD) type 2 enzyme, encoded by HSD3B2, converts the Δ^5 steroids, pregnenolone, 17-hydroxypregnenolone, and DHEA, to the respective Δ^4 steroids, progesterone, 17-hydroxyprogesterone and androstenedione in the adrenal cortex, the ovary, and the testis. The 3β-hydroxysteroid dehydrogenase (3β-HSD) type 1 enzyme encoded by HSD3B1 is expressed primarily in skin, placenta, and adipose tissue. In the ovary, androstenedione is synthesized in the theca cells

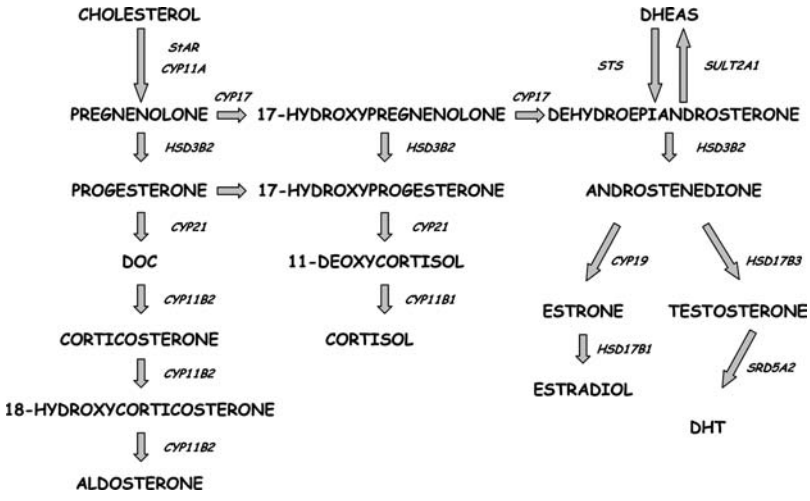

Figure 1 Steroid hormone biosynthesis. Steroidogenic enzymes and intermediates for adrenal, testicular, and ovarian steroidogenesis are shown. *Abbreviations*: 3β-HSD2, 3β-hydroxysteroid dehydrogenase type 2; 17β-HSD1, 17β-hydroxysteroid dehydrogenase type 1; 17β-HSD3, 17β-hydroxysteroid dehydrogenase type 3; CYP11A, cholesterol desmolase; CYP11B1, 11β-hydroxylase; CYP11B2, aldosterone synthase; CYP17, 17α-hydroxylase/17,20-lyase; CYP19, aromatase; CYP21, 21-hydroxylase; StAR, StAR; SULT2A1, DHEA sulfotransferase; STS, steroid sulfatase.

and diffuses to the granulosa cells where aromatase (P450arom), encoded by CYP19, converts androstenedione to estrone. Expression of cell-specific aromatase transcripts in placenta, adipose tissue, skin, and brain is governed by different promoters. In the gonads, the final steps in sex steroid biosynthesis are catalyzed by members of the 17β-hydroxysteroid dehydrogenase (17β-HSD) enzymes. The type 1 17β-HSD converts estrone to estradiol in granulosa cells. In Leydig cells, the type 3 17β-HSD catalyzes the conversion of androstenedione to testosterone. The type 5 17β-HSD is expressed in the ovary and other peripheral tissues where it converts androstenedione to testosterone.

Following secretion, most circulating sex steroid hormones are bound to a carrier protein, sex hormone-binding globulin (SHBG) which is synthesized in the liver. The free or unbound hormone is the biologically active form. SHBG concentrations vary inversely with androgen and insulin concentrations. Increased SHBG concentrations occur with pregnancy, exogenous estrogen treatment, and hyperthyroidism. In androgen target tissues, testosterone is converted to DHT by 5α-reductase. Two different genes, SRD5A1 and SRD5A2, encode the type 1 and type 2 5α-reductase isozymes, respectively. The type 1 isozyme is expressed in the skin and the type 2 is expressed in the prostate and male genital structures. Androgen effects in skin are also modulated by the cutaneous expression of 17β-HSD.

Clinical investigations and laboratories studies have confirmed that insulin promotes steroidogenesis. Treatment of the polycystic ovary syndrome (PCOS) demonstrates the importance of insulin action in steroidogenesis. Lowering insulin concentrations by weight loss or pharmacologic agents lowers circulating androgen concentrations (6,7). Insulin acts through its cognate receptor; the downstream pathway affecting 17,20-lyase activity for ovarian steroidogenesis appears to involve the phosphatidylinositol-3-kinase pathway (8–10).

Steroid Hormone Metabolism

Several mechanisms exist to inactivate steroid hormones. For androgens, the 3α-HSD isozymes convert potent androgen to less active metabolites. The hepatic 3α-HSD inactivates DHT by converting it to 3α-androstanediol. Sulfotransferases catalyze steroid sulfation. Estrogen sulfotransferase (SULT1E1) encoded by the STE gene is expressed in liver, adrenal, kidney, muscle, fat, and uterus where it inactivates estrogen by conversion to estrogen sulfate.

Steroid sulfatase, encoded by the STS gene located on the short arm of the X chromosome, cleaves the sulfonate function on the steroid molecule. Placental expression of this enzyme plays a major role in placental estrogen biosynthesis. Loss of function mutations in the STS gene are associated with impaired placental estrogen biosynthesis and congenital X-linked ichthyosis; this disorder affects one in 2000 to 6000 liveborn males (11). Despite the hypothesis that DHEAS is readily converted to DHEA, recent data suggests that there is minimal hepatic conversion of DHEAS to DHEA. Rather, DHEA sulfotransferase rather than steroid sulfatase activity appears to regulate DHEA bioavailability (12).

Another mechanism for inactivation of steroids involves glucuronidation. Glucuronidation mediated by the UDP-glucuronosyl transferase (UGT) enzymes increases the water solubility to enhance urinary excretion of steroids. There are two families of UGT enzymes. The UGT1 enzymes are encoded by a single gene, give rise to alternatively spliced transcripts, and preferentially act on estrogens. The UGT2 enzymes consist of two subgroups, UGT2A and UGT2B. Multiple UGT2B genes and enzymes have been identified; each has different steroid substrate specificity. The proteins encoded by the UGT2B15 and UGT2B17 genes preferentially glucuronidate androgens (13).

The circulating concentration of a steroid is governed by rate of secretion and rate of tissue uptake and metabolism. Sampling venous effluent over time has been used to calculate secretion rates. Metabolic clearance rate is defined as volume of blood cleared of hormone in a specific time period.

Androgen Receptor

The androgen receptor (AR) is a ligand-dependent transcription factor. The AR encoded by the AR (AR) gene is comprised of four functional domains, the N terminal transactivation, DNA binding, hinge, and ligand binding domains. The gene is located on the proximal long arm of the X chromosome at Xq11 to Xq12. Three microsatellite trinucleotide repeats are located in exon 1 of the AR gene. The highly polymorphic CAG repeat, encoding a polyglutamine region shows much variability. Transcriptional activity varies inversely with the length of the CAG repeat (14). In the absence of ligand, the AR is located in the cytoplasm of its target cells in association with specific chaperone proteins. In the presence of ligand, the receptor assumes a different conformation, dimerizes, and moves into the nucleus where it binds to DNA response elements.

PUBERTY: ADRENARCHE AND GONADARCHE

Adrenarche is a phenomenon limited to humans and a few non-human primates (15). No apparent changes in cortisol or ACTH concentrations have been identified at adrenarche (16). At adrenarche, expression of 3β-HSD decreases while cytochrome b_5, cytochrome P450 oxidoreductase, and DHEA sulfotransferase increase (17). These changes favor the 17,20-lyase activity of P450c17 to promote conversion of 17-hydroxypregnenolone to DHEA and DHEAS. Although the findings have been inconsistent, clinical studies suggest that insulin, IGF-I, and GH concentrations influence the timing, onset, and progression of

adrenarche (18). Comparison of IGF-I concentrations among prepubertal children have shown higher concentrations in African American children (19). Nevertheless, the molecular mechanisms responsible for the onset of adrenarche continue to evade clarification.

To date, the hormone responsible for regulation of adrenal androgen biosynthesis remains to be identified. GATA-6 is a transcription factor found in the adrenal cortex, which influences transcription of steroidogenic enzymes including DHEA sulfotransferase (20,21).

EXCESSIVE BODY HAIR

Hirsutism

The medical definition of hirsutism is the presence of excessive terminal hair growth that appears in a male pattern (i.e., sexual hair) in women (22,23). Hirsutism presents as the growth of coarse terminal hairs in androgen-dependent areas in a female, represents the relative sensitivity of the hair follicle to androgen exposure, and is a common clinical manifestation of hyperandrogenemia. The modified Ferriman–Gallwey score provides a semi-subjective method to quantitative the extent of hair growth in nine androgen-dependent areas such as the the mustache area, chin, upper chest, abdomen, and back (24). Although a Ferriman–Gallwey score of 8 or more is usually considered to indicate hirsutism, variation occurs between ethnic groups. The prevalence of hirsutism varies between 2% to 8% depending on the scoring criteria used with no significant difference between whites and blacks (25). The Ferriman–Gallwey scoring system loses value following cosmetic treatments. Virilization and masculinization are terms used to describe the presence of more severe symptoms of androgen excess. Specifically, these terms refer to the presence of clitoromegaly, masculine body habitus, male-pattern hair loss, and voice changes. Menstrual irregularities may also occur.

Physicians often consider hirsutism as a purely cosmetic problem and may fail to asses potential hyperandrogenic causes. Conversly, expensive laboratory assessments may be performed that are not necessary for diagnosis.

Pathogenesis

During gestation, the precise distribution of the approximate 5 million hair follicles is established

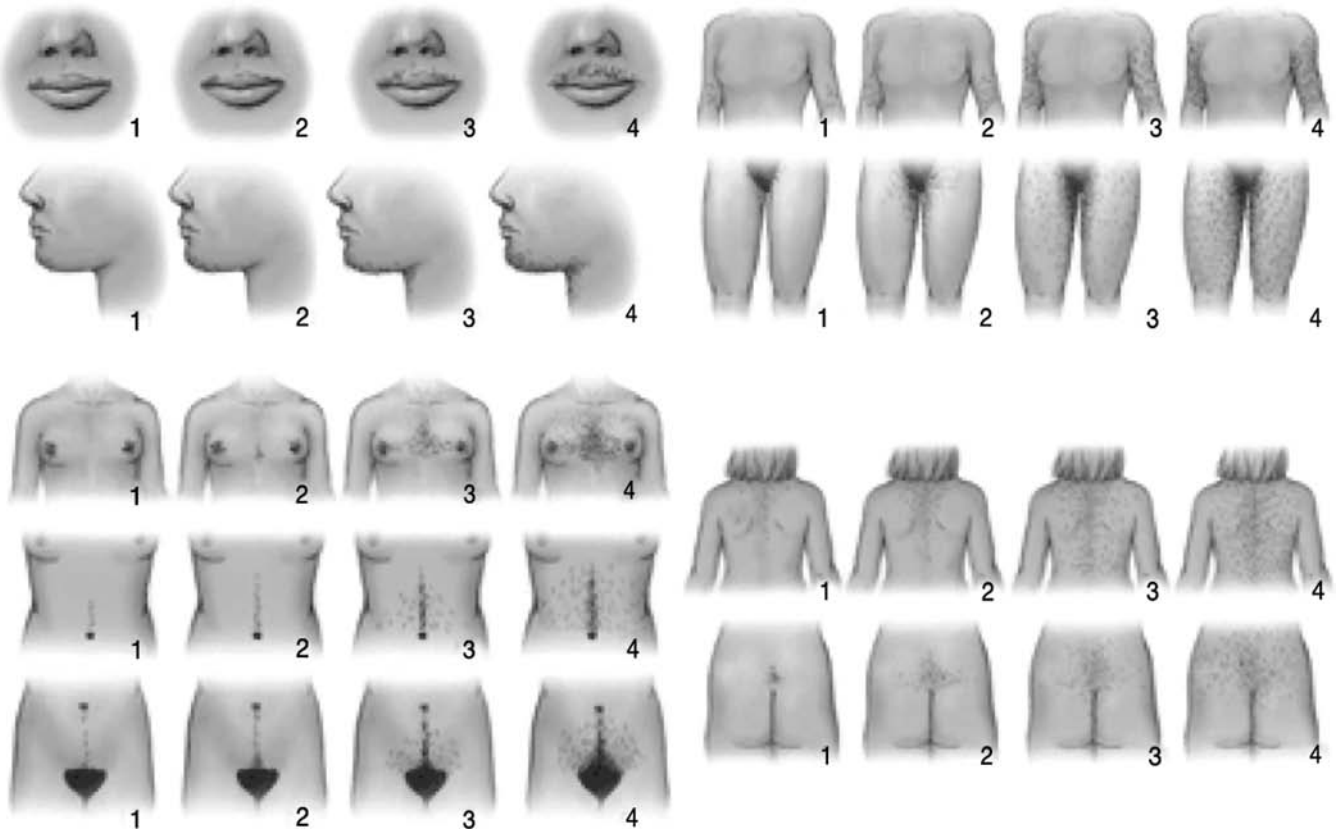

Figure 2 The Ferriman–Gallwey Scoring System for hirsutism. Each of the nine body areas most sensitive to androgen is assigned a score, from 0 (no hair) to 4 (frankly virile), and these are summed to provide a hormonal hirsutism score. *Source*: Adapted from Ref. 24.

over the body and most people lose 50 to 150 scalp hairs each day (27). Hair follicles contain stem cells that migrate to the hair matrix for division and differentiation. Hair follicles undergo several phases including telogen (resting/shedding), anagen (growth), and catagen (apoptosis-driven regression) (Fig. 3). The dermal papilla secretes IGF-1 and fibroblast growth factor 7 (FGF7); both factors influence hair follicle development and cycling (28). Hair length is proportional to the duration of the anagen phase. On the scalp with longer, denser hair, there are more hair follicles in the anagen stage (22). Cessation of the anagen phase is modulated by FGF5 (29).

Lanugo hairs are long, unmedullated hairs that grow during fetal life and are shed during the first few post-natal months of life (30). During the prepubertal years, sexual hair is small, straight, and light in color and is considered to be vellus. Vellus hairs are produced by follicles that penetrate only into the papillary dermis (31). The sebaceous glands associated with androgen-sensitive hair follicles are small. Hair growth on the face, neck, trunk, pubic, and axillary regions are influenced by androgens. With androgen exposure, vellus hairs become longer, darker, and curlier and are considered to be terminal hairs. Estrogens prolong the anagen phase.

The growth of sexual hair is dependent on the action of androgens on the hair follicles and the sebaceous glands. Testosterone and DHT act through ARs in the dermal papilla to increase hair follicle size. Under their influence vellus follicles develop terminal hairs that are larger, curlier, and darker, thereby they become more visible. Almost paradoxically, excess androgens can cause miniaturization of follicles leading to male-pattern baldness. Studies in normal skin and in acne-prone skin have demonstrated expression of the type 1 5α-reductase in the sebaceous gland and of the type 2 5α-reductase in the companion layer of the hair follicle (32). Skin has the capacity to synthesize sex steroid de novo from cholesterol as well as to interconvert specific steroids (33).

The degree of hirsutism is not directly related to the circulating levels of androgens. Moreover excess sexual hair may or may not be accompanied by skin

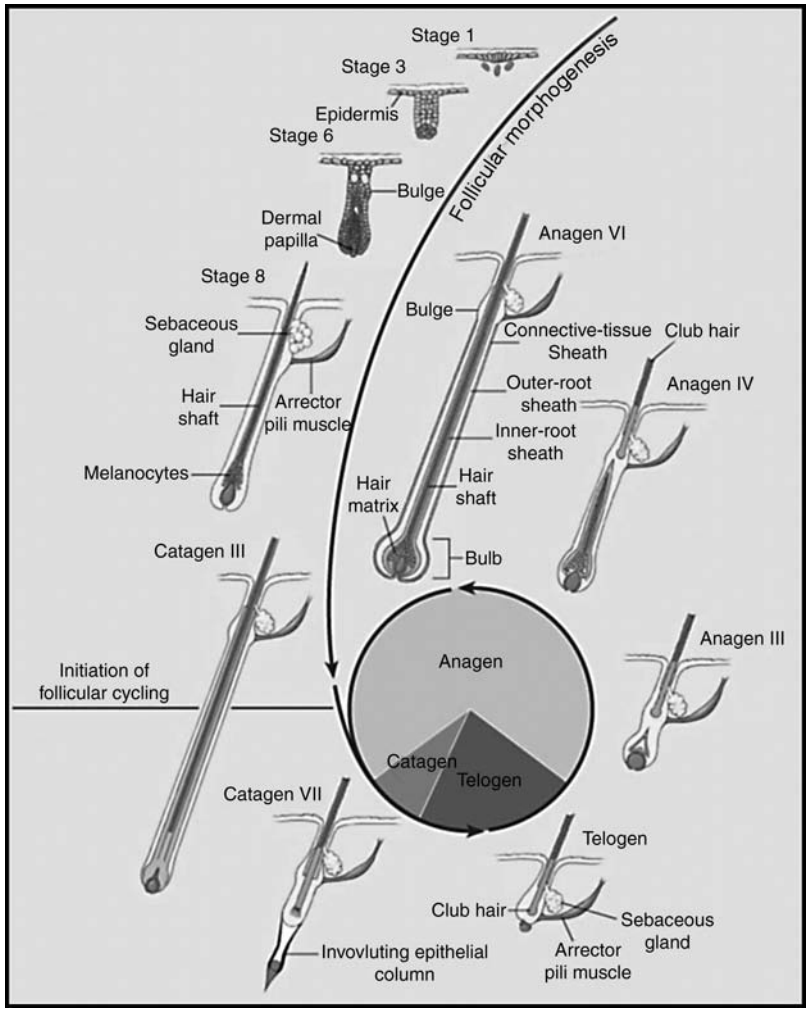

Figure 3 Development and cycling of hair follicles. Selected stages of the morphogenesis of hair follicles and the three stages of follicular cycling (anagen, catagen, and telogen) are shown. The roman numerals indicate morphologic substages of anagen and catagen. The pie chart shows the proportion of time the hair follicle spends in each stage. *Source*: Adapted from Ref. 27.

manifestations such as seborrhea, acne, or alopecia as the sebaceous glands may also vary in their sensitivity to the hyperandrogenemia. Testosterone is the main circulating androgen (34–37). It arises as a by-product of ovarian and adrenal function, either by secretion or by the metabolism of secreted prohormones (mainly androstenedione or DHEAS) in peripheral tissues, such as fat (24,38). Testosterone levels are slightly lower in the premenstrual phase and slightly higher in midcycle (39), during the midfollicular phase of the menstrual cycle the levels vary by about 25% above and below the mean and are highest in the early morning.

Free testosterone seems to be the main bioactive portion of plasma testosterone (40,41). The level of free testosterone is often elevated when the total testosterone level is normal in hirsute women. This reflects the relatively low levels of SHBG in such patients, which determines the fraction of plasma testosterone that is free or bound to albumin (42). Levels of SHBG are suppressed by the hyperinsulinemia and by androgen excess itself (40,43), so that the total testosterone level may be normal despite elevated androgen levels. The level of SHBG is also low in persons with hypothyroidism; rarely, it is congenitally absent (44).

Differential Diagnosis

The reason for the hirsutism can be distinguished between the various causes such as hypertrichosis and polycystic ovary syndrome (PCOS) (37,45). Approximately half of women with mild hirsutism with a score of 8 to 15 on the Ferriman–Gallwey scale have the idiopathic form of this condition (34), described below; whereas in the remaining of these women and in most of those with more marked hirsutism, elevated androgen levels are usually found. Hyperandrogenism is most often caused by the presence of PCOS (46). However, many of the cases of hirsutism due to PCOS are atypical in that the patients lack some of the important features usually associated with the syndrome, i.e., menstrual irregularity, polycystic ovaries, and obesity (46,47). Thus the absence of some of such features in a hirsute patient does not rule out the diagnosis of PCOS, but if risk factors such as obesity and menstrual irregularity are present, even mild degrees of excess sexual hair growth are usually associated with androgen excess (48).

There are other more infrequent causes of hirsutism that need to be considered. Non-classic congenital adrenal hyperplasia is present in only 1.5% to 2.5% of women with hyperandrogenism (37,45) (Vol. 2; Chap. 9). Androgen-secreting tumors are present in about 0.2% of women with hyperandrogenism (37); more than half of such tumors are malignant (49). Cushing's syndrome, hyperprolactinemia, acromegaly, and thyroid dysfunction must be considered as causes of androgen excess, but these conditions usually present because of symptoms other than hirsutism. About 8% of women with hirsutism have mild, often asymptomatic, idiopathic hyperandrogenism (23) due to abnormal peripheral metabolism of prohormones. Androgenic medications also may cause hirsutism. The medical history and physical examination often clarify the risk factors associated with virilizing disorders, PCOS, other endocrinopathies. The assessment of the use of androgenic medications is particularly important in ascertaining the etiology of hirsutism, as most such drugs are not detected by testosterone assays, with the exception of valproic acid, which raises plasma testosterone levels (50). A rapid pace of development or progression of hirsutism or evidence of virilization (such as clitoromegaly or increasing muscularity) should raise concern that an androgen-secreting neoplasm is present. However, tumors producing only moderately excessive levels of androgen have indolent presentations (49,51).

In patients with moderate or severe hirsutism (with a score of more than 8 in the above-mentioned scale) or in patients with rapid progression and masculinization, an assessment of the circulating androgen levels should be performed. Determinations of androgen levels are most accurately performed by a specialty laboratory (52). The normal upper limit for total plasma testosterone levels in women varies from about 70 to 90 ng/dL (2.43–3.12 nmol/L). The normal levels and the large variation is due to systematic differences among assays (53), and because many laboratories provide excessively broad normal ranges as the general population tested includes women with unrecognized androgen excess (54,55). Testing for plasma free testosterone is 50% more sensitive than that for total testosterone in detecting androgen excess and is the best single indicator of hyperandrogenism. However, there is no uniform laboratory standard for measuring free testosterone levels, so assay-specific results differ widely. Methods that purport to assay free testosterone directly are particularly suspect. The most reliable assays for measuring free testosterone compute the level of free testosterone from the levels of total testosterone and SHBG (56).

Routine testing for other circulating androgens is of lesser value (34,36,37). The level of DHEAS is increased in approximately 15% of women who have normal levels of total and free testosterone. A mildly elevated level in a woman with a normal free testosterone level is unlikely to be clinically relevant aside from being associated with acne (57–59). Very high levels of total testosterone [>200 ng/dL (6.94 nmol/L)] or DHEAS [>700 μg/dL (19 μmol/L)] heighten the likelihood of an underlying neoplasm (with elevated levels of DHEAS indicating an adrenal source) (60); however, lesser elevations were present in women with androgenic tumors (49).

A total testosterone level that is normal or marginally elevated (within about 20 ng/dL [0.69 nmol/L] of the upper limits of normal) in the absence of other features of concern may indicate idiopathic hirsutism or idiopathic hyperandrogenism. Further workup is

generally not necessary unless the hirsutism progresses despite therapy or if it presents with other worrisome features, such as menstrual irregularity, obesity, or increasing masculinization. An assessment of the free testosterone level is warranted in patients with features of other disorders, even if the total testosterone level is not clearly elevated.

If androgen excess or signs and symptoms that suggest a secondary cause of hirsutism are present, the patient should be referred to an endocrinologist. Even if hirsutism is mild (i.e., modified Ferriman–Gallwey score between 8–12) and menses are regular, measurement of androgen concentrations may be warranted. Ultrasonographic examination of the ovaries, the adrenal glands, or both is a useful screening procedure if the symptoms suggest the presence of a neoplasm (61); pelvic ultrasonography may be useful if PCOS is suspected (62,63).

Hypertrichosis

Hirsutism is distinct from hypertrichosis which refers to generalized increased hair growth in androgen-dependent and -independent body regions (Table 2). Hypertrichosis, in which hair is distributed in a generalized, non-sexual pattern, is not caused by excess androgen, although associated hyperandrogenism of any cause may aggravate this condition.

Generalized excessive hair growth usually occurs as the result of genetic/ethnic factors. Hypertrichosis can also be due to and can represent a cutaneous sign of a systemic disorder, or may develop as a side effect of specific medications, e.g., phenytoin, diazoxide, minoxidil, and cyclosporine. Specific situations such as malnutrition and starvation can be associated with hypertrichosis. Excessive hair over the spine may be a sign of an underlying spinal abnormality such as myelomeningocele, vertebral anomalies, or lipoma (64).

Table 2 Hypertrichosis

Congenital
Congenital nevi
Hemihypertrophy
Spinal dysraphism
Congenital hypertrichosis lanuginose
Cornelia de Lange syndrome
Leprechaunism/insulin receptor gene mutations
Mucopolysaccharidoses
Porphyrias
Rubinstein–Taybi syndrome
Acquired
Skin irritation (friction, atopic eczema)
Cerebral disturbances (post-encephalitis, head injury, etc.)
Acrodynia
Malnutrition
Dermatomyositis
Thyroid hormone abnormalities
Lawrence–Seip syndrome
Malignancy
Drug-induced

Congenital disorders such as Cornelia de Lange, mucopolysaccharidoses, and some forms of porphyria are associated with hypertrichosis.

Idiopathic Hirsutism

The term idiopathic hirsutism is typically reserved for women with excessive body hair and normal serum androgen concentrations and regular menses. Using this definition, less than 20% of hirsute women have idiopathic hirsutism (65). The pathophysiology presumably reflects increased 5α-reductase activity in the skin or, perhaps, altered AR activity (23). Excess hair may reflect an ethnic or familial trait, which is not due to hyperandrogenism. Usually these patients have a low hirsutism score, between 8 and 15 on the Ferriman–Gallwey scale; when the score exceeds these values, increased androgen levels are usually present.

Management of Excessive Body Hair

Treatment of hirsutism involves two therapeutic avenues. The cause of the androgen excess needs to be identified and treated with an appropriate therapeutic intervention to lower the androgen concentration(s). Reduction of androgen concentrations decreases the number of hairs progressing to terminal hairs. In the absence of lowered androgen concentrations/effects, terminal hair growth will continue and the cosmetic modalities used to treat hirsutism may be ineffective. Cosmetic hair removal is necessary to eliminate the undesired terminal hair growth. Shaving and bleaching are commonly used. Waxing and chemical depilating agents can be used, but often lead to skin irritation. Two types of chemical depilating agents are available, sulfides of alkali metals and thioglycolate salts. Both work by breaking disulfide bonds to dissolve the hair shaft. The cell-cycle inhibitor, elfornithine hydrochloride, 13.9% (Vaniqua), has been helpful for some women because it slows hair growth (Table 3)(66).

Electrolysis and laser treatment provide longer lasting treatments. By inserting an electrode into individual hair follicles, hair follicles are destroyed. Electrolysis involves destruction of the hair follicle through production of hydroxide ions by a direct electric current. For laser treatment, hair follicles are destroyed by a combination of selective heat absorption by darker hairs and deeper penetration of the light into the dermis. Laser hair removal works using one of the two mechanisms. The energy from ruby, alexandrite, and diode lasers is absorbed by melanosomes and released as heat, which damages the hair follicle. The Q-switched NdL:YAG laser works in conjunction with a carbon particle suspension applied to the skin prior to the treatment; the carbon penetrates the follicle, absorbs the laser energy, and generates damaging heat. Both electrolysis and laser methods require trained personnel and are expensive, time-consuming, and painful. Deleterious

Table 3 Medications Commonly Used for Hirsutism

Drug type	Active ingredient	Example	Major mechanism	Indication	Contraindications	Dose	Major side effects
Cell-cycle inhibitor	Eflornithine hydrochloride, 13.9%	Vaniqa	Irreversible inhibitor of ornithine decarboxylase	Focal hirsutism	Pregnancy, breastfeeding	Topical, twice daily	Rash, potential systemic toxicity with widespread application
Oral contraceptives[a]	Ethinyl estradiol 30 μg + drospirenone 35 μg + norgestimate 35 μg + ethynodiol diacetate	Yasmin Ortho-Cyclen Demulen 1–35	Suppresses ovarian function	Generalized hirsutism	Breast cancer, smoking (absolutely if age >35 yr), cardiovascular disease, uncontrolled hypertension, thromboembolic disorders, clotting dysfunction, cerebral-vascular disease, liver tumors, and pregnancy	One tablet by mouth at bedtime (the larger estrogen doses may be necessary in heavier women for menstrual regularity)	Irregular vaginal bleeding, venous thrombosis
Estrogen/progestin patch		Ortho-Evra	Suppresses ovarian function	Generalized hirsutism		One patch weekly for three weeks followed by one week off free of patch	
Antiandrogens[b]	Spironolactone		Competitive Inhibitor of androgen receptor binding	Moderate or severe hirsutism	Lack of contraception, kidney or liver failure	50–100 mg by mouth, twice daily	Male pseudohermaphroditism in fetus, irregular menstrual bleeding unless oral contraceptive administered, decreased libido, nausea, hyperkalemia, hypotension, liver dysfunction
	Cyproterone acetate		Competitive inhibitor of androgen receptor binding	Moderate or severe hirsutism	Lack of contraception	Induction: 50–100 mg by mouth at bedtime, days 5–15 Maintenance: 5 mg by mouth at bedtime, days 5–15	Male pseudohermaphroditism in fetus, irregular menstrual bleeding unless estrogen administered cyclically, decreased libido, nausea
	Flutamide		Nonsteroidal competitive inhibitor of androgen receptor binding	Severe hirsutism	Lack of contraception, liver disease	125–250 mg, twice daily	Male pseudohermaphroditism in fetus, hepatotoxicity
Glucocorticoids	Glucocorticoid	Prednisone	Suppress adrenal function	Congenital adrenal hyperplasia	Uncontrolled diabetes, obesity	5–7.5 mg by mouth at bedtime	Changes typical of Cushing's syndrome, adrenal atrophy
Gonadotropin-releasing agonists	Leuprolide acetate, depot suspension	Lupron Depot	Suppress gonadotropins	Alternative to oral contraceptive	Osteoporosis	7.5 mg monthly intramuscularly, with 25–50 μg transdermal estradiol	Osteoporosis without estrogen-progestin replacement

[a]The oral contraceptives included here are examples of preparations with low androgenic activity.
[b]Use of anti-androgens alone may be associated with unplanned pregnancy in sexually active adolescent girls who are not actively using effective means of contraception.
Source: From Ref. 26.

consequences include local reactions such as burns, scarring, and dyspigmentation (33).

In addition to the cosmetic therapies the suppression of androgen production or the blocking of the action of androgens within the skin should be considered (22). The suppression or blockage of androgen prevents progression from vellus to terminal hair growth, though this is a long-term effect of the medication. The maximal effect requires 9 to 12 months of treatment because of the long duration of the hair-growth cycle. The assessment of efficacy of the treatment is therefore difficult as is often based on subjective assessment and lack of randomized, controlled trials. The drugs often used for the treatment of hirsutism are off-label agents. Oral contraceptives (OCPs) suppress plasma-testosterone levels, particularly the level of free testosterone, mainly by inhibiting ovarian function and raising the levels of SHBG levels (22). This method of treatment can reduce by half the need for shaving (67) and can arrest the progression of hirsutism from various causes, but it will not reverse hirsutism (23,68); cosmetic measures should also be used. Although any combination pill will suffice, contraceptives with non-androgenic progestins (Table 3) are preferable because of their potentially favorable effects on lipid levels (69), acne (70), and in the case of drospirenone, salt retention (71). It is unclear whether the newer low-dose pills included in the table carry a risk of venous thromboembolism (72).

Antiandrogens are usually required to achieve the best result reflected by a substantial reduction of hirsutism. Competitive inhibitors of androgen binding to the AR are superior to drugs that interfere with testosterone metabolism (73–76). They are effective regardless of the cause of hyperandrogenism and are helpful in the treatment of idiopathic hirsutism (23). Spironolactone is the antiandrogen of choice utilized in the United States. Cyproterone acetate is a progestational antiandrogen available in Canada, Mexico, and Europe but not in the United States. The long-term use of either spironolactone or cyproterone acetate can be expected to reduce the Ferriman–Gallwey score by 15% to 40% within six months after the start of therapy, although there is considerable variation among individual women. Contraceptives containing estrogen and progestin should be used concomitantly with these agents, as they complement antiandrogenic actions while ensuring menstrual cyclicity and preventing pregnancy. Flutamide, an antiandrogenic drug marketed for the treatment of prostate cancer, is rarely used for hirsutism because of its expense and risk of hepatocellular toxicity. The 5α-reductase inhibitor finasteride is less effective for hirsutism than are antiandrogens (76). Gonadotropin-releasing hormone agonists are an alternative to OCPs, but long-term use is undesirable due to effects on bone mineralization (68). Androgen blockade modalities have been limited by concerns related to the risk of pseudohermaphroditism in male fetuses.

ADRENAL CAUSES OF HYPERANDROGENISM

Congenital Adrenal Hyperplasias

The virilizing congenital adrenal hyperplasias are a group of autosomal recessive disorders characterized by impaired cortisol biosynthesis (Vol. 2; Chap. 9). The loss of negative feedback inhibition leads to subsequent increases in ACTH and adrenal androgen secretion. The most common type of CAH is 21-hydroxylase deficiency due to mutations in the 21-hydroxylase (CYP21A2) gene. Less commonly, CAH is due to 3β-HSD deficiency associated with mutations in the type 2 3β-HSD (HSD3B2) gene and 11β-hydroxylase deficiency associated with mutations in the 11β-hydroxylase (CYP11B1) gene. The phenotypic spectrum ranges from classical forms with presentation during infancy or early childhood to the non-classical forms. Typical presentations for the non-classical forms include premature development of pubic hair, hirsutism, irregular menses, infertility, and clitoromegaly. Accelerated linear growth velocity and advanced skeletal maturation, assessed by bone age radiograph, are typical findings.

The incidence of 21-hydroxylase deficiency varies from 1 in 15,000 livebirths for the classical forms to approximately 1 in 1000 for the milder forms (77). The CYP21A2 gene is located at chromosome 6p12 in close proximity to a highly homologous pseudogene, CYP21A1P. The coding regions of CYP21A2 and CYP21A1P show 98% homology (78,79). To data, over 100 mutations have been identified (80). However, the vast majority of affected alleles carry one of the more common mutations. These common mutations represent gene conversion events in which the functional gene has acquired deleterious sequences from the pseudogene. Diagnosis of the milder forms of 21-hydroxylase deficiency CAH often requires pharmacologic stimulation with ACTH. After the blood sample for basal hormone determinations has been obtained, 0.25 mg synthetic ACTH is administered by intra-muscular or intravenous bolus injection. Stimulated levels can be obtained at either 30 or 60 minutes. Available data suggests that mutations will be found on both CYP21A2 alleles if the ACTH-stimulated 17-OHP value is greater than 1500 ng/dL (45 nmol/L) (81). Although molecular genotype results are necessary for certainty, ACTH-stimulated 17-OHP values between 500 and 1200 ng/dL suggest heterozygosity for CYP21A2 mutations. However, 50% of heterozygous mutation carriers show ACTH-stimulated 17-OHP values less than 500 ng/dL (82).

Mutations in the HSD3B2 gene cause 3β-HSD deficiency. Affected infants of both sexes can present with genital ambiguity. In females, the accumulation of DHEA is sufficient to virilize the external genitalia. Decreased HSD3B2 activity in males interferes with testosterone biosynthesis resulting in undervirilization. Correlation of ACTH-stimulated 17-Preg and DHEA levels with HSD3B2 genotyping showed that

non-classical 3β-HSD deficiency is uncommon. Indeed, similar to 21-hydroxylase deficiency, ACTH-stimulated 17-hydroxypregnenolone concentrations are 9 to 10 standard deviations above age and pubertal stage matched values for subjects with HSD3B2 mutations on both alleles (83–85). Mild forms of 11β-hydroxylase deficiency due to mutations in CYP11B1 are extremely rare.

Individuals with classical salt-losing CAH require both glucocorticoid and mineralocorticoid replacement therapy. Glucocorticoid therapy without mineralocorticoid treatment is generally sufficient for patients with simple virilizing and late onset forms of CAH. However, mineralocorticoid therapy may be beneficial in patients with simple virilizing CAH. The goal of treatment is suppression of adrenal androgen concentrations without excessive glucocorticoid exposure. Both undertreatment and overtreatment can be associated with short stature. Hydrocortisone replacement therapy, 7 to 16 mg/m^2/day, administered approximately every eight hours is generally adequate. Adolescents and adults may prefer prednisone and dexamethasone because of the dosing schedule. Affected individuals need to know when to increase their dose because of acute physiological stress, how to administer a short-acting intramuscular glucocorticoid such as Solu-Cortef, and wear a medical alert ID badge.

Cushing's Syndrome

This term is used to describe the clinical features associated with excessive exposure to endogenous or exogenous glucocorticoids (Vol. 2; Chap. 8). Exogenous Cushing's syndrome represents the consequences of pharmacologic doses of glucocorticoids used for their anti-inflammatory properties to treat a variety of disorders, i.e., inflammatory bowel diseases, connective tissue disorders, asthma, etc. The endogenous forms of Cushing's syndrome are due to increased corticotropin-releasing hormone (CRH), ACTH, or cortisol secretion. Cushing's disease refers specifically to increased pituitary ACTH secretion often due to a pituitary tumor, but this disorder may also involve excessive hypothalamic CRH secretion. Patients with multiple endocrine neoplasia type I due to loss of function mutations in the menin (MEN-1) gene and those with Mc-Cune Albright syndrome due to activating mutations in the GNAS1 gene can develop ACTH-secreting pituitary adenomas.

Ectopic tumors secreting ACTH or CRH can also cause excessive glucocorticoid secretion. Typically, ectopic tumors occur in adults in association with anorexia, weight loss, muscle wasting, and hyperpigmentation. Although extremely rare, ectopic ACTH-secreting tumors have been found in association with bronchial/thymic carcinoids, malignant paraganglioma, carcinoids, islet cell pancreatic tumors, medullary thyroid cancer, and pheochromocytomas (86–88).

In children under eight years of age, ACTH-independent glucocorticoid excess is often due to adrenal tumors. Approximately 10% to 30% of adrenal adenomas and carcinomas secrete other steroid hormones such as androgens in addition to cortisol (89). Adrenocortical neoplasms can be associated with Beckwith–Wiedemann syndrome, MEN type II, Li–Fraumeni syndrome, and Carney complex. Carney complex is a heterogenous disorder with autosomal dominant inheritance pattern is associated with spotty skin pigmentation, cardiac myxomas, thyroid tumors, and testicular tumors. In approximately 40% of families, Carney syndrome is associated with loss of function mutations in the protein kinase A regulatory subunit 1-α (PRKARIA) gene. The typical adrenal histology is primary pigmented nodular adrenocortical disease (PPNAD) in which the adrenal glands contain dark nodules scattered within atropic adrenal glands. The micronodules are usually visible on imaging studies giving an irregular contour. PPNAD can be associated with ''periodic'' Cushing syndrome characterized by intermittent excessive glucocorticoid secretion.

Menstrual changes and/or hirsutism occur in approximately 70% of post-pubertal females with Cushing's syndrome, but only approximately 2% to 4% of hirsute women have Cushing's syndrome (90,91). Impaired linear growth velocity is a characteristic feature of excessive glucocorticoid exposure in children. Pseudo-Cushing's syndrome associated with mildly elevated cortisol concentrations can be caused by depression, anxiety, morbid obesity, and alcoholism. Thus, the diagnosis of Cushing's syndrome requires confirmation of inappropriate cortisol secretion with loss of both physiological negative feedback inhibition and diurnal variation. Atypical presentations and pseudo-Cushing's syndrome confound the diagnostic evaluation. The quest for the etiology of Cushing's syndrome is best postponed until laboratory data verifying excessive endogenous glucocorticoid secretion is unequivocal. In some instances, screening tests may need to be repeated (92).

Elevated urinary free cortisol excretion is often the first diagnostic study to confirm endogenous hypercortisolism. Cortisol concentrations are best measured by high-performance liquid chromatography, gas chromatography coupled with mass spectroscopy, or tandem mass spectroscopy. Loss of diurnal variation can be documented by midnight plasma or salivary cortisol determinations (93). The 1 mg overnight dexamethasone suppression test can be used, but both false negative and false-positive results can be obtained (94). Sensitivity is improved when the cut-off level used is 1.8 mcg/dL. Once the diagnosis of Cushing's syndrome is confirmed by demonstration of excessive endogenous glucorticoid secretion, the next step is to identify the specific etiology.

Approximately 70% of cases are due to Cushing's disease. Measurement of serum ACTH is helpful to distinguish ACTH-independent causes such as adrenal tumors from ACTH-dependent causes such as Cushing's disease or ectopic ACTH/CRH secretion. As ACTH is rapidly degraded, careful attention must

be paid to blood collection and use of two-site immunoradiometric assay. Typically, ACTH concentrations are extremely elevated with the presence of an ectopic ACTH-secreting tumor. High-dose dexamethasone suppression tests and CRH stimulation testing can be useful to confirm Cushing's disease. In approximately 60% of patients with Cushing's disease, pituitary magnetic resonance images (MRIs) with gadolinium enhancement will demonstrate a discrete pituitary adenoma (95). For some patients, bilateral inferior petrosal sinus sampling for ACTH is beneficial to confirm the diagnosis of Cushing's disease (96). The majority of ectopic ACTH-secreting tumors are due to neuroendocrine tumors. Additional studies such as computed tomography (CT), MRI, and somatostatin analog scintigraphy with ^{111}In-pentetreotide may identify occult ACTH-secreting neoplasms (97).

Glucocorticoid Resistance

The inherited glucocorticoid resistance syndrome is characterized by symptoms due to hyperandrogenism such as premature pubarche, hirsutism, oligomenorrhea, infertility, and hypokalemic hypertension. Despite elevated cortisol concentrations, symptoms typical of Cushing's syndrome are absent. This autosomal dominant disorder is due to loss of function mutations in the glucocorticoid receptor (GCCR) gene. Impaired glucocorticoid signal transduction interferes with negative feedback inhibition resulting in increased ACTH and cortisol secretion. In some instances, mineralocorticoid secretion is also increased resulting in hyperkalemic hypertension. Treatment with dexamethasone is often beneficial to lower adrenal mineralocorticoid and androgen secretion.

Androgen-Secreting Tumors

Androgen-secreting tumors are uncommon causes of hyperandrogenism among children and adolescents. In girls, the tumor may originate in an ovary, adrenal cortex, or, rarely, in an ectopic location (98). Ovarian androgen-secreting tumors such as Sertoli–Leydig and steroid cell tumors are extremely uncommon in the pediatric age group. They should be considered when there is a rapid virilization and elevated concentrations of Δ^4-androstenedione and/or testosterone. The majority of ovarian tumors derive from germ cells. Sertoli–Leydig cell tumors (arrhenoblastoma) are usually unilateral and benign (99). Less than 20% of the tumors arise from the surface epithelium of the ovary in children as opposed to 70% in adults (100).

Adrenocortical tumors occur most commonly in older adults. However, the second peak incidence of adrenocortical tumors occurs in children less than 10 years of age. Among children, the incidence varies across geographic regions and is approximately 10 times greater in Southern Brazil than elsewhere in the world (101). Over 90% of childhood adrenocortical tumors, both adenomas and carcinomas, secrete hormones, primarily androgens (61). Thus, virilization often prompts referral and evaluation (102).

Typically, DHEA or DHEAS concentrations are elevated. Because the clinical characteristics due to excessive adrenal hormone secretion are similar, differentiation between benign and malignant lesions requires histological examination of the tissue. Generally, adrenocortical adenomas are small whereas carcinomas are large. Both show lipid-depleted cells with granular cytoplasm and varying degrees of mitotic activity. Carcinomas often contain areas of hemorrhage, necrosis, and calcification. Features such as invasion into the capsule or blood vessels indicate malignancy. Expression profiling may prove to be helpful to predict the natural history of the tumor (103).

Adrenocortical tumors have been associated with Beckwith–Wiedemann syndrome, Li–Fraumeni, Carney complex, and MEN-1 syndrome. Unrelated patients with adrenocortical tumors in Southern Brazil carry the identical TP53 tumor suppressor mutation, R337H (104). Loss of 17p and structural rearrangement of 11p15 are associated with malignancy and provide useful markers to predict natural history of the adrenocortical tumor (105).

The diagnosis of an adrenocortical tumor is suggested by the presence of virilization, elevated hormone concentrations, and the presence of a suprarenal mass. Surgical excision is the primary modality for treatment. Prior to surgery, ultrasound, MRI, and CT are essential to assess for invasion of adjacent structures, metastases, and involvement of the vena cava. Fluorodeoxyglucose positron emission tomography imaging may be helpful to identify metastases and recurrences (106). Adrenocortical carcinomas tend to be extremely friable increasing the chance of intra-operative rupture and spillage. Given this characteristic and risk for tumor rupture, percutaneous biopsy is best avoided (107).

Cytotoxic adjuvant therapy with mitotane and other agents may be beneficial (108). Mitotane has significant toxicities including vomiting, anorexia, mental confusion, renal and hepatic dysfunction. Because it is adrenolytic, patients receiving this agent should be considered to have adrenal insufficiency and treated with glucorticoid and mineralocorticoid replacement therapy (102). Nevertheless, the prognosis is poor for metastatic adrenocortical carcinoma especially with large tumors, extensive necrosis, capsular invasion, and marked mitotic activity. Development of agents that inhibit angiogenesis or adrenocortical carcinoma (ACC)-signaling pathways offers prospect of expanded therapeutic options and improved outcome (109).

POLYCYSTIC OVARY SYNDROME

Definition and Diagnosis

PCOS is a polygenic multi-factorial heterogeneous disorder characterized by chronic anovulation and

hyperandrogenism. This disorder affects approximately 5% of reproductive aged women (110). Among women with PCOS, the prevalence of PCOS is 24% in mothers and 32% in sisters (111). Clinical features of hyperandrogenism include hirsutism, acne, and androgenic alopecia. Approximately 70% of affected women of White, Black, South Asian, or Pacific Island descent manifest hirsutism (112,113). Hirsutism is less common among affected women of East Asian ethnic origin.

The diagnosis of PCOS is based on clinical features indicative of hyperandrogenemia with laboratory confirmation of hyperandrogenism and exclusion of other disorders such as late onset congenital adrenal hyperplasia. However several factors contribute to difficulties in the diagnosis of this entity. The 1990 NIH/NICHD consensus conference defined the diagnostic criteria to include (i) hyperandrogenism/hyperandrogemia, (ii) oligo-ovulation, and (iii) exclusion of other known disorders (114). The proceeding of the Rotterdam Consensus Conference in 2003 recommended that the definition of PCOS should be modified to include two of the following three features: (i) oligo and/or anovulation, (ii) clinical and/or biochemical signs of hyperandrogenism, and (iii) polycystic ovaries (115). As described above, differing methodologies in different laboratory for determination of androgen concentrations necessitates knowledge of the normal ranges for the laboratory. Although free testosterone determination may be the best single marker indicative of hyperandrogenism, assay differences confound the use of this hormone marker (56). While abdominal or transvaginal ultrasonography often demonstrate the presence of polycystic ovaries in women with PCOS, polycystic ovaries can be found in normal women (116,117). Multi-cystic ovaries can be detected in normal adolescent girls and in women with hypothalamic amenorrhea. Ultrasound criteria used to evaluate for polycystic ovaries include follicle number ($\geq$12 follicles measuring 2 to 9 mm in diameter), ovarian volume ($>10\,cm^3$), and ovarian area (118). For obese adolescent girls with PCOS, pelvic MRI may provide better visualization of the structural components of the ovary than pelvic ultrasound (119). Polycystic ovaries can occur in women with untreated nonclassical CAH or inadequately treated classical CAH suggesting that hyperandrogenism influences ovarian morphology. Because the development of polycystic ovaries presumably represents a consequence of chronic hyperandrogenism, ovarian ultrasonography may be relatively unrevealing for adolescent girls with incipient PCOS. Polycystic ovaries need not be present to make a diagnosis of PCOS (45) and conversely, their presence alone does not establish the diagnosis (54,117).

The symptoms of PCOS usually begin around menarche (120). Premature pubarche, the result of early secretion of adrenal steroids, may be a harbinger of the syndrome (121). In addition, an aberrant intrauterine environment has been implicated in the condition's pathogenesis, particularly its metabolic components (122–125). Women with PCOS often manifest LH hypersecretion (126). They present with chronic anovulation most often manifested as oligomenorrhea (fewer than nine menses per year) or amenorrhea. Anovulatory cycles may lead to dysfunctional uterine bleeding and decreased fertility. However, in routine clinical practice, abnormal gonadotropin concentrations provide little diagnostic sensitivity (an elevated LH level or an elevated LH/FSH ratio) as a single value of these hormones does not always reflect the gonadotropin concentrations that vary over the menstrual cycle and are released in a pulsatile fashion into the circulation, throughout a 24-hour period. Thus, single measurements of these hormones do not diagnose PCOS.

Insulin and Polycystic Ovary Syndrome

A substantial proportion of women with PCOS are overweight; many are obese (127). Although obesity itself is not considered the inciting event in the development of the syndrome, excess adiposity can exacerbate the associated and metabolic derangements that characterize PCOS (Vol. 1; Chap. 1 and 11). Insulin plays both direct and indirect roles in the pathogenesis of hyperandrogenemia in PCOS. Insulin acts synergistically with LH to enhance the androgen production of theca cells. Insulin also inhibits hepatic synthesis of SHBG, the key circulating protein that binds testosterone, and thus increases the proportion of testosterone that circulates in the unbound, biologically available, or free state. Because women with PCOS typically have hyperinsulinemia, the concentration of free testosterone may be elevated when the total testosterone concentration is at the upper range of normal or only modestly elevated. Cutaneous manifestations of hyperandrogenemia in PCOS include hirsutism, acne, and male-pattern hair loss (androgenic alopecia), whereas acanthosis nigricans is a cutaneous marker of hyperinsulinemia and is often detected in patients with this disorder.

Insulin resistance and hyperinsulinemia are often associated with PCOS. The central role of insulin resistance/hyperinsulinemia in the pathogenesis of PCOS has been validated by clinical studies involving weight loss, pharmacologic suppression (e.g., diazoxide), and insulin sensitizing agents (128–130). The term ''insulin resistance'' generally refers to decreased insulin action in regards to carbohydrate and lipid metabolism (Vol. 1; Chap. 11). Findings indicative of insulin resistance include decreased insulin stimulated glucose uptake, increased hepatic glucose production, and increased lipolysis. Irrespective of the molecular mechanism(s) responsible for the insulin resistance and hyperinsulinemia in PCOS, the mitogenic (growth) actions of insulin are generally

preserved. Insulin also inhibits hepatic synthesis of SHBG resulting in increased bioavailable testosterone. The elevated insulin concentration acts synergistically with LH and/or ACTH, respectively to provoke excessive ovarian and/or adrenal androgen biosynthesis (131,132). In this sense, the ovary becomes a target of the metabolic derangements.

Adolescent and adult women with PCOS have an increased risk to develop the metabolic syndrome (133,134). The metabolic syndrome consists of a constellation of features including insulin resistance, dyslipidemia, obesity, and hypertension. It is associated with increased risks for impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM). The metabolic syndrome appears to represent the consequences of chronic activation of the proinflammatory pathway in obesity (135,136). With increasing obesity, macrophages invade adipose tissue (137,138). The changes in adipokine and cytokine secretion associated with obesity have led to the hypothesis that macrophages directly influence adipocyte biology favoring development of insulin resistance. To maintain euglycemia, the beta cells of the pancreas secrete more insulin resulting in hyperinsulinemia. The impaired insulin-mediated suppression of lipolysis leads to increased free fatty acids and ectopic lipid accumulation in atypical locations such as liver, muscle, and pancreatic beta cells (139). If beta cell failure occurs, hyperglycemia and T2DM develop.

Thirty percent to forty percent of women with PCOS have IGT, and as many as 10% have type 2 diabetes by their fourth decade (127,140). These prevalence rates are among the highest known among women of similar age (141), An enhanced rate of deterioration in glucose tolerance is also evident in PCOS (127,142). Women with PCOS are more insulin resistant than are unaffected counterparts matched for body mass index, fat-free body mass, and body-fat distribution (143–145). A defect in the insulin-signaling pathway appears to be present in both the adipocyte and skeletal muscle, the primary target tissues of insulin action (144,145). However insulin resistance alone cannot fully account for the predisposition to and development of type 2 diabetes among PCOS patients (146,147). Most women with PCOS are able to compensate fully for their insulin resistance, but a substantial proportion (particularly those with a first-degree relative with type 2 diabetes (148) have a disordered and insufficient beta-cell response to meals or a glucose challenge (148–152). Before the development of frank glucose intolerance, defects in insulin secretion may be latent and revealed only in circumstances that augment insulin resistance, as with the development of gestational diabetes in pregnancy (153) or glucose intolerance associated with glucocorticoid administration (151).

Insulin resistance has also been shown to be a key player in the pathophysiology of complications present in obese and PCOS patients. Included are hypertension, dyslipidemia, microvascular disease, and sleep apnea. There is also an increased prevalence of endometrial hyperplasia and carcinoma in women with PCOS (154,155). This increase has been attributed largely to the persistent stimulation of endometrial tissue by estrogen (mainly estrone) without the progesterone-induced inhibition of proliferation and differentiation to secretory endometrium that occurs after ovulation. Endometrial carcinoma has also been associated with obesity and type 2 diabetes, both of which are common in PCOS. Breast and ovarian cancer have been variably associated with PCOS (155).

Pathophysiology

Theca cells obtained from women with PCOS have an innate propensity to synthesize and secrete excessive amounts of androgens (156,157). Using a variety of strategies such as cell culture, transient transfection, suppressive subtractive hybridization, and cDNA micro-array analyses, it appears that that increased CYP17, CYP11A1, and HSD3B2 expression contribute to the excessive androgen biosynthesis (158–160).

Many women with PCOS exhibit LH hypersecretion with increased LH/FSH ratio, LH pulse amplitude, LH pulse frequency, and LH responses to GnRH stimulation. Two plausible explanations for this LH hypersecretion are that it represents: (i) an intrinsic abnormality of the GnRH pulse generator or (ii) a consequence of chronic anovulation and/or the abnormal insulin, androgen, estrogen and progesterone concentrations (161–164). Regardless of the inciting event, the GnRH pulse generator in some women with PCOS is more resistant to negative feedback inhibition by estradiol and progesterone leading to persistently increased GnRH pulsatility (165,166). This relative insensitivity to progesterone can be attenuated by anti-androgens (167). Other factors that modulate LH secretion in women with PCOS include ethnicity, duration of androgen exposure, body mass index, estradiol concentration, and insulin concentration (168–170). The persistent hyperandrogenism and LH hypersecretion appear to evolve into a self-perpetuating vicious cycle resulting in chronic anovulation (171). Studies performed both in vivo and in vitro (in cultured theca cells) consistently suggest that ovarian theca cells in affected women are more efficient at converting androgenic precursors to testosterone than are normal theca cells (157,172).

Whereas LH regulates the androgenic synthesis of theca cells, FSH is responsible for regulating the aromatase activity of granulosa cells, thereby determining how much estrogen is synthesized from androgenic precursors.

For some women with PCOS, the first manifestation is the development of pubic hair (premature pubarche) at an atypically early age (173,174).

Premature pubarche represents the physical manifestation of early adrenal pubertal maturation (adrenarche). Although the pubertal changes associated with adrenarche such as pubic hair, axillary hair, adult-type apocrine odor, and acne slowly progress, breast development and menarche occur slightly earlier than expected for population norms (175). Detection of hyperinsulinemia, lipid abnormalities, and decreased SHBG and IGFBP-1 concentrations among some girls with premature pubarche provides circumstantial evidence that premature adrenarche represents the initial manifestation of PCOS for some girls (176,177).

Genes and Polycystic Ovary Syndrome

The familial pattern of PCOS indicates involvement of genetic factors (111). Hereditability for hyperandrogenemia has been demonstrated (55,178). Family members, of women with PCOS, often have T2DM, IGT, insulin resistance, and/or hyperinsulinemia (179–182) Numerous linkage analysis and candidate gene association studies have been performed, but phenotypic and genetic heterogeneity, inconsistent diagnostic criteria, imprecise male phenotype, relative infertility, incomplete penetrance, and epigenetic factors hinder both approaches in the quest for the PCOS genes (183). Despite the multiple impediments, some progress has been made to identify relevant genes (184). For example, some women with PCOS have ACTH-stimulated 17-OHP and molecular genotype results indicating heterozygosity for CYP21A2 mutations (185–187). These women differ from obligate heterozygote CYP21A2 mutation carriers ascertained through studies of families with classical CAH where heterozygotic carriers are asymptomatic despite having mildly elevated serum androgen concentrations (188). Candidate genes have included those associated with androgen biosynthesis, androgen metabolism, insulin signal transduction, insulin secretion, and serum inflammatory markers (189).

Representative candidate genes involved in PCOS are shown in Table 4.

Treatment of Polycystic Ovary Syndrome

The most frequently used medications for the treatment of hirsutism are shown in Table 3. These are applicable for the treatment of PCOS, too. The most common types of menstrual dysfunction in PCOS patients are oligo/amenorrhea, menorrhagia, and chronic anovulation. An increased risk of endometrial hyperplasia occur. Amenorrhea refers to absence or abnormal cessation of menses. Absence of menarche by age 16 years in the presence of normal breast and pubic hair development is defined as primary amenorrhea. Following a period of relatively regular menses, absence of menses for three consecutive months is considered to be secondary amenorrhea. Oligomenorrhea is defined as intermenstrual interval greater than 35 days. Normal menstrual cycles range from 28 to 35 days, with approximately 40 ± 20 mL blood loss (216). In adolescent girls, the disorders associated with oligo/amenorrhea encompass a

Table 4 Representative Candidate Genes with Evidence of Linkage, Association, or Both, with The PCOS

Pathway and Protein (Gene)	Comments
Insulin secretion and action	
Insulin receptor (INSR) region	D19S884, an anonymous marker 1 cm centromeric to INSR; evidence for linkage and association with PCOS (190,191)
Insulin variable-number tandem repeats (VNTR)	Region involved in transcriptional regulation of insulin gene; evidence for linkage and association with class III allele (192–194)
Insulin receptor substrate 1 (IRS-1)	Post-receptor molecule in insulin insulin-signaling pathway; association with PCOS (195,196)
Insulin receptor substrate 2 (IRS-2)	Post-receptor molecule in insulin insulin-signaling pathway; association with PCOS (195,196)
Calpain 10 (CAPN10)	Cysteine protease with effect on insulin action and secretion; linkage and association with type 2 diabetes (197,198)
Peroxisome-proliferator-activated receptor γ (PPARγ)	The Pro12Ala polymorphism in the PPARγ gene is a modifier of insulin resistance in PCOS (199–201)
Protein phosphatase 1 regulatory subunit (PPP1R3)	Variant of regulatory subunit of the glycogen-associated form of protein phosphatase-1 derived from skeletal muscle; associated with insulin resistance (202)
Gonadotropin secretion and action	
Follistatin (FST)	Acts to inhibit ovarian follicular maturation and androgen production; enhances FSH and insulin secretion (190,203)
Androgen biosynthesis, secretion, transport, and metabolism	
Androgen receptor (AR)	Number of CAG repeats associated with androgen levels in PCOS (204)
Sex hormone-binding globulin (SHBG)	Association of pentanucleotide (TAAAA)n repeat polymorphism with PCOS (205,206)
Cytochrome P-450C17 (CYP17)	Possible association with PCOS (207–211)
Cytochrome P-45011α (CYP11α)	Early analyses revealed association with hyperandrogenemia and PCOS (212,213); more recently, strength of association has been questioned (214)
11β-Hydroxysteroid dehydrogenase (11β-HSD) and hexose-6-phosphate dehydrogenase (H6PD)	Mutations in both 11β-HSD and H6PD in a triallelic digenic model of inheritance result in low 11β-HSD expression and NADPH generation with loss of 11β-HSD1 oxo-reductase activity (215)

Abbreviations: FSH, follicle-stimulating hormone; PCOS, polycystic ovary syndrome.
Source: From Ref. 46.

broader range than among adult women with secondary amenorrhea because disorders affecting sexual differentiation are included in the differential diagnosis (Vol. 2; Chap. 11) Causes of delayed puberty with absence of secondary sexual characteristics should also be considered. Disorders associated with oligo/amenorrhea can be categorized into four groups: (i) anatomic defects; (ii) primary gonadal failure; (iii) hypothalamic–pituitary dysfunction; and (iv) other causes. It should always be kept in mind that adolescent girls tend to use contraception sporadically and become sexually active before using contraception. Thus, pregnancy should be excluded as the cause of amenorrhea. Abnormal bleeding due to miscarriage, incomplete miscarriage, or ectopic pregnancy can occur. Physical examination, β-human chorionic gonadotropin determination, and transvaginal ultrasound are helpful.

Anovulatory dysfunctional bleeding, characterized by irregular and prolonged bleeding, occurs during the peri-pubertal and peri-menopausal years; it is also associated with PCOS. In this disorder, unopposed estrogen action on the uterus leads to development of dilated veins and suppression of spiral arteriole development causing irregular breakdown of the endometrium. Withdrawal of progesterone during ovulatory cycles stimulates a series of events in the endometrium. Inflammatory mediators such as interleukin-8, monocyte chemotactic peptide-1, and cyclooxygenase 2 are upregulated; prostaglandin concentrations increase followed by an influx of leukocytes (217). Menorrhagia, defined as blood loss greater than 80 mL, may occur in 10% to 30% of women (218). Impaired platelet aggregation and excessive local fibrinolysis likely contribute to excessive menstrual bleeding; spiral arteriolar vasoconstriction, and localized hemostatic plug formation within endometrial vessels play important roles in menstrual hemostasis (219).

A small proportion of women with excessively heavy menses have a coagulopathy such as von Willebrand's disease, idiopathic thrombocytopenia purpura, platelet dysfunction, or leukemia (220). Other causes of heavy menstrual bleeding include liver dysfunction, hypothyroidism, vaginal foreign bodies, vaginal or cervical inflammation, polyps, or tumors (Vol. 2; Chap. 14). The diagnosis of von Willebrand's disease depends on clinical features and laboratory confirmation of subnormal von Willebrand factor antigen and/or activity (221). Thyroid function studies, exclusion of pregnancy, blood count, and clotting studies are appropriate laboratory studies. Transvaginal sonography may be helpful to assess for polyps, submucous fibroids, and endometrial hyperplasia. For severe bleeding associated with hemodynamic instability, blood transfusion and high-dose estrogen therapy may be necessary. When the patient is actively bleeding and hemodynamically stable, a monophasic OCP should be administered as follows: one pill t.i.d. for three days, two pills b.i.d. for two days, and one pill daily until the pack is completed followed by routine OCP use. Iron treatment is advisable when anemia occurs (222).

Non-steroidal anti-inflammatory drugs (NSAIDs) can reduce menstrual flow. Published recommendations are: (i) mefenamic acid, 250 to 500 mg two to four times per day; (ii) naproxen, 250 to 500 mg two to four times per day; or (iii) ibuprofen, 600 to 1200 mg/day (223). Progestogens have been used traditionally based on the unsubstantiated notion that menorrhagia is associated with anovulatory cycles. High doses of oral progestogens, 30 mg/day, may control torrential bleeding within 24 to 48 hours at which time the dose can be reduced over a few day (224). Cyclical oral progestogens for 5 to 10 days are less effective at controlling heavy menstrual bleeding compared to NSAIDs, danazol, or progestogen-containing intra-uterine systems (225,226). Combined OCPs can be used. Another option is the levonorgestrel intrauterine system (LNG-IUS) which provides sustained release of levonorgestrel, 20 mcg/day, for up to five years (227). The LNG-IUS has been demonstrated to reduce menstrual flow by inducing endometrial atrophy and provides effective contraception (228–230). In adolescent girls, medical treatment, as opposed to surgical intervention, is preferred to preserve fertility.

Whereas OCPs are helpful to suppress the ovarian hyperandrogenism, they have minimal effect on insulin sensitivity. Benefits of OCPs include decreased androgen concentrations, regularization of menstrual periods and decreased risk for endometrial hyperplasia, decreased terminal hair growth, and decreased acne. Ethinyl estradiol and mestranol are the estrogens most often used in OCPs. The choice of a contraceptive pill is largely based on the progestational component because of differences in androgenic potential. Ovarian suppression is usually achieved at the end of the third week of treatment with an effect on acne within one to two months (231). Due to hair growth cycles, the effect on hair growth generally takes approximately 6 to 12 months. However, terminal hair growth is not reversed, merely slowed in rate. Cosmetic treatments such as electrolysis, laser hair removal, waxing, bleaching, or topical chemicals are necessary to remove terminal hair growth as discussed in the section of hirsutism.

Spironolactone, used primarily for its antimineralocorticoid action, also is an anti-androgen that interferes with the binding of androgens to their receptors. It can be used at dosages of 100 to 200 mg/day. Side effects are generally minimal with the most common side effect being menstrual irregularity. Other side effects can include hyperkalemia, postural hypotension, breast tenderness, polyuria, polydipsia, fatigue, and headaches. Because the potential for teratogenic effects (undervirilization of a male fetus) exists with spironolactone or any anti-androgen, concomitant use of OCP is strongly recommended for the adolescent girl.

Recently, insulin sensitizers such as metformin and members of the glitazone family have become therapeutic options especially for adolescents with IGT or T2DM (7,128,232,233). Hence, adolescents with PCOS should undergo periodic screening for abnormal glucose tolerance. In girls with PCOS, metformin therapy was also associated with attenuation of the adrenal steroidogenic response to ACTH (234). Although metformin appears to influence ovarian steroidogenesis directly (235–237), this effect does not appear to be primarily responsible for the attenuation of ovarian androgen production in women with PCOS. Rather, metformin acting through AMP-activated protein kinase (AMPK) inhibits the output of hepatic glucose, necessitating a lower insulin concentration and thereby probably reducing the androgen production of theca cells. The thiazolidinediones improve the action of insulin in the liver, skeletal muscle, and adipose tissue and have only a modest effect on hepatic glucose output. As with metformin (238,239), the thiazolidinediones are reported to affect ovarian steroid synthesis directly (238–240), although most evidence indicates that the reduction in insulin levels is responsible for decreased concentrations of circulating androgen. Obese women with PCOS who took troglitazone had consistent improvements in insulin resistance, hyperandrogenemia, and glucose tolerance (7,130). In addition, troglitazone treatment was associated with a relative improvement in pancreatic beta-cell function and a reduction in levels of the prothrombotic factor plasminogen-activator inhibitor type 1 (PAI-1) (130). Although troglitazone is no longer available, subsequent studies using rosiglitazone (239,240) and pioglitazone (241) have had similar results.

Weight reduction is important in treating overweight patients with PCOS. No unique weight-loss regimen targets excess adiposity specific to the syndrome. Restricting carbohydrates as compared with fats is generally perceived to be advantageous in this patient population. However, several recent studies designed to address this issue have not shown a distinct benefit from calorie-restricted diets limiting carbohydrates rather than fat (242,243).

Flutamide is a potent non-steroidal agent with selective antiandrogenic actions. The administration of flutamide (250 mg/day), in conjunction with an estrogen–progestin, improved acne, seborrhea, and hirsutism in adolescent women (244). Among non-obese adolescent girls with hyperandrogenism, combined use of metformin and flutamide resulted in greater improvements in insulin sensitivity, decreases in androgen concentrations, and resumption of ovulation compared to monotherapy (245). However, flutamide is associated with an increased risk for fulminant hepatitis (246). Cyproterone acetate, a synthetic progestagen with moderate anti-androgen activity, has been used in conjunction with estradiol. Cyproterone decreases testosterone and increases SHBG concentrations, but does not improve insulin resistance and is not currently available in the United States (247). Finasteride, a 5α-reductase inhibitor, has been reported to decrease hirsutism with variable effects on androgen concentrations. Although the majority of adolescent girls are not actively trying to conceive, issues related to potential infertility are a concern to them and their parents. Thus, a brief discussion regarding assisted reproductive technologies is relevant.

SUMMARY AND CONCLUSIONS

A thorough past medical history is the first step in the evaluation of a patient with hirsutism, amenorrhea, and/or irregular menstrual cycles. The age at onset of thelarche, adrenarche, and menarche should be obtained. The presence of a past or current chronic medical disorder and medication use are relevant. Detailed history regarding the development and progression of acne and terminal hair growth is relevant. Because some patients are so embarrassed that they shave the hair growth prior to the evaluation, questions regarding cosmetic treatment are necessary to estimate the true extent of hair growth. Questions to obtain information regarding food intake, exercise, past growth parameters, polyuria, nocturia, and galactorrhea are helpful for diagnosis and management. For the patient with primary or secondary oligo/amenorrhea, questions regarding symptoms of thyroid dysfunction, sexual activity, emotional/psychologic issues, and family dynamics can be extremely relevant. For patients with secondary amenorrhea, a detailed menstrual history regarding menstrual flow, duration, intervals, and dysmenorrhea will help identify the etiology of the menstrual disorder.

History of pubertal development, menarche, hirsutism, and fertility should be obtained from female family members. Because abnormalities of glucose tolerance are common in families of women with PCOS, it is important to ascertain if family members have diabetes.

Height, weight, and body mass index should be recorded. On the physical examination, the extent of terminal hair growth on the face, chest, abdomen, back, buttocks, and thighs should be noted. Extent of breast and pubic hair development should be documented. Presence of additional clinical features such as acne, striae, oily hair, masculine body habitus, clitoral enlargement, acanthosis nigricans, Turner syndrome stigmata, and labial masses should be assessed.

The choice of laboratory studies is driven by the medical history and physical examination. Rapid progression, of the clinical features associated with hyperandrogenism, suggest the diagnosis of an androgen-secreting tumor. When signs and symptoms of androgen excess are present, total and free testosterone, androstenedione, 17-hydroxyprogesterone,

DHEAS, SHBG, prolactin, LH, and FSH concentrations should be determined in a blood sample obtained during the morning hours. In some instances, 3α-diol conjugates, either glucuronides or sulfates, can be elevated in hirsute women.

The finding an elevated morning 17-OHP (>200 ng/dL) may necessitate an ACTH stimulation test to assess for late onset CAH (248). Elevated DHEAS concentrations suggest an adrenal source such as an adrenal tumor for which imaging studies would be helpful. Ovarian androgen-secreting tumors need to be considered when testosterone concentrations are extremely elevated (>200 ng/dL). For patients with features suggestive of Cushing's syndrome, determination of 24-hour urinary-free cortisol excretion, evening cortisol concentrations, and dexamethasone suppression are necessary to confirm excessive glucocorticoid secretion. A pituitary imaging study, MRI or CT, should be obtained in the patient with hyperprolactinemia in the absence of causative medications.

Ultrasonography can provide helpful information regarding uterine anatomy and ovarian function. Endometrial appearance and ovarian volume change throughout the menstrual cycle on transvaginal ultrasound (249). Because the common pathophysiology for anorexia nervosa, chronic illness, and gonadotropin deficiency is decreased gonadotropin secretion, transvaginal ultrasound will show a thin atrophic looking endometrium; the ovaries may appear to be multifollicular with normal size and contain six or more cysts 4 to 10 mm in diameter. For women with PCOS, the ovaries may appear large with large follicles; the endometrium is generally thickened reflecting hyperestrogenization. However, as criteria to define polycystic ovaries are inconclusive, it is important to use clinical features and hormone levels to make diagnosis (194).

The large variety of estrogen–progestin OCPs currently available offer a range of estrogen dosages and contain different progestins (250). Ethinyl estradiol in doses ranging from 20 to 50 mcg is the most commonly used estrogen; mestranol is sometimes used. The effects of the progestins used in OCP reflect interactions with multiple steroid hormone receptors including the progesterone receptor, AR, estrogen receptor, and GCCR. Small structural changes in the progestin molecule may be associated with differences in effects. Many of the early progestins were derived from the testosterone molecule and, therefore, have been associated with the undesirable side effects of acne, water retention, and bloating (251). Drospirenone, a newer progestin available as a combined OCP (30 mcg ethinyl estradiol/3 mg drospirenone, Yasmin®), is derived from spironolactone (252).

The risk of venous thromboembolism is increased by a factor of three to four among the users of low-dose OCPs containing norethindrone acetate, norethindrone, ethynodiol diacetate, levonorgestrel, norgestrel, and lynestrenol (253,254). Recent meta-analyses suggested that OCPs containing desogestrel or gestodene have an increased risk for venous thromboembolic events compared to those on low estrogen formulations containing levonorgestrel (255). The risk for thromboembolic events is increased among women who have protein C deficiency, protein S deficiency, factor V Leiden variant, or prothrombin G20210A mutation (256). Women with hypertension or migraines have an increased risk for cardiovascular complications when taking combined OCP (257). Guidelines published by the American College of Obstetricians and Gynecologists list smoking, uncontrolled hypertension, history of stroke, ischemic heart disease, or venous thromboembolism, and active breast cancer as conditions associated with an increased risk for adverse effects with OCP use (258).

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14

Adolescent Menstrual Abnormalities

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INTRODUCTION

The normal developmental progression from a prepubertal stage to ovulatory menstruation requires an intact hypothalamic–pituitary–ovarian axis. In addition, normal female reproductive structures and adequate nutritional status are necessary for normal menses to occur. Central, gonadal, structural, or systemic abnormalities can lead to menstrual dysfunction. Menstrual abnormalities can be defined as lack of menstruation (primary or secondary amenorrhea), irregular menstruation (oligomenorrhea or dysfunctional uterine bleeding (DUB)) or abnormalities associated with menstruation (dysmenorrhea). Therefore, a discussion of menstrual abnormalities must begin with a description of the current knowledge regarding normal female pubertal development and menarche.

NORMAL PUBERTAL DEVELOPMENT AND MENARCHE

Physiologic Maturation

The progression from the suppressed hormonal axis of the prepubertal period to the pubertal pulses of gonadotropins requires an intact hypothalamic–pituitary–gonadal axis, as well as an appropriate metabolic milieu(1). Alterations in nutritional content, illness states, endocrinologic function, or stress can alter the progression in a previously normal axis. Once the pulsatile gonadotropin response is activated, a normal gonad with appropriate hormone production, functional theca and granulosa cell development, and adequate, responsive oocytes are required to begin menstrual cycling and ovulatory function. Nonovulatory menstrual flow may occur prior to the establishment of regulated gonadotropin surges due to unregulated hormonal fluctuations. These stages may represent a normal progression in pubertal development, prior to the development of cyclical ovulatory cycles. Once established, menstrual cycles can be disrupted by a variety of endocrinologic, systemic, and environmental etiologies. The normal development of the vaginal outflow tract is essential for normal menstrual flow, and the lack of menses in the setting of normal pubertal development may provide evidence for a previously undiagnosed developmental anomaly (Fig. 1) (3).

Pubertal Timing

The progression of female pubertal development and menarche has remained unchanged over the last century (4). However, the age of puberty and menarche has been an area of recent research and debate. The suggestion of a trend toward earlier pubertal development in girls was raised by the Pediatric Research in Office Settings (PROS) that analyzed a large sample of 3 to 12 year old girls. This study sample found the average age of menarche of 12.16 years in African American girls and 12.88 years in white girls (5,6). An elevated body mass index in this population was correlated with younger age of thelarche and menarche. For the American adolescent, the average age of menarche as defined by the Third National Health and Nutrition Examination Survey (NHANES, 1988–1994) is 12.54 years (7), 12.43 years (8), or 12.40 (9), depending on the type of statistical analysis used. There remains agreement among these studies that the African American girls had earlier pubertal development on average 12.13 years, compared to 12.6 years in the white girls (7–9). According to the PROS data (5), 62.1% of African American girls had menarche by age 12 years, and only 35.2% of the white girls. Approximately, 28% of 11 year old African American girls and 13.4% of white girls, and 6.3% of 10 year old African American girls and 1.8% of white girls had reached menarche.

There is an average of two years between the start of thelarche, generally the first sign of puberty,

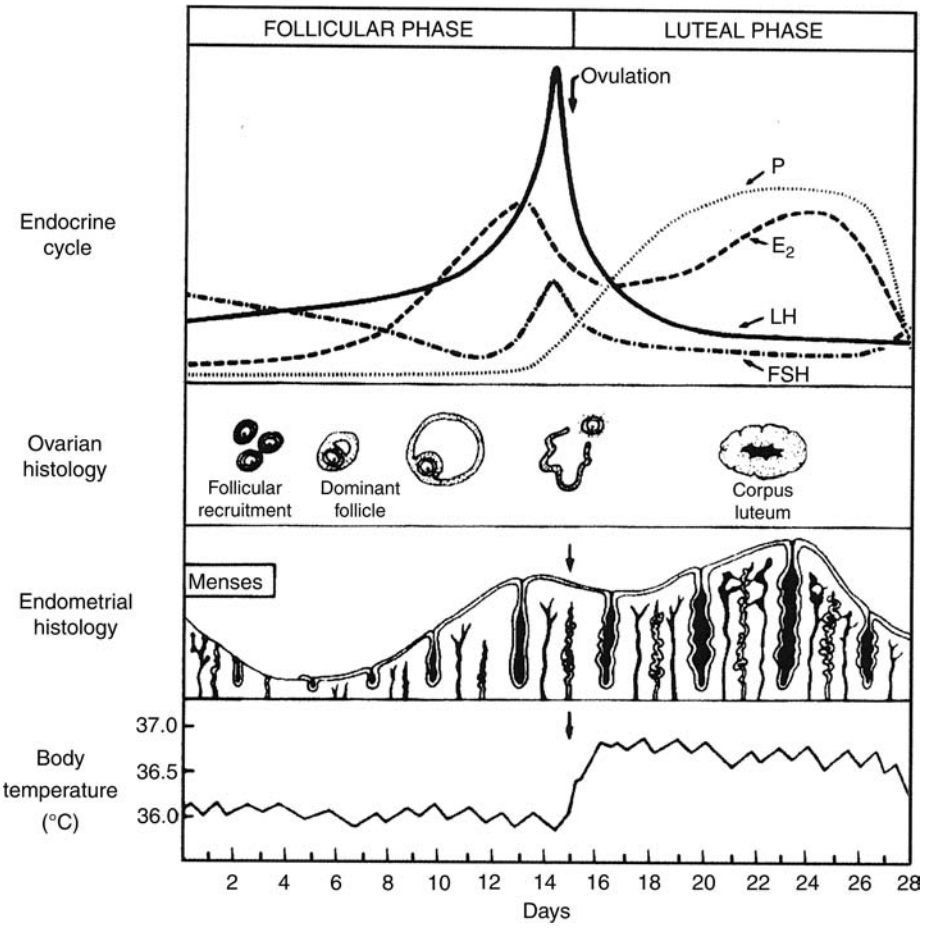

Figure 1 Normal menstrual cycle. *Abbreviations*: P, progesterone; E_2, estradiol; LH, luteinizing hormone; FSH, follicle stimulating hormone. *Source*: Adapted from Ref. 2.

and the onset of menarche. The onset of menarche is fairly constant in adolescent development, with about two thirds of females reaching menarche at a Tanner sexual maturity rating (SMR) of 4. Menarche occurs at SMR 2 in 5% of girls, SMR 3 in 25%, and not until SMR 5 in 10%. The normal duration of menstrual cycles can vary between 25 and 41 days for a given individual, and an adolescent may have anovulatory cycles for the initial two years post menarche. Most menstrual periods last approximately two to seven days, with 30 to 40 ml of bleeding (3,10).

Longitudinal data have confirmed a relationship between age at menarche and final height. Although those with early maturation (e.g., menarche in white girls below 11.7 years and in African American girls below 11.0 years) had the highest peak height velocity, final height was decreased compared to those with average or late maturation (11). The increased height velocity and height gained after menarche in early maturing girls is counterbalanced by a shorter duration of prepubertal growth. In addition, the early maturing girls tended to be heavier, with higher body mass index. Thus, those girls who mature early will be taller and heavier during pubertal development, but obtain a shorter final adult height.

Puberty and Weight

The association between weight and pubertal progression has been well documented. Obesity-related pubertal precocity has been an area of research for half of a century (12). Subsequent literature proposed the theory of a critical weight required for pubertal progression and menarche, or the Frisch hypothesis (13–16). However, data from analyses of girls with altered pubertal progression suggested that there maybe other essential regulators of puberty and menarche (17). More recent literature suggests that leptin and other metabolic regulators may be involved in the association of weight and pubertal development. Leptin, described in Vol. 1; Chap. 1, is a product of adipocytes (18) and is involved in metabolic signaling at the level of the hypothalamus (19–21). Leptin deficiency, due to mutations in leptin or its receptor, leads to an obese phenotype associated with hypogonadotropic hypogonadism (22,23). The supplementation of leptin in a leptin-deficient patient led to both an elevation in gonadotropins and an early pubertal pattern of follicle stimulating hormone (FSH) and luteinizing hormone (LH) secretion (24), Investigations of young women with eating disorders (25,26)

and healthy subjects (27,28) have suggested that leptin may serve as a permissive factor for puberty and menstruation, perhaps acting as a signal of an adequate metabolic state (29).

Genetic Regulators of Puberty

Both rodent and human data have identified the G protein-coupled receptor, GPR54 and its endogenous ligands from the KISS-1 gene to be regulatory factors in pubertal development. Humans and mice with mutations in these genes were found to have hypogonadotropic hypogonadism associated with lack of pubertal development (30–32). The KISS-1 gene encodes for a 145 amino acid peptide that is cleaved into a family of peptides called kisspeptins. Data suggest that these peptides may play a role in the gonadotropin-releasing hormone (GnRH) regulation of LH secretion (33–36). There are a number of neuronal modulators that are also important in GnRH signaling (37). Kallmann syndrome is a cause of isolated pituitary gonadotropin insufficiency associated with anosmia resulting from a lack of or altered development of GnRH and olfactory neurons. The heterogeneity of the genetic defects in this syndrome is becoming evident. The syndrome is inherited in a sex-linked or autosomal manner and it is known that mutations in the KAL-1 gene contribute to this phenotype. More recent evidence indicates that genetic defects in the fibroblast growth factor receptor 1 gene can result in this phenotype (38). Other genetic defects cause isolated congenital hypogonadotropic hypogonadism. They include GnRH receptor mutations and defects in the GPR54 gene (39). Abnormalities in the genes for leptin, the leptin receptor, Prop-1, LHX-3, Dax-1, and LH and FSH are also involved in hypogonadotropic hypogonadism (38,40). FSH deficiency is caused by a homozygous deletion in the β subunit (41). LH and FSH receptor defects have also been characterized (42). The genetic etiology for congenital lipoid adrenal hyperplasia, defined as a mutation in the steroidogenic acute regulatory (Star) protein has been identified. This protein, involved in the translocation of cholesterol into the mitochondrial inner membrane, is essential for steroidogenesis. Individuals with this disorder who are supplemented with glucocorticoids and mineralocorticoids can develop ovarian failure (43). Other steroid pathway defects, such as a deficiency of 3β-hydroxysteroid dehydrogenase type II and 17α-hydroxylase deficiency have been characterized molecularly as etiologies of menstrual dysregulation (Table 1) (42).

There has been a recent expansion of knowledge about the specific genetic contributors to normal pubertal development and menarche. This enhanced understanding may lead to molecular diagnostic tools to categorize more definitively the clinical diagnoses of delayed pubertal development and amenorrhea.

MENSTRUAL DISORDERS

Amenorrhea

Definitions

Primary amenorrhea can be defined by chronologic criteria as the lack of spontaneous uterine bleeding by the age of 14 years, without secondary sexual characteristics, or by the lack of episodes of spontaneous menstrual periods by age 16 years, regardless of normal secondary sex characteristics. In addition, developmental criteria can be used. These criteria include the lack of episodes of spontaneous uterine bleeding, despite having attained a Tanner SMR 5 for at least one year or despite the onset of breast development four years previously. In the specific cases of girls with phenotypic and/or genotypic evidence of Turner syndrome, the lack of spontaneous uterine bleeding by age 14 is suggestive of primary amenorrhea in the setting of primary ovarian failure due to gonadal dysgenesis (Table 2).

Secondary amenorrhea is defined as the cessation of menses for six months, or a length of time equal to three previous cycles that occurs after periodic uterine bleeding has been established.

Etiology

Primary Amenorrhea without Secondary Sexual Characteristics

Amenorrhea in an adolescent who has not experienced thelarche suggests an alteration in gonadotropin stimulation or ovarian response to pulsatile hypothalamic activation. Alterations in genetic coding or abnormal

Table 1 Single Gene Mutations that Influence Pubertal Development on Menstrual Function

Phenotype	Gene	Mode of inheritance
Kallmann syndrome	KAL-1 FGFR1	Autosomal recessive or autosomal dominant
Hypogonadotropic hypogonadism	GPR54 KISS-1	Autosomal resessive
Gonadotropin releasing hormone resistance	GNRHR	Autosomal recessive
Isolated follicle stimulating hormone deficiency	FSHβ	Autosomal recessive
Hypergonadotropic hypogonadism	FSHR	Autosomal recessive
Luteinizing hormone resistance	LHR	Autosomal recessive
Congenital lipoid adrenal hyperplasia	STAR	Autosomal recessive
Galactosemia	GALT	Autosomal recessive
3β hydroxysteroid dehydrogenase type II deficiency	HSD3β2	Autosomal recessive
17α-hydroxylase deficiency	CYP17	Autosomal recessive

Source: Adapted from Ref. 42.

Table 2 Causes of Menstrual Abnormalities

Primary amenorrhea	Secondary amenorrhea	Oligomenorrhea
Primary ovarian failure	*Causes of primary amenorrhea*	*Causes of primary amenorrhea*
(Hypergonadotropic hypogonadism)	Pregnancy	Pregnancy
Congenital	Hyperandrogenic states	Hyperandrogenic states
46, X Turner syndrome	Turner syndrome mosaicism	
Turner syndrome mosaicism		
17α-hydroxylase deficiency		
FSH or LH receptor mutations		
Acquired	Abnormal thyroid function	Abnormal thyroid function
Autoimmune oophoritis		
Irradiation and chemotherapy		
Galactosemia		
Hypogonadotropic hypogonadism:	Hyperprolactinemia	Hyperprolactinemia
Congenital	Hypothalamic amenorrhea	Hypothalamic anovulation
GnRH deficiency (Kallman syndrome)		
GnRH receptor mutation		
GPR54/KISS-1 mutations		
Hypopituitarism		
Prader Willi syndrome		
Bardet Biedel syndrome		
Acquired		
Trauma		
Infiltration (histiocytosis X, suprasellar tumors)		
Anorexia nervosa		
Excessive exercise		
Hyperandrogen disorders		
PCOS		
Congenital adrenal hyperplasia		
Androgen producing tumor		
Structural abnormalities		
Agenesis of Müllerian structures		
Abnormal androgen response/ production in 46 XY individuals		
Complete androgen insensitivity		
17α-hydroxylase. 17, 20-lyase deficiency		
Acquired uterine synechiae		
Pregnancy		

Source: Adapted from Ref. 43.

ovarian hormone response can lead to a normal prepubertal female phenotype without secondary sexual characteristics or menarche.

Primary Ovarian Failure. Primary ovarian failure leads to elevation in central stimuli, causing hypergonadotropic hypogonadism. Approximately 30% of primary amenorrhea is caused by genetic abnormalities. Turner syndrome, 45X genotype, is the most common genetic cause of gonadal failure (see Vol. 2; Chap. 12). In addition, related genotypes (45,X, 45 XX/X, or 45 XY/X) can also cause altered ovarian function. Stigmata are variable and can be limited, but classically include short stature; streaked gonads; sexual infantilism; and somatic anomalies (webbed neck, short fourth metacarpal, cubitus valgus, and coarctation of the aorta). Individuals with Turner syndrome mosaicism: (45X/46XX) exhibit a variety of phenotypes. Eighty percent of such individuals are short, 66% have some somatic anomaly, and 20% have spontaneous menses. The characteristics for X/XXX and X/XX/XXX individuals are similar to those for X/XX individuals. The lack of aneuploidy or a normal peripheral karyotype does not exclude the possibly of genetic causes of ovarian dysgenesis. A structurally abnormal X chromosome with microdeletions or mutations can lead to variable phenotypes. A long-arm deletion commonly causes normal stature, no somatic abnormalities, streaked gonads, and sexual infantilism. Short-arm deletion defects lead to a phenotype similar to that of Turner syndrome (45).

Pure gonadal dysgenesis with a normal female peripheral karyotype, mosaicism (46 XX/XY), or male karyotype can present as ovarian failure with streaked gonads, sexual infantilism, normal stature, and normal somatic features. Pure gonadal dysgenesis with a 46 XY karyotype carries a risk for gonadoblastoma due to the Y chromosome component of gonadal tissue.

Premature ovarian failure can be caused by more subtle genetic abnormalities or heritable metabolic abnormalities. A fragile X premutation in a female carrier can cause ovarian failure. This diagnosis can be important for prognostication and for familial genetic counseling about the most common form of genetic mental retardation (46). Galactosemia, an inborn error of metabolism, is diagnosed by newborn screening evaluation. Untreated elevations in galactose can lead to altered ovarian function (47). Rare LH and FSH receptor defects can cause a failure of ovarian response to elevated gonadotropin levels. A variety of pubertal changes, and both primary and secondary amenorrhea have been reported with these abnormalities. Females are less affected than males by LH receptor mutations, but can present with altered ovarian function, such as polycystic ovary syndrome (PCOS), and/or primary amenorrhea (48–50).

Acquired ovarian failure can be caused by autoimmune oophoritis, galactose accumulation, or gonadal injury by radiation and chemotherapeutic agents for the treatment of oncologic disease. Gonadal trauma may also result in ovarian failure. Evidence of autoimmunity, such as ovarian and/or adrenal antibodies, suggests the need to evaluate adrenal status and consider clinical evidence of other autoimmune phenomenon, such as, hypoparathyroidism, thyroiditis, pernicious anemia, or type 1 diabetes. In individuals with premature ovarian failure without adrenal autoimmunity the prevalence of thyroid autoantibodies has been documented to be 14%, parietal cell antibodies 4% and pancreatic antibodies 2% (51). Autoimmune polyglandular syndromes (chap. 26 for details) can be categorized into type 1, the association of autoimmune adrenalitis, hypoparathryoidism, and mucocutaneous candidiasis. This syndrome, also termed the autoimmune polyendocrinopathy–candidiasis–ectodermal dysplasia complex, can be associated with ovarian failure in up to 60% of individuals. Autoimmune polyglandular type 2 includes Addison's disease in association with hypothyroidism or type 1 diabetes. The prevalence of ovarian failure in APS type 2 is significantly lower, at 10% to 25% (52,53). The prevalence of ovarian antibodies is related to the association with other autoimmune disorders, particular Addison's disease. Some studies have reported 60% to 100% of patients with primary ovarian failure and Addison's disease will have detectable autoantibodies, while those with ovarian failure alone have antibodies detected in less than 1% of cases (54). However, limitations in the techniques for isolation of ovarian autoantibodies have hindered consistent analyses of prevalence (55). The prevalence of ovarian autoantibodies in primary ovarian failure has been reported to range from 20% to 69% (56–60).

Ovarian failure can be caused by radiation and/or chemotherapy for cancer treatment. With the increasing success of therapy for childhood cancers, there is a growing population of individuals with secondary ovarian failure from these therapies (61–65). Individuals treated before puberty may present with lack of pubertal development, primary or secondary amenorrhea.

Enzymatic deficiencies can cause alterations in ovarian function resulting in amenorrhea. 17α-hydroxylase deficiency with a normal female karyotype can present with a phenotype of normal stature, sexual infantilism, amenorrhea, hypertension, and hypokalemia. Laboratory evaluation displays elevated progesterone levels, low 17α-hydroxyprogesterone concentrations, and elevated serum deoxycorticosterone levels. Androgen and estrogen production is decreased due to the enzymatic blockade (66,67).

Hypogonadotropic Hypogonadism. Central causes of the lack of secondary sexual development and menstruation are abnormal hypothalamic stimulation or pituitary response. The resulting deficiency of gonadotropins is called hypogonadotropic hypogonadism. Identified genetic causes are discussed previously (Table 1).

Other congenital syndromes are associated with hypogonadotropic hypogonadism and obesity, such as Prader–Willi syndrome and Bardet–Biedel syndrome (68–70). Developmental anomalies of the hypothalamus and/or pituitary, often with other midline defects, can be associated with deficient gonadotropin production (40).

Chronic illnesses, particularly those with systemic inflammation and failure to thrive, can affect the hypothalamic–pituitary axis. Examples include cystic fibrosis (71,72) and chronic renal disease (73).

Anorexia nervosa and excessive exercise can be associated with primary amenorrhea and lack of secondary sexual characteristics if these issues arise manifested at a prepubertal age. More commonly, they result in primary or secondary amenorrhea associated with normal secondary sexual development. In addition, inflammation, tumor or trauma can interrupt the hypothalamic pituitary communication and result in a hypogonadotropic state.

Primary Amenorrhea with Normal Breast Development and Abnormal Female Reproductive Structures

Complete androgen insensitivity (formerly testicular feminization) is present in individuals with a XY-karyotype in whom the Wolffian ducts fail to develop and the external genitalia develop as female in the absence of a response to testosterone stimulation. The underlying defect is a mutation in the androgen receptor, rendering it insensitive to testosterone's actions. Because Müllerian inhibitory factor (MIF) continues to be made by the Sertoli cells of the male gonads, the Müllerian ducts regress, and there is lack of formation of internal female genitalia. At puberty, the low levels of endogenous gonadal and adrenal estrogens, unopposed by androgens, result in thelarche and progressive breast development. Because of the end-organ insensitivity to androgens, there is sparse or absent pubic and axillary hair (74).

Developmental abnormalities of the Müllerian structures result in amenorrhea due to the absence of the uterus or an outflow obstruction. An obstruction in the vaginal canal with normal hormonal status leads to premenstrual symptoms often diagnosed as cyclical abdominal pain. The young woman may develop hematocolpos or hematometra. Agenesis of the Müllerian structures (Mayer–Rokitansky–Hauser syndrome) causes vaginal agenesis and/or uterine anomalies. It can be associated with renal and skeletal anomalies (75,76).

Frasier syndrome and Denys–Drash syndrome caused by mutations in the Wilm's tumor, WT1 are characterized by a female phenotype with a 46XY karyotype and rapidly progressive glomerulopathy. Dysgenetic gonads are variable and may allow for some pubertal progression with subsequent amenorrhea. These individuals are at risk for the development of gonadoblastoma (77).

Primary Amenorrhea with No Breast Development and No Uterus

The combination of abnormal female reproductive structures and lack of pubertal progression is a rare cause of primary amenorrhea. The individual usually has a male karyotype, elevated gonadotropin levels, and testosterone values that are either equal to or less than a normal female level. The gonads produce enough MIF to inhibit the development of female internal genital structures, but not enough testosterone to develop male internal and external genitalia. The causes include 17,20-lyase/17α-hydroxylase deficiency, and agonadism with the lack of development of Müllerian or Wolffian structures.

Secondary Amenorrhea

Pregnancy should be considered as the most common cause of secondary amenorrhea. In addition, it must be considered on the differential diagnosis of primary amenorrhea.

The large majority of the genetic, structural, and acquired causes of primary amenorrhea can cause secondary amenorrhea due to the variable presentation of these disorders. For example, the presentation of primary gonadal failure due to Turner syndrome can have a variable phenotype consistent with slowly progressive ovarian failure, and normal early pubertal changes and menarche.

Hypothalamic dysfunction can be caused by structural lesions, medications, environmental stressors, chronic illnesses, or other endocrinopathies. Infiltrative lesions can include suprasellar tumors such as craniopharyngiomas, germinomas, teratomas, or gliomas. Inflammatory lesions including tuberculous granulomas, meningoencephalitis, histiocytosis, and sarcoidosis can cause hypothalamic and/or pituitary dysfunction. The pituitary can be the site of infiltration, infarct, or tumor. Traumatic injury of the pituitary stalk due to sheer stress can cause hypopituitarism and amenorrhea. Medications that can influence hypothalamic activity include phenothiazines, estrogen and progestin contraceptives, glucocorticoids, and heroin, among others. Other endocrinopathies such as hyperthyroidism or hypothyroidism, cortisol excess in Cushing syndrome, or hyperprolactinemia from medication use or a prolactinoma can dysregulate the hypothalamic stimulation of LH and FSH secretion. Emotional and/or physical stress can also result in hypothalamic dysfunction.

Excessive exercise is a common cause of secondary or primary amenorrhea. Athletes, particularly runners, gymnasts, competitive divers, figure skaters, and ballet dancers, have high rates of amenorrhea associated with high rates of disordered eating. The female athlete triad has been described as the combination of disordered eating, amenorrhea, and decreased bone density (78–80). The prevalence of secondary amenorrhea in adult athletes ranges from 3.4% to 66% depending on the sport studied (81). As many as 18% of female recreational runners, 50% of competitive runners training 80 miles/wk, and 47% to 79% of ballet dancers may be amenorrheic (78,82–84).

The pulsatile nature of LH, and thus normal menstrual function, appears dependent on energy availability. Low energy availability appears to result in a hypometabolic state that can include the metabolic alterations, hypoglycemia, hypoinsulinemia, euthyroid sick syndrome (low total triiodothyronine, T_3), hypercortisolemia, and suppression of leptin secretion (81,85). Leptin has been shown to be a permissive factor for menstruation, likely due to its correlation with adequate fat mass (25). Although both amenorrheic athletes and regularly menstruating athletes have reduced LH pulsatile secretions and reduced 24-hour mean leptin levels, amenorrheic runners have more extreme suppression and disorganization of LH pulsatility. The specific level of energy availability needed to maintain normal reproductive function is not known.

Significant weight loss with or without excess exercise can cause amenorrhea. The mechanism of amenorrhea appears to be hypothalamic derangement. The estradiol levels in patients with weight loss and anorexia nervosa can vary from low to normal, depend on the stage of amenorrhea. Consequently, such individuals may or may not respond to progesterone withdrawal with uterine bleeding.

Hyperandrogenic states are known to cause amenorrhea due to a variety of proposed mechanisms. PCOS is the most common cause of hyperandrogenism in both women and adolescent girls (86). Many researchers believe PCOS to be primarily a hypothalamic disorder with dysregulation of LH and FSH secretion. A 1990 US NIH consensus conference identified key features for the diagnosis of PCOS (87): hyperandrogenism, menstrual dysfunction, clinical evidence of hyperandrogenism, and the exclusion of congenital adrenal hyperplasia. Probable criteria for PCOS

included insulin resistance and perimenarchal onset. The 2003 Rotterdam consensus workshop defined PCOS more broadly, recognizing ovarian dysfunction as the primary component, without mandatory anovulation. The revised definition included two of the three criteria: oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries by ultrasonography, with the exclusion of other etiologies of hyperandrogenism (88). The consensus definitions are broad, allowing for a clinical and biochemical diagnosis of a wide spectrum of phenotypes. Recent data have contributed to the theory that PCOS is a heritable, developmental abnormality, manifesting in the peripubertal period (89,90). Other pathologic forms of hyperandrogenism such as late onset congenital adrenal hyperplasia, exogenous androgen use or an androgen-producing tumor can also cause primary or secondary amenorrhea.

Oligomenorrhea, Menometrarrhagia, and Dysfunctional Uterine Bleeding

The etiologies of secondary amenorrhea can also cause irregular menstrual flow. The most common etiology in adolescents is anovulatory DUB. Some other common etiologies include pregnancy, hyperandrogenism, particularly PCOS, hyperprolatinemia, or thyroid abnormalities. Adolescents can present with vaginal bleeding as a sign of a sexually transmitted disease, non-compliance with hormonal therapies, or sexual abuse or assault. Although rare, vulvovaginitis, trauma, skin lesions, foreign bodies, or tumors can present as irregular bleeding, particularly in the preadolescent female.

DUB is irregular or prolonged vaginal bleeding seen in the absence of structural pathology or systemic disease (91,92). Menorrhagia (or ''hypermenorrhea'') is defined as prolonged ($>$ seven days) or excessive (>80 ml) uterine bleeding at regular intervals. The most common cause of DUB in adolescents is immaturity of the hypothalamic–pituitary–ovarian axis. Menorrhagia that presents at menarche may be associated with underlying bleeding abnormalities. Possible etiologies include abnormalities in platelet number and/or function, a coagulation defect, or von Willibrand's disease (93).

Dysmenorrhea and Premenstrual Syndrome

Dysmenorrhea, or pain with menses, is common among adolescents. Twenty to ninety percent of female adolescents report dysmenorrhea (91). Pelvic pain secondary to anatomic or organic causes must also be considered (e.g., endometriosis; outflow tract obstruction; gastrointestinal, urogenital, reproductive, or musculoskeletal abnormalities or disease). The symptoms are incapacitating for some young women, resulting in absence from school or work (94). A study of college students found an association between dysmenorrhea and anxiety, depression, and loss of social support networks (95).

Behavioral and somatic symptoms occurring during the luteal phase that are severe and incapacitating to some degree have been termed the premenstrual syndrome. This syndrome has a prevalence of up to 2.5% of women (96). This array of predictable physical and behavioral symptoms occurs cyclically and resolves quickly with the onset of menstruation. The etiology is unknown, but may be secondary to reduced serotonergic function during the luteal phase and/or an altered γ-aminobutyric acid receptor complex response (97).

DIAGNOSIS AND HISTORY

Diagnosis

The evaluation of amenorrhea, oligomenorrhea, menorrhagia, or DUB can be carried out through a thorough history, physical examination, and performance of laboratory tests in a logical sequence. It is essential to rule out the diagnosis of pregnancy before conducting an extensive evaluation.

History

The initial history should include an assessment for evidence of a systemic disease. Diseases associated with secondary amenorrhea such as anorexia nervosa, inflammatory bowel disease, diabetes mellitus, and a pituitary adenoma can be ascertained by identifying fluctuations in weight, bowel habits, urination patterns, headaches, or visual changes. A history of thyroid dysfunction is particularly important, because even mild thyroid dysfunction can lead to menstrual abnormalities. The family history should include parental pubertal timing and growth patterns, as well as mother's and sister's ages of onset of menarche. In addition, a family history should include evidence of any thyroid disease, diabetes mellitus, eating disorders, menstrual, or bleeding problems. A thorough past medical history is essential, including a discussion of childhood development, and that individual's age of onset of thelarche and adrenarche, and the presence or absence of a growth spurt. As psychiatric illness and stress can contribute to menstrual irregularities, an assessment of the patient's emotional status is important. In addition, a sensitive history of medications, including illicit drug use, can lead to identification of the cause of menstrual dysfunction. For example, heroine and methadone are strongly correlated with menstrual dysfunction (98). A nutrition and exercise history should be completed, particularly, questions about sports that may predispose to amenorrhea. The history should include a sexual history with questions about contraception use and symptoms of pregnancy. A complete history of menarche and early menstrual patterns can assist in the diagnosis of secondary amenorrhea. In addition, evidence of androgen excess such as hair distribution and acne, can be suggestive of PCOS or another ovarian or adrenal abnormality.

Physical Examination

A complete physical evaluation should include height, weight, and sexual maturity staging. The physical examination should include an evaluation for signs of systemic disease or malnutrition. A clinician should check for signs of androgen excess, such as acne or hirsuitism. Physical evidence of other endocrinopathies, such as a goiter, acanthosis nigricans, cushingoid habitus, or galactorrhea should be sought. Stigmata of Turner syndrome such as short stature, webbed neck, low-set ears, broad shieldlike chest, short fourth metacarpal, and increased carrying angle of the arms can be visualized. Petechiae, echymoses, or other signs of bleeding would be noteworthy, especially in the setting of menorrhagia at the time of menarche. The evaluation can include a test for anosmia. The pelvic examination may not be necessary in all individuals, but can include a search for a stenotic cervix, vaginal agenesis, imperforate hymen, transverse vaginal septum, absent uterus, or pregnancy.

Laboratory Evaluation

The laboratory evaluation can be divided into those adolescents with the following conditions (Fig. 2).

Primary and Secondary Amenorrhea with Normal Secondary Sex Characteristics and Normal Genitalia

For primary and secondary amenorrhea with normal secondary sex characteristics, the laboratory evaluation depends on the presence of specific signs and symptoms. If there is evidence of galactorrhea, the adolescent should be evaluated for a prolactinoma or an exogenous source of prolactin, such as medication use. If there is evidence of hyperandrogenism, an evaluation of androgen levels and source should be initiated. A pregnancy test should always be considered. If clinically indicated, diabetes mellitus and/or thyroid abnormalities should be evaluated with measurements of blood glucose levels and thyroid function tests. Uterine synechiae or Asherman syndrome, should be considered if there is a history of dilation and curettage or endometritis. This condition may cause partial or total obliteration of the uterine cavity. If this problem is suggested by the history, a gynecological referral for evaluation by hysteroscopy or hysterosalpingography is indicated. If no underlying endocrinologic or systemic illness is diagnosed, one can consider and assessment of the uterine estrogen exposure with the administration of a progesterone withdrawal test. A positive response correlates with circulating estradiol levels adequate to prime the endometrium. A positive response (ranges from minimal brown staining to normal menstrual flow) indicates a serum estradiol concentration of greater than 40 pg/mL.

If there is no response to progesterone, then either hypothalamic-pituitary dysfunction or ovarian failure is likely. Measurement of gonadotropin concentrations can be informative. A high FSH level indicates ovarian failure, whereas, a normal or low FSH level suggests a hypothalamic-pituitary disturbance. If ovarian failure is suspected, a karyotype, antiovarian antibodies, and screening for autoimmune endocrinopathies should be considered. If hypothalamic-pituitary failure is suspected, a magnetic resonance imaging (MRI) scan, visual fields, and pituitary stimulation tests should be considered. If the LH:FSH ratio is elevated (>1.5–2) in the setting of secondary amenorrhea or oligomenorrhea, PCOS should be placed higher in the differential diagnosis. Evidence of biochemical or physical signs of hyperandrogenism would be further supportive evidence.

Individuals with weight loss, anorexia nervosa, heavy substance abuse, or heavy exercise may or may not withdraw to progesterone. If they do not experience withdrawal bleeding within 10–14 days after discontinuing the progesterone, it is indicative of low estradiol levels.

Primary Amenorrhea and Absent Secondary Sex Characteristics or Absent Uterus or Vagina

In general, breast development should be at least at SMR 4 to be considered indicative of full gonadal function. A breast stage of 2 or 3 may indicate adrenal function alone without gonadal function. If the examination reveals normal breast development, but an absent uterus and blind vaginal pouch, a karyotype and a test for serum testosterone concentrations are indicated. The diagnoses can include 46,XX female with congenital absence of the uterus, or a 46,XY female with complete androgen insensitivity.

If the examination reveals absent secondary sex characteristics but a normal uterus, an FSH test is ordered. A low or normal FSH level suggests a hypothalamic or pituitary abnormality, and a careful neuroendocrine evaluation is necessary. A high FSH level and a blood pressure within the reference range suggest a genetic disorder or gonadal dysgenesis. A karyotype should be ordered. A high FSH level and hypertension suggest 17α-hydroxylase deficiency. This is confirmed by an elevated progesterone level (>3 ng/mL), low 17α-hydroxyprogesterone level (<0.2 ng/mL), and an elevated serum deoxycorticosterone level (66,67).

The absence of both breast development and uterus or vagina is very rare. These findings suggest gonadal failure and the presence of MIF secretion from testicular tissue. This could arise from anorchia occurring after MIF activity was present or an enzyme block, such as a 17,20-lyase defect. The evaluation should include LH, FSH, progesterone, and 17-hydroxyprogesterone measurements, and a karyotype (99).

Menorrhagia

The physical examination should also consider evidence of trauma (e.g., vaginal laceration). The laboratory evaluation for menorrhagia or DUB should include a

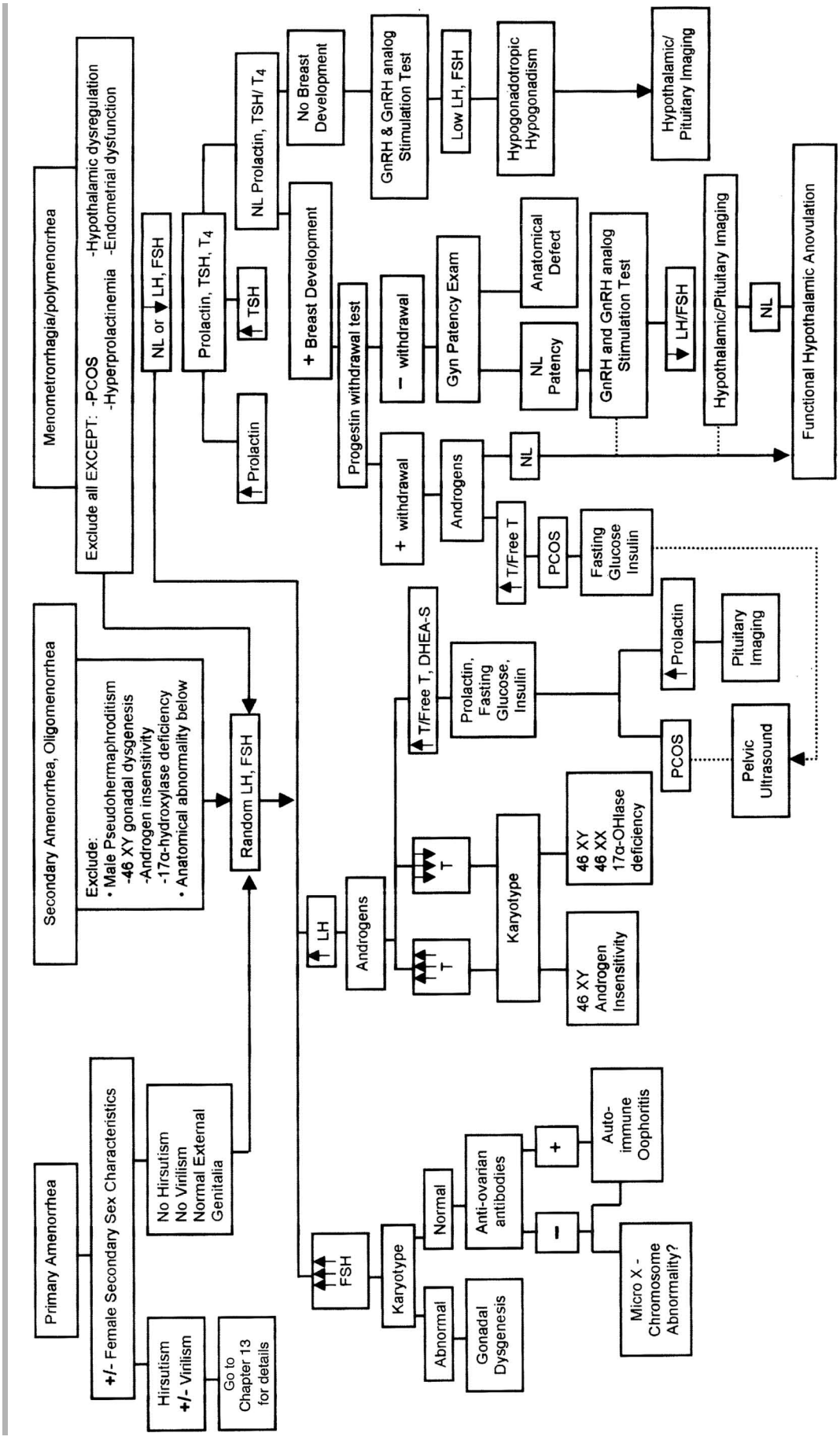

Figure 2 Evaluation of Amenorrhea. *Abbreviations*: T; testosterone; FSH; follicle stimulating hormone; LH; luteinizing hormone; NL, normal; TSH, thyroid stimulating hormone; Ty, thyroxine.

Table 3 Medical Therapy for Menstrual Disorders in Adolescent Females

Amenorrhea	
Development of secondary sexual characteristics *Incremental increase of*: Conjugated estrogen (Premarin) 0.3–0.625 mg × po × qd × 1–2 yr or estrone sulfate (Estratab) 0.3 mg starting dose or micronized estradiol (Estrace) 0.5 mg starting dose or transdermal estradiol (Vivelle dot) 0.025 mg starting dose	**Menstrual cycle regulation** *Estrogen preparation*: 0.625 mg of conjugated estrogen or 0.1 mg of transdermal estradiol or 20 mcg of ethinyl estradiol days 1–25 +Medroxyprogesterone (Provera) 5–10 mg days 16–25 or low dose combined oral contraceptive pills with 20–35 mcg ethinyl estradiol + progestin
Dysfunctional uterine bleeding:	
Menstrual cycle regulation (see above)	**Reduction in pain or volume of menstrual flow:** Nonsteroidal antiinflammatory drugs Cyclical progestins Oral contraceptive pills SSRI Alprazolam GnRH agonists **Heavy bleeding:** Combined oral contraceptive pills 30–35 mcg ethinyl estradiol every 6–12 hours, decreasing by one pill per day over 4–5 days **Severe bleeding:** High dose oral contraceptive pills 50 mcg every 6 hr plus anti-emetic or conjugated equine estrogen 25 mg IV every 4–6 hr
PCOS (See Chapter 13 for details): Combined oral contraceptive pills *Insulin sensitizing agents*: Metformin 500 mg daily, increasing to maximum of 2 g/day *Antiandrogen therapy*: Spironolactone 25 mg daily, increasing to 100–200 mg/day	

urine pregnancy test, complete blood count, and if present at the time of menarche, a reticulocyte count and coagulation studies (91–93). A relatively new test, PFA-100, can also be helpful as a preliminary screen for a bleeding disorder (100).

Medical Treatment

Primary Amenorrhea

Therapy for hypogonadotropic hypogonadism should begin with estrogen therapy (0.3 mg conjugated estrogen, Premarin, or equivalent, per day or less to avoid premature epiphyseal closure if the adolescent has short stature). A transdermal patch can be used, starting at 0.025 mg of estradiol per day, or 1/2 of the 0.3 mg premarin pill can be administered. Other options include estrone sulfate, Estrab, at 0.3 mg daily or micronized estradiol, Estrace, at 0.5 mg daily. Patients with normal height can receive up to 0.625 mg/day of conjugated estrogen (Premarin) or 0.1 mg of estradiol via transdermal patch. High doses of estrogen and premature introduction of progesterone can cause increased subareolar breast development and abnormal contours. A typical maintenance schedule would include 0.625 to 1.25 mg/day of conjugated estrogens on days 1 through 25 of each month, or twice weekly estrogen patch application at 0.1 mg of estradriol with 10 mg of medroxyprogesterone acetate on days 12 through 25. The progestin is added to induce withdrawal bleeding and thus avoid endometrial hyperplasia. Some patients will opt to continue combination estrogen/progestin therapy with oral contraceptive pills with the ethinyl estradiol dose ranging from 20 to 35 μg or combination patch therapy (Table 3). If pregnancy is desired, pulsatile GnRH administration is an option.

Genetic abnormalities causing gonadal failure can be treated with similar hormonal supplementation. If a Y chromosome is present in an XX-karyotyped individual, gonadal removal is necessary due to the risk of gonadoblastoma.

Enzymatic defects such as 17α-hydroxylase deficiency, 17,20-lyase deficiency or 21-hydroxylase deficiency, late onset congenital adrenal hyperplasia, require both glucocorticoid and estrogen–progestin replacement. If complete androgen insensitivity is diagnosed, the individual requires gonadal removal due to the risk of gonadal malignant transformation. However, the gonadal tissue may provide sufficient hormone production to allow for the development of secondary sexual characteristics and therefore, the appropriate timing for removal should be individualized for each patient. After the testes are removed, maintenance estrogen therapy is needed.

In structural abnormalities of the female reproductive structures with normal female gonads, the young women may not require hormonal replacement therapy. However, some patients may require a vaginoplasty for normal sexual function

and an MRI or intravenous pyelogram to rule out renal anomalies.

Primary and Secondary Amenorrhea with Normal Secondary Sex Characteristics

PCOS is a common cause of amenorrhea and the treatment of this condition is an area of active research. Medroxyprogesterone acetate (10 mg) can be given alone for 10 days every one to two months to induce withdrawal bleeding and avoid a vicious cycle of DUB. Estrogen and progestin, given as oral contraceptive pills is a first-line therapy for primary or secondary amenorrhea. Antiandrogens, most commonly prescribed as spironolactone, are helpful in addition to an oral contraceptive in adolescents who have hirsutism. Insulin sensitizing agents should be considered for adolescent girls with clinical (acanthosis nigricans) or biochemical (elevated serum insulin level) evidence of hyperinsulinism. The threshold to prescribe these agents should be lowered if there is a family history of type 2 diabetes mellitus and/or early cardiovascular disease. When pregnancy is desired, referral for use of clomiphene citrate and or insulin sensitizing agents, such as metformin can be recommended (Chap. 13).

In hypothalamic-pituitary dysfunction and ovarian failure, the precipitating cause must be diagnosed and treated, if possible. Hormonal therapy with combined estrogen and progestin, often provided as an oral contraceptive, is recommended to induce uterine bleeding every one to two months. The diagnosis of uterine synechiae requires referral to a gynecologist for possible transhysteroscopic lysis of the adhesions.

Treatment of menstrual dysfunction caused by eating disorders or excess exercise should include counseling on nutrition and physical activity in order to address the primary etiology. Provision of estrogen replacement therapy to preserve bone density is a common practice in these patients, although data are conflicting regarding the skeletal benefits of this therapy (101). Conjugated estrogens, in doses that improve bone mineral density (BMD) in postmenopausal women, in combination with medroxyprogesterone, has not been shown to improve BMD in young women with anorexia nervosa (102). Adrenal and gonadal androgens, growth hormone, IGF-I, and bisphosphonates are being investigated in clinical trials (103–106). Calcium intake should be increased to 1500 mg/day (as elemental calcium) and vitamin D provided at the recommended daily allowance (400 IU/day) in these young women. If a young woman does not withdraw to a ''progestin challenge'' (short course of progesterone) or has a documented low circulating estradiol level, estrogen/progestin replacement therapy should be considered, particularly in amenorrheic athletes who show no signs of gaining weight or reducing activity after six months.

The practitioner should evaluate these individuals, as outlined previously, to eliminate the possibility of pregnancy, thyroid dysfunction, prolactinoma, or a disorder of androgen excess. It should not be assumed that amenorrhea is simply secondary to exercise or weight status.

Menorrhagia

The treatment for excessive menstrual flow is determined by the severity, etiology, and underlying condition of the affected individual (91). Treatment goals include provision of estrogens (to halt endometrial bleeding) and progestins (to induce endometrial stability). Severe menorrhagia can necessitate a hospitalization for stabilization of hemodynamic status if the hematocrit is severely low. In such instances, higher doses of oral contraceptive pills (50 μg of ethinyl estradiol) or intravenous estrogen therapy (conjugated equine estrogen at 25 mg every 4–6 hours) may be necessary to reduce the menstrual flow. More commonly, outpatient therapy with the use of low dose combined oral contraceptive pills, 30 to 35 μg of ethinyl estradiol, can be utilized to halt excessive flow. The use of up to 4 pills per 24-hour period can be initiated for several days with a subsequent wean to once daily therapy. Resulting anemia can be treated with dietary and iron supplementation and a close follow up evaluation (107). Avoidance of DUB can be achieved by administration of oral medroxyprogesterone, 5 to 10 mg daily for 10 days every 6–8 weeks, to induce withdrawal bleeding in patients with prolonged oligomenorrhea in the absence of estrogen deficiency (e.g., PCOS).

Dysmenorrhea and Premenstrual Syndrome

The use of non-steroidal anti-inflammatory preparations is the most common therapy to alleviate symptoms of dysmenorrhea (92). Hormonal therapy, most commonly provided as oral contraceptive pills, can also be used in the treatment of dysmenorrhea as ovulation is suppressed. Because of this underlying therapeutic mechanism, some authorities consider provision of oral contraceptives to be the first-line recommendation. There are some data to support the use of serotonin-reuptake inhibitors (SSRIs) (108), alprazolam (109), and GnRH agonists (110). The former are effective in stabilizing mood during the premenstrual phase, and the latter in reducing disabling cramps (96). SSRIs are the agents of choice for severe premenstrual syndrome (111). Lifestyle changes such as improved stress management, vitamin supplementation (e.g., provision of vitamin B6 and E, magnesium, calcium), and increased exercise are other treatments that may alleviate symptoms (97).

Contraception

In evaluating amenorrhea or irregular menstrual bleeding, pregnancy must be considered. Prevention of pregnancy in the adolescent population includes education, as well as family and community support. Abstinence should be stressed in this population as a means to avoid pregnancy and sexually transmitted

Table 4 Options for Emergency Contraception

Brand	Pills per dose	Ethinyl estradiol per dose in μg	Levonorgestrel per dose in mg	Antinausea medication recommended
Plan B	1	0	0.75	No
Preven	2	100	0.50	Yes
Alesse	5	100	0.50	Yes
Aviane	5	100	0.50	Yes
Levora	4	120	0.60	Yes
Lo/ovral	4	120	0.60	Yes
Nordette	4	120	0.60	Yes
Ovral	2	100	0.50	Yes
Triphasil	4	120	0.50	Yes

Source: Adapted from Ref. 113.

disease. In addition, education regarding contraceptive methods is important. Contraception for adolescents can include hormonal strategies, such as combined oral contraceptive pills, combined contraceptive patches, or medroxyprogesterone injections. In addition, barrier methods such as condoms are essential for the prevention of sexually transmitted diseases.

The use of emergency or post-coital contraception has gained more attention in recent years, although the practice has been described in the literature since 1974 (112). Historically, combined oral contraceptive pills have been used in combination to provide a minimum of 100 μg of ethinyl estradiol and a minimum of 0.50 mg of levonorgestrel, or 1.0 mg of norgestrel. The pills are given as two doses, 12 hours apart, within 72 hours of unprotected sexual intercourse (Table 4). There are two FDA approved regimens for use as emergency contraception. These include Preven (ethinyl estradiol, 50 μg and levonorgestrel, 0.25 mg), similar to the hormones in a combined oral contraceptive formulation, and plan B (levonorgestrel, 0.75 mg), a progestin-only formulation. Preven is prescribed as two pills taken at once and repeated 12 hours later. A pregnancy test prior to use is prescribed and an antiemetic is necessary (114). Plan B is prescribed as one pill per dose given 12 hours apart. There is less nausea and vomiting with this preparation and it may be more effective. This preparation has recently been approved for over-the-counter use in adults (115).

CONCLUSIONS

Normal female pubertal development includes the attainment of secondary sexual characteristics and menarche in a relatively constant sequence. Recent literature has questioned the ''normal'' timing of puberty in the U.S. population. However, variation from the normal sequence of events still necessitates a thorough evaluation.

New research has identified specific genes and metabolic factors involved in regulating pubertal progression. Thus, the definition of normal puberty and menarche has expanded from the clinical to the molecular level. This may lead to the development of new tools to characterize pubertal signals and menstrual irregularities.

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15

Disorders of Sexual Differentiation

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INTRODUCTION

The establishment of chromosomal sex (46,XY or 46,XX) is considered the first step in normal sexual differentiation. However, the first sexual structures that form may be considered as either primordial or neutral. The term sex determination refers to the process in which the primordial gonad is committed to development as testis or ovary. Sex differentiation refers to the development of the internal accessory duct structures and external genitalia (1–5).

GONADAL DIFFERENTIATION

Embryology

Bipotential Gonad

The bipotential gonad develops from an out pouching of tissue on the posterior aspect of the celomic cavity on either side of the aorta. This structure is termed the urogenital ridge. It consists of three regions: the pronephros, the mesonephros, and the metanephros. The central region, the mesonephros, gives rise to the bipotential gonad and the first renal system. The primitive gonad forms on the medial portion of the urogenital ridge and is apparent at about four weeks of gestation. It consists of cells from the underlying mesonephric mesenchyme, cells of the celomic epithelium, and germ cells. Germ cells migrate from the yolk sac and enter the gonadal ridge during the fourth and sixth week of gestation. They undergo mitotic division during migration and continue to divide after arrival in the gonadal ridge (4,6).

Testis Determination

Testis determination occurs at about six weeks of gestation. The first histological evidence is the proliferation of cells from the celomic epithelium. These cells migrate into the underlying mesenchyme and give rise to Sertoli cells (7). Sertoli cells encircle germ cells, form the testis cords, cause mitotic arrest of germ cells, and secrete both antiMüllerian hormone (AMH) and inhibin. Leydig cells develop later than Sertoli cells and begin to secrete testosterone at seven to eight weeks of gestation (8). Peak serum levels of testosterone are reached by the 16th week of gestation. After testis determination, a second migration of cells from the mesonephros occurs. This migration is necessary for the development of the celomic vessel and the formation of the peritubular myoid cells that stabilize the testis cords (9–11).

Ovarian Determination

Ovarian determination takes place at about seven weeks of gestation. It involves formation of follicular cells, which are thought to arise from the same progenitors as those that give rise to Sertoli cells. Follicular cells surround germ cells in a loose configuration and form primordial follicles. This process is associated with arrest of germ cells in meiotic prophase (10). In the absence of germ cells, the development of ovarian follicles is abnormal (12).

Genetic Control of Gonadal Differentiation

Genes Involved in Formation of the Gonadal Ridge

The homeobox gene LIM1 plays an important role in formation of the gonadal ridge (13,14). Mice homozygous for deletions in Lim1 lack proper development of the head, gonadal ridge, and the ureteric bud of the metanephros. The human LIM1 gene has been identified, but no mutations have been reported. A related gene termed Lhx9 is expressed in the urogenital ridge in mice, and the absence of Lhx9 causes gonadal agenesis (15). However, no mutations in LHX9 have been found in humans who have abnormal gonadal differentiation (16).

The steroidogenic factor 1 (SF1) gene located on chromosome 9p33, is a member of the orphan nuclear receptor family. SF1 was first identified as an activator of genes involved in biosynthesis of adrenal steroids. Later it was found that mice missing SF1 lack adrenal glands and gonads, and have impaired development of the ventromedial nucleus of the hypothalamus (17).

The Wilms tumor suppressor (WT1) gene is located on chromosome 11p13, and encodes a transcription factor necessary for the development of the

bipotential gonad and the kidney. WT1 activates transcription of sex-determining region Y (SRY), and also plays a role in testis determination. Mutations in the coding region can result in Wilms tumor and 46,XY gonadal dysgenesis. Alternative splicing of WT1 results in two isoforms. Mutations that alter the proportion of the two isoforms result in the Denys–Drash syndrome, a condition characterized by 46,XY gonadal dysgenesis and severe renal dysfunction (18,19).

Genes Involved in Testis Determination

The SRY gene initiates the process of testis determination. SRY is located on chromosome Yp11.3, close to the pseudoautosomal boundary. It belongs to the family of high-mobility group (HMG) proteins, which contain a related DNA-binding motif termed the HMG box. The SRY gene product bends DNA and the change in chromatin results in increased transcription of target genes. SRY is expressed in cells from the celomic epithelium that become Sertoli cells, and also directs the migration of cells from the mesonephros (7).

The SOX9 gene, located on chromosome 17q24.3–25.1 encodes an SRY-like protein expressed by Sertoli cells and cartilage. An increase in expression of SOX9 follows expression of SRY, and SOX9 may be a target gene of SRY. Overexpression of SOX9 in humans (20) and mice (21) results in XX sex reversal. SOX9 also interact with SF1 in regulating the expression of the AMH gene (22,23). Mutation of SOX9 results in campomelic dysplasia and 46,XY gonadal dysgenesis.

GATA4 is a transcription factor expressed in both the urogenital ridge and in the testis. GATA4 plays a role in embryonic testis by interacting with SF1 during transcriptional activation of AMH. It also plays a critical role in testis differentiation because disruption of GATA4 results in absence of testicular cords and steroidogenic enzymes (24).

DAX1 is a member of the nuclear hormone receptor superfamily. Like SF1, DAX1 is expressed in the bipotential gonad, the embryonic adrenal cortex, pituitary gonadotropes, and in the ventromedial nucleus of the hypothalamus. Expression of DAX1 is repressed in testis but continues in the ovary after gonadal determination. It was initially considered to be an ''antitestis'' gene because duplication causes sex reversal in males and antagonizes the synergy between SF1 and WT1 in vitro (25). Mutations of DAX1 result in X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism (26). Curiously, DAX1 also appears to have a role in testis differentiation because mutations of DAX1 also result in abnormalities of testicular tubules.

Signaling proteins such as Fgf9 induce proliferation of the celomic epithelium. Mice with targeted disruption of Fgf9 failed to develop Sertoli cells or testicular cords (27). Knock out of the insulin receptor (Insr), IGF1 receptor (28), and Insr-related receptor in mice results in XY sex reversal (29). Platelet-derived growth factor is also involved in the migration of cells from the mesonephros following expression of SRY. The Pdgfr-α gene appears to influence the formation of Leydig cells (30). Desert hedgehog (DHH) is another signaling protein linked to Leydig cell formation (31). Mice with disruption of DHH have abnormal Leydig cell formation. The influence of DHH in humans is more profound as a heterozygous mutation in the gene for DHH has been associated with mixed gonadal dysgenesis (32).

In summary, various genes such as LIM1, LHX9, SF1, and WT1 are involved in the formation of the gonadal ridge. SRY is the trigger of testis determination. Subsequent steps involve the expression of SOX9, SF1, WT1, FGF9, Pdgfr, and DHH (33,34).

Genes Involved in Ovarian Determination

Although ovarian determination is still poorly understood, some progress has been made in recent years. This has come largely from the elucidation of the role of Wnt4. Wnt4 is expressed in somatic cells and induces expression of follistatin. Together they block the migration of mesonephric cells and so antagonize male development. Lack of the Wnt4 gene expression impairs ovarian development and promotes expression of testis specific markers such as AMH and testosterone. Wnt4 and follistatin also support survival of germ cells. The transcription factor Figα (factor in germ line α) recruits granulosa cells in the formation of follicles and promotes expression of the zone pellucida proteins in the oocyte. In addition, Foxl2 promotes appropriate differentiation of granulosa cells during folliculogenesis (35).

ANATOMIC SEX DIFFERENTIATION OF THE REPRODUCTIVE TRACT

The Undifferentiated Stage

Müllerian and Wolffian Ducts

The reproductive tract of the male and female fetus is identical until eight weeks of gestation. They consist of bilateral Wolffian and Müllerian ducts and neutral external genitalia (Figs. 1 and 2). The Wolffian ducts, or mesonephric ducts, are the primordia of the epididymis, vas deferens, and seminal vesicles. Wolffian ducts are excretory canals of the primitive kidney, and are incorporated into the genital system when renal function is assumed by the definitive kidney. The Müllerian ducts, or paramesonephric ducts, are the primordia of the fallopian tubes, uterus, and upper two-thirds of the vagina. They originate from a cleft between the gonadal ridge and the mesonephros, and grow parallel to the Wolffian ducts, crossing them ventrally to form a single structure. By week 8 of gestation, the ducts reach the dorsal wall of the urogenital sinus, where they form the Müllerian tubercle, which separates the cranial vesicourethral canal from the caudal urogenital sinus. The fused tips of the Müllerian ducts are separated from the dorsal wall

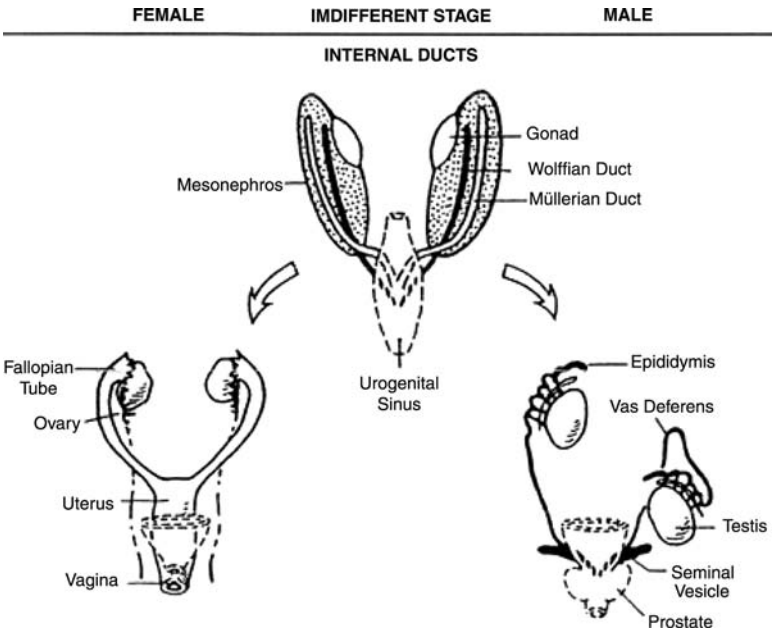

Figure 1 Phenotypic differentiation of internal ducts in male and female embryos.

of the urogenital sinus by a solid mass, termed vaginal cord (Figs. 1 and 2) (36).

Several transcription factors are involved in the differentiation of intermediate mesenchyme that forms the sex ducts. Lim1, which plays a role in formation of the gonadal ridge, is also necessary for expression of transcription factors such as Pax2 and Hox6 which mediate formation of the accessory sex ducts. Pax2 is expressed in the epithelium of Wolffian ducts. By contrast Hox6 and other members of the Hox gene family are expressed in the fallopian tubes (Hoxa 9), uterus (Hoxa 10,11), cervix (Hoxa 11), and upper vagina (Hoxa 13) (37).

Wnt4, which plays a critical role in ovarian determination, is also necessary for development of Müllerian structures, but not for development of Wolffian ducts. Disruption of Wnt4 results in a complex phenotype in mice. In addition to poor ovarian

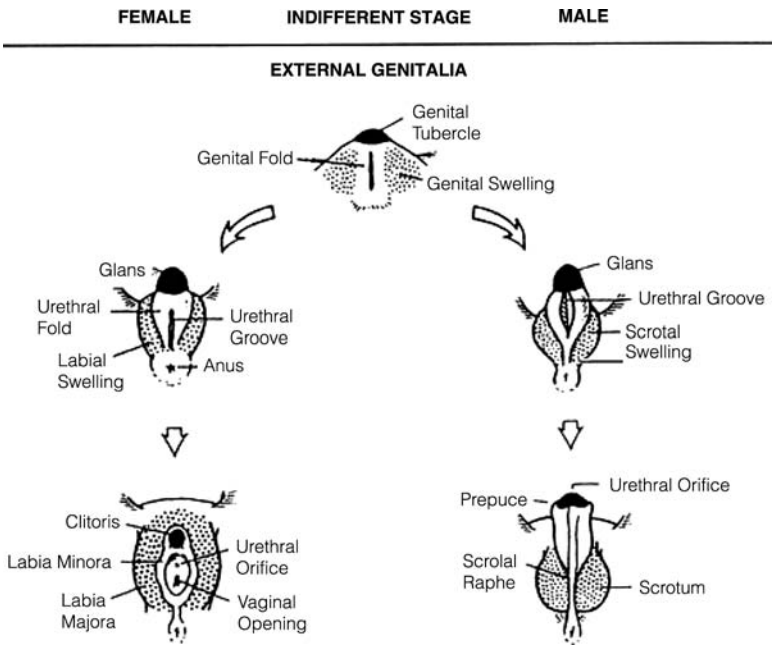

Figure 2 Phenotypic differentiation of external genitalia in male and female embryos.

development, there is production of testosterone and development of testes cords. Müllerian duct formation is abnormal, and Wolffian ducts proliferate in response to the ovarian secretion of testosterone. Wnt4 is also involved in formation of the kidney and mice with knock out of Wnt4 die of renal agenesis. Wnt5, Hoxa 13, and Wnt7a regulate growth of the Müllerian ducts (38).

External Genitalia

Development of the neutral external genitalia involves formation of the genital membrane, which closes the ventral part of the cloaca. The cloacal membrane forms the urogenital and anal membranes at week 6 of gestation. By week 8 the cloaca is divided into an anterior urogenital sinus and a posterior anorectal canal. The genital tubercle is located ventrally, the urethral folds are located medially, whereas the labioscrotal swellings are located laterally.

Male Differentiation

Regression of Müllerian ducts begins at eight weeks of gestation in response to secretion of AMH. The regression of the Müllerian ducts represents an unusual pattern of cell loss. Few cells actually die. Instead, they lose both polarity and orientation, and cease to divide. The basement membrane dissolves, and a tight ring of connective tissue forms around the cells (39). By 10 weeks of gestation Müllerian ducts have nearly completely disappeared. Wolffian ducts are stabilized and subsequently differentiate into epididymis, vas deferens, and seminal vesicles. Mucous epididymal secretion is demonstrable from 25 weeks of gestation.

With respect to differentiation of the urogenital sinus, the prostatic bud appears at approximately 10 weeks of gestation at the site of the Müllerian tubercle, and grows into solid branching cords. Maturation of the prostatic gland is accompanied by development of the prostatic utricle, the male remnant of the vaginal pouch. Two buds of epithelial cells, the sinoutricular bulbs, develop from the urogenital sinus close to the opening of the Wolffian ducts, grow inward, and fuse with the medial Müllerian tubercle to form the sinoutricular cord. This structure makes contact with the caudal tip of the fused Müllerian ducts and is cannulized at 18 weeks of gestation to form the prostatic utricle (40).

Masculinization of the male external genitalia begins in the nine-week-old fetus with lengthening of the anogenital distance, fusion of the labioscrotal folds, and closure of the rims of the urethral groove. This results in formation of a penile urethra. Penile organogenesis is completed by 11 weeks of gestation. The male and female phallus are the same size until week 16 of gestation (41).

The gubernaculum develops from the ligaments that extend from the developing gonads to the abdominal wall. Transabdominal movement brings the testis into the internal inguinal ring at around 22 weeks. Enlargement of the gubernaculum results from secretion of insulin-like hormone 3 (INSL3), a development that keeps the testes close to the inguinal ring as the abdomen grows. The process is referred to as the first phase of testicular descent. Androgens promote the release of calcitonin gene-related peptide (CGRP) from the genital femoral nerve. This permits the second phase of descent in which CGRP mediates growth of the gubernaculum and its migration into the scrotum along with the testis. There must also be an increase in the intraabdominal pressure to promote movement of the testis through the inguinal canal. The actual passage of the testis through the inguinal canal into the scrotum does not occur until after week 28, and may be delayed until the immediate postnatal period (42,43).

Female Differentiation

As mentioned previously, Müllerian ducts give rise to the fallopian tubes, uterus, and upper two-thirds of the vagina. Beginning at 10 weeks of gestation, the Wolffian ducts are incorporated into the wall of the Müllerian derivatives.

In the female, the genital tubercle becomes the clitoris, the labio-scrotal folds become the labia majora, and the urethral folds become the labia minora. At 15 weeks, the lower pole of the Müllerian ducts fuses with the upper portion of the vaginal pouch to form the vagina. Later the urogenital sinus is divided by a tissue plane that results in separate urethral and vaginal openings on the perineum (Fig. 3).

HORMONAL CONTROL OF SEX DIFFERENTIATION

AntiMüllerian Hormone

AMH causes the cranial–caudal regression of Müllerian ducts during week 8 to 10 of gestation. AMH is secreted by Sertoli cells. Granulosa cells also secrete AMH, but not until the critical period of Müllerian regression has passed. AMH is a dimeric glycoprotein and a member of the transforming growth factor β (TGFβ) family, which includes inhibin, activins, and other factors. AMH is cleaved by plasmin at a site located 109 amino acids away from the carboxyl terminus, generating the TGFβ-like carboxyl-terminal fragment that is thought to contain the biologically active site of AMH. The human AMH gene has been cloned and has been mapped to the short arm of chromosome 19 (5,44).

The AMH receptor is a complex transmembrane serine/threonine kinase, and is a heterodimer made up of type I and type II receptors. Ligand binding to the receptor results in phosphorylation and activation of the type I receptor. The gene for the type I receptor is termed ALK2, and the gene product signals through the bone morphogenic protein pathway (45). Wnt7a plays a role in development of the type II receptor. Hence, the mutation of Wnt7a in male

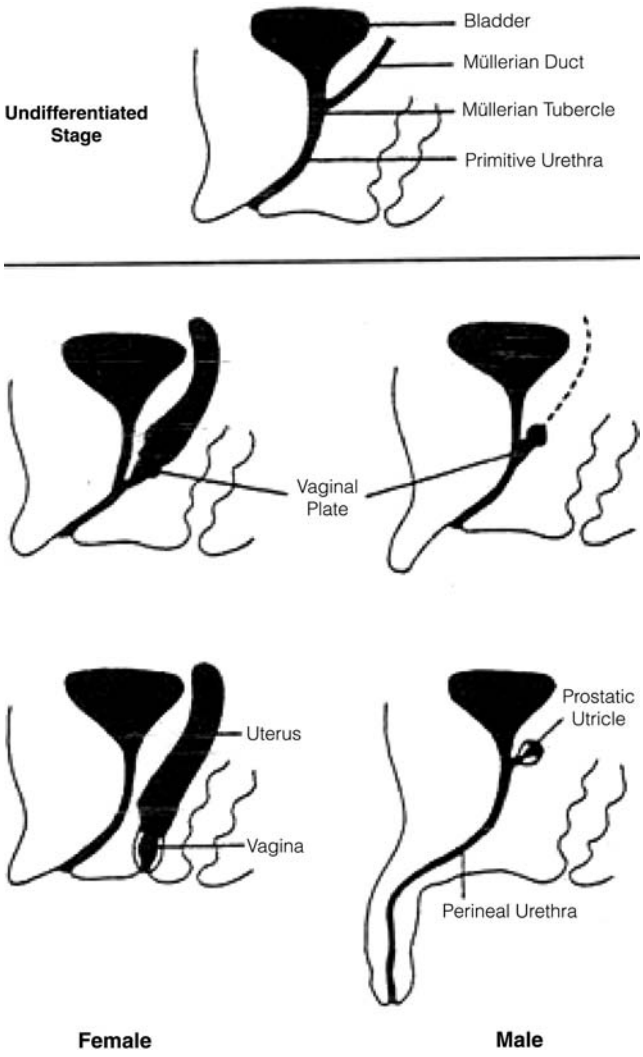

Figure 3 Sex differentiation of the urogenital sinus and external genitalia.

mice results in the persistence of Müllerian ducts (38). The gene for the type II receptor has been cloned and has been mapped to chromosome 12. This gene is expressed in the mesenchyme surrounding the Müllerian structures (46).

Serum AMH is also a marker of testicular function in infancy, and serum levels are useful in the diagnosis and management of patients with a variety of intersex and gonadal abnormalities (47). Granulosa cells of the postnatal ovary also produce AMH and elevated serum levels have been reported in granulosa cell tumor. AMH is produced by Sertoli cells until puberty occurs, and is also secreted by postnatal granulosa cells. However, its effect in either sex after birth has not been determined.

The effect of AMH on ovarian function is controversial. AMH has been reported to inhibit progression of meiosis in oocytes, and to result in masculinization of the fetal ovary. AMH also stimulates testosterone secretion (5). However, AMH is not required for testicular development because mutations impairing AMH production do not appear to affect testicular organogenesis.

Testosterone

Leydig cells appear between weeks 7 and 8 in the human fetus, and proliferate until the 18th week of gestation. Receptors for human chorionic gonadotropin (hCG) are present on fetal Leydig cells by at least 12 weeks of gestation and help to insure continued testosterone secretion. Biosyntheses of testosterone requires five enzymes: steroidogenic acute regulatory protein (StAR), side chain cleavage (P450scc), 3β-hydroxysteroid dehydrogenase (3β-HSD), 17-hydroxylase/17,20 desmolase (P45017), and 17β-hydroxysteroid dehydrogenase (17β-HSD). Leydig cell number decreases after 18 weeks of gestation and, indeed, few Leydig cell number are visible by that time. Testicular and serum testosterone concentrations reach a peak of about 300 ng/dL (200–600) at 15 to 18 weeks of gestation. After 15 weeks, the low level of testosterone production is maintained by hCG. A decrease in testosterone synthesis later in gestation is correlated with a decrease in luteinizing hormone (LH)/hCG receptors. During the second half of pregnancy, LH becomes the principal regulator of testosterone secretion, and LH secretion is regulated in part by testosterone through negative feedback at the level of the hypothalamus. In male infants, serum testosterone levels rise again during the first two days of life, decline until the end of the first week, and then rise with a peak at two months of age. By six months, testosterone levels reach prepubertal levels (48).

Mechanism of Testosterone and Dihydrotestosterone Action

Testosterone enters most cells by passive diffusion, although it has been suggested that the Wolffian duct takes up testosterone by pinocytosis. In target tissues, testosterone is converted to DHT by steroid 5α-reductase. The conversion of testosterone to DHT is necessary for sex differentiation of male external genitalia, and there are two explanations for this. First, DHT binds to the androgen receptor (AR) with greater affinity than does testosterone. Second, DHT cannot be aromatized, and thus its action is purely androgenic. Although masculinization of prostate, urogenital sinus, and external genitalia depend on DHT, proliferation of Wolffian ducts required testosterone alone. This may be possible because the internal ducts are exposed to high local concentrations of testosterone.

There are two forms of steroid 5α-reductase, one having optimal activity at pH 5.5 and the other at pH 8. The former enzyme, which is the predominant form in the prostate, is called steroid 5α-reductase 2, and mediates conversion of testosterone to DHT in the external genitalia. It has been mapped to chromosome 2. The

enzyme termed steroid 5α-reductase 1 has been mapped to chromosome 5p15. Found largely in liver, brain and nongenital skin, it does not appear to play a role in differentiation of the reproductive tract.

Binding of DHT to the AR is necessary for androgen effect (49). The AR gene is located on the long arm of the X chromosome near the centromere (Xq11–q12) (50). The AR is a member of a superfamily of nuclear receptors that includes the progesterone, glucocorticoid, mineralocorticoid, estrogen, thyroid hormone, vitamin D, vitamin A, and v-Erb receptors. It consists of eight exons encoding a protein of approximately 919 amino acids. Exon 1 encodes the N-terminal transcription activation domain; exons 2 and 3 the DNA-binding domain, and exons 4 to 8 the steroid-binding domain (18). The DNA-binding domain consists of two cysteine-rich zinc fingers, and also encodes a region necessary for translocation of the receptor into the nucleus. The transcriptional activation domain contains variable trimeric repeats. The variable number of GGN repeats encodes 16 to 27 glycine residues, whereas CAG repeats encode 11 to 31 glutamine repeats. Heat shock proteins—HSP 56, 70, and 90—are bound to the inactive receptor. Binding of androgen results in their release. Conformational changes expose nuclear localization signals that permit interaction of the AR with transport proteins. Within the nucleus, activated ARs localize to specific nuclear compartments (51).

Various coregulators interact with transcriptional activation domains, AF1 and AF2. The term coactivator refers to proteins that interact with steroid hormone receptors to increase transcription of target genes, whereas corepressors reduce transcription. Members of the p160 family of proteins increase transcription by recruiting other proteins necessary for remodeling of chromatin. By contrast, coregulators such as nuclear receptor corepressor repress transcription (52).

Estrogen

The biological effects of estrogens are mediated by estrogen receptors (ERs). Estrogen diffuses freely into cells where it binds to ER in the nucleus. The ER-ligand complex dimerizes, binds to the estrogen response elements, and hence modulates transcription of estrogen-regulated genes. Two ERs, α and β, have similar affinity and specificity. The human ER β has been detected as early as 13 to 20 weeks of gestation with highest levels being found in the adrenals, and in the male reproductive tract (53). Expression of the ER α and ER β has been identified in the uterus at the beginning of the second trimester and plays a role in uterine development.

Estrogen has also been implicated in several pathological states of male sexual differentiation. For example, prenatal exposure to estrogens can result in cryptorchidism, possibly by inhibiting insulin-like factor 3. Hypospadias, epididymal cysts, persistent Müllerian ducts, and prostate disease have also been related to prenatal exposure to estrogen in males. ER α is essential for fluid reabsorption in the efferent ductules and its absence results in infertility (54). In the female embryo, ER α and ER β are detected on epithelium of the oviduct and may modulate oviduct development. ER gene expression has also been identified in the uterus beginning in the early second trimester of fetal development, suggesting a role for ER in differentiation of primitive uterine mesenchyme in stromal and myometrial compartments.

Environmental Factors

An increase in the prevalence of developmental abnormalities of the reproductive tract including cryptorchidism, hypospadias, and micropenis has been noted over the past 20 to 30 years, and has raised great concern. This change has been attributed in part to fetal exposure to environmental compounds with estrogenic effects (xenoestrogens), such as herbicides, pesticides, PCBs, plasticizers, and polysterenes, as well as exposure to antiandrogens such as the polyaromatic hydrocarbons, linuron, vinclozolin, and *pp*′-DDE. Such environmental agents are referred to as endocrine disruptors (55).

Xenoestrogens and antiandrogens can disrupt androgen effect by competition for binding to the AR, diminished conformational change, as well as suboptimal nuclear transfer, an abnormal DNA binding and transcriptional activation. Most antiandrogens interact directly with the AR. Environmental antiandrogens such as the fungicide vinclozolin disrupt male sex differentiation by blocking AR binding to androgen response elements. DDE, a DDT metabolite that accumulates in the environment, inhibits human AR transcriptional activation (56). Exposure of the male fetus to antiandrogens results in diminished masculinization. Xenoestrogens influence male differentiation by interacting indirectly with either ER α or ER β. Hypospadias has been related to estrogen exposure during the first trimester, and the occurrence of abnormalities of the sex organs in sons of women exposed to DES is well documented. At least 20% of these men had epididymal cysts, hypospadias, and cryptorchidism (57). Phthalates, which are chemicals used in the manufacture of cosmetics and other industrial products cause incomplete masculinization of male rats in utero by decreasing Sertoli cell adhesion to germ cells (58).

AMBIGUOUS GENITALIA

Conditions with ambiguous genitalia are classified into three major categories: (i) abnormalities of gonadal differentiation, (ii) abnormal sex differentiation in subjects with a 46,XY karyotype, and (iii) abnormal sex differentiation in subjects with a 46,XX Karyotype.

Abnormalities of Gonadal Differentiation

Gonadal Dysgenesis in Subjects with 46,XY Karyotype

These abnormalities can be subdivided as: 46,XY complete gonadal dysgenesis, 46,XY partial gonadal dysgenesis, and 46,XY true hermaphroditism. Although each of these conditions has a distinct phenotype, there is considerable overlap. An additional condition, termed testicular regression sequence, has a range of etiologies, but some evidence suggests that this condition is also related to 46,XY partial gonadal dysgenesis (Table 1).

46,XY Complete Gonadal Dysgenesis

46,XY complete gonadal dysgenesis is defined by absence of testis determination despite nonmosaic male karyotype. The condition is characterized by normal female external genitalia and streak gonads. The terms Swyer syndrome and 46,XY pure gonadal dysgenesis have also been used to describe this condition.

Clinical Presentation. Most subjects with 46,XY complete gonadal dysgenesis present for evaluation of delayed puberty or amenorrhea. Subjects are usually normal in appearance and most have normal height. However, a few subjects have stigmata of Turner syndrome. Breast development is present in some subjects and may be related to persistent ovarian function. However, breast tissue and/or menses in subjects with 46,XY complete gonadal dysgenesis is concerning because it may also signal the presence of an estrogen secreting tumor. There is normal development of pubic hair in women with 46,XY complete gonadal dysgenesis, and in some individuals the clitoris may be slightly enlarged (59). Physical examination and ultrasonography indicate normal vagina, uterus, and fallopian tubes, but absence of Woffian structures. When subjects present as adolescents or adults, serum levels of LH and follicle stimulating hormone (FSH) are abnormally elevated. Serum estradiol levels are usually low, but may be elevated in the presence of an estrogen secreting tumor. Plasma levels of testosterone are normal or slightly elevated due to the effect of abnormally elevated serum LH on residual theca cells in the streak gonad. The latter observation provides an explanation for the presence of clitoromegaly in some subjects (60).

Table 1 Classification of Genetic Abnormalities of Gonadal Differentiation

46,XY Gonadal dysgenesis
46,XY complete gonadal dysgenesis
46,XY partial gonadal dysgenesis
46,XY true hermaphroditism
Embryonic testicular regression sequence
Mosaicism or chimerism involving Y chromosome
46,XX sex reversal

Gonadal Histology. Gonadal histology is usually characterized by a wavy fibrous connective tissue, which in part resembles ovarian stroma. The streak gonad of 46,XY complete gonadal dysgenesis was likely to have been an ovary in utero, which then degenerated into a streak (59).

Gonadal tumors occur in 30% of subjects with 46,XY complete gonadal dysgenesis. Although tumors usually develop after puberty, they may be present earlier. The most common tumor is a gonadoblastoma. About half of subjects with gonadoblastoma also have coincidental dysgerminoma. Although gonadoblastoma is generally considered a carcinoma in situ, dysgerminoma is a malignant tumor. Other malignant tumors also occur, but are unusual. They include embryonal carcinoma, endodermal sinus tumor, choriocarcinoma, and immature teratoma (61).

Management of Women with 46,XY Gonadal Dysgenesis. Management of women with 46,XY gonadal dysgenesis involves gonadectomy to prevent malignancy followed by cyclical hormonal therapy to promote normal pubertal development and normal maturation of bones. Because the uterus is normal, pregnancy can be achieved following in vitro fertilization of a donor egg and implantation of the embryo.

46,XY Partial Gonadal Dysgenesis

46,XY partial gonadal dysgenesis is defined by incomplete testis determination in an individual with a nonmosaic 46,XY karyotype. This condition has also been referred to as dysgenetic male pseudohermaphroditism and 46,XY mixed gonadal dysgenesis. It is distinguished from 45,X/46,XY mixed gonadal dysgenesis in which pathology is related to mosaicism.

Clinical Presentation. Individuals with this condition usually present ambiguous genitalia with various degrees of masculinization. An utriculo-vaginal pouch is present in a large number of subjects. Proliferation of Wolffian ducts depends on the extent of embryonic testosterone secretion, whereas the extent of Müllerian duct development depends on the secretion of AMH. Gonads are usually intraabdominal, but in some individuals, they may be found in the inguinal canal or in the scrotum. Serum levels of plasma testosterone are low in the newborn period, as are levels following hCG stimulation. If the diagnosis is made after puberty, serum levels of LH and FSH are abnormally high, whereas plasma levels of testosterone are normal or low (59).

Stigmata of Turner syndrome are present in approximately one-quarter of subjects with 46,XY partial gonadal dysgenesis. The explanation for this physical finding is unknown, but many of these cases may have hidden mosaicism with a 45,X cell line.

Gonadal Histology. Gonadal abnormalities in 46,XY partial gonadal dysgenesis include formation of streak gonads and dysgenetic testes. Streak gonads are similar to those found in 46,XY complete

gonadal dysgenesis, except that they may be more fibrotic. The dysgenetic testes in these individuals are characterized by disordered, poorly formed seminiferous tubules and incomplete formation of the tunica albuginea (59).

Gonadal Tumors. The risk of gonadal tumors in subjects with 46,XY partial gonadal dysgenesis is roughly 16% to 30%. The types of tumors are like those of 46,XY complete gonadal dysgenesis. However, caution is necessary in management of these patients, as tumors have occurred as early as 15 months of life (62).

Management. Surgical correction of the external genitalia should be performed. There is also need to remove internal duct structures that correspond to those of the opposite sex of rearing. If the child is raised as a boy, streak gonads should be removed, and dysgenetic gonads should be brought into the scrotum or, if this is not possible, they should be removed. Although the risk of malignancy remains even if the gonads are in the scrotum, the development of tumors can be ascertained by careful and regular physical examination. Dysgenetic testes may produce testosterone at puberty, but testosterone therapy is often necessary. If the individual is raised as a female, gonadal tissue should be removed to avoid the possibility of malignancy and to prevent virilization later in life.

Subjects raised as females will require cyclical hormonal therapy at puberty for development of secondary sexual characteristics. Menses can be achieved by hormonal supplementation if the uterus is intact.

46,XY True Hermaphroditism

Clinical Presentation. The 46,XY true hermaphroditism is characterized by the presence of gonadal tissue that contains both ovarian follicles and normal appearing seminiferous tubules in a patient with a male karyotype. Patients usually have ambiguous genitalia, although genitalia range in appearance from completely female to completely male. There is a mix of Wolffian and Müllerian duct structures depending on development of testicular tissue. Management is the same as that of subjects with 46,XY gonadal dysgenesis (63).

Gonadal Histology. The presence of an ovary on one side of the abdomen and a testis on the other side is found in about 50% of patients. In other cases, one or both of the gonads can be ovotestis. The risk of gonadal tumors in subjects with 46,XY true hermaphroditism is approximately 10% (61).

Embryonic Testicular Regression Sequence

This condition is characterized by loss of testicular tissue on one or both sides of the abdomen in an individual with 46,XY karyotype. Patients have ambiguous or female genitalia with abnormal differentiation of internal sex ducts. It is thought that loss of the testicular tissue in this syndrome occurs during the critical period of sex differentiation. Early loss of testicular tissue may be associated with female external genitalia, whereas ambiguous genitalia implies loss of testis at a slightly later stage (64).

Some evidence suggests that the etiology of the testicular regression sequence is related to 46,XY partial gonadal dysgenesis. This is based on cases in which there is testicular loss on one side, and evidence of dysgenesis on the opposite side. Other etiologies of embryonic testicular regression sequence include teratogenic effect and vascular accidents (62,65,66).

Embryonic testicular regression sequence is distinguished from the Vanishing Testis syndrome in which there is normal sex differentiation in a 46,XY individual and loss of testicular tissue on one or both sides. In this condition the loss of testicular tissue is likely to have occurred in a later part of gestation and may be related to fetal testicular torsion (62).

Etiology of 46,XY Gonadal Dysgenesis

Abnormalities of the SRY Gene. Deletions of the distal region of the short arm of the Y chromosome including SRY have been associated with 46,XY complete gonadal dysgenesis (Table 2). Physical findings of Turner syndrome are present in most of these patients (67). Mutations of SRY account for approximately 15% of patients with 46,XY complete gonadal dysgenesis. Most of the mutations are in the HMG box and some of them have been shown to reduce binding of SRY to DNA or to reduce the SRY-induced bending of DNA (18). Nonetheless several missense mutations of SRY outside of the HMG box have been reported in association with 46,XY complete gonadal dysgenesis. Mutations outside the coding region have also been detected in a few patients, including large deletions 5′ and 3′ to the SRY gene (68,69) and a point mutation in the promoter region that interferes with the binding of Sp1 transcription factor (70). With respect to 46,XY partial gonadal dysgenesis, SRY mutations are unusual.

The etiology of 46,XY true hermaphroditism has been attributed to a point mutation of SRY as well as to a postzygotic somatic mutation in the gene (71). These observations underscore the relationship between 46,XY partial gonadal dysgenesis and 46,XY true hermaphroditism.

Mutations of WT1, SF1, SOX9, and DHH have also been implicated in 46,XY gonadal dysgenesis. Large deletions of DNA involving the WT1 gene can result in the WAGR syndrome. Mutations in a coding region of WT1 can result in syndromes characterized by Wilms tumor and 46,XY gonadal dysgenesis or syndromes with Wilms tumor, 46,XY gonadal dysgenesis, and progressive nephropathy. Frasier syndrome is characterized by 46,XY gonadal dysgenesis and renal dysfunction, but not Wilms tumor. It is associated with mutations in the splice variant of WT1 (+KTS) which

Table 2 Genetic Factors Involved in Human Disorders of Sexual Differentiation

Gene	Chromosomal location	Genetic defect	Clinical presentation
Sex-determining region Y	Yp11.3	Point mutations and small deletions	46,XY CGD; 46,XY PGD; 46,XY true hermaphroditism
		Deletion of distal Yp	46,XY CGD and Turner stigmata
		Translocation to X chromosome	46,XX sex reversal
SOX9	17q24	Mutation	Campomelic dysplasia and 46,XY PGD
		Duplication	46,XX sex reversal
Steroidogenic factor 1	9q33	Mutation	46,XY GD, adrenal hypoplasia, and hypogonadotropic hypogonadism
DAX1	Xp21.3	Deletion	46,XY and 46,XX: hypogonadotropic hypogonadism and adrenal hypoplasia; 46,XY: abnormal testis development
		Duplication	46,XY sex reversal
WT1	11p13	Deletion	WAGR
		Mutation	46,XY: GD, Denys–Drash syndrome, Frasier syndrome
AMH	19p13.3p13.2	Mutation	Persistent Müllerian duct syndrome
DHH	12q13.1	Mutation	46,XY CGD; 46,XY PGD
Wnt4	1p31p35	Duplication	46,XY GD
		Point Mutation	46,XX: absence of Müllerian duct derivatives, renal agenesis, androgen excess
XH2	Xq13	Mutation	46,XY GD, mental retardation, and α-thalassemia

Abbreviations: AMH, antiMüllerian hormone; CGD, complete gonadal dysgenesis; DHH, desert hedgehog; GD, gonadal dysgenesis; PGD, partial gonadal dysgenesis; WT1, Wilms tumor suppressor.

is necessary for transcriptional activation of SRY. Subjects typically have female external genitalia. By contrast, the Denys–Drash syndrome is characterized by 46,XY gonadal dysgenesis, Wilms tumor, and severe glomerulosclerosis (19). This condition is caused by mutations in the WT1 gene that alter the normal proportion of the +KTS and −KTS splice site variants.

Haploinsufficieny of the SF1 gene has been associated with complete gonadal dysgenesis and adrenal failure. However, 46,XY gonadal dysgenesis without adrenal failure has been reported in some patients with SF1 mutations (72,73). Individuals with an XY karyotype but duplication of the short arm of the X chromosome that includes DAX1 also have 46,XY gonadal dysgenesis suggesting that DAX1 is an antitestis gene. However, mutations of DAX1 result in subtle abnormalities of testes histology in addition to adrenal hypoplasia and hypogonadotropic hypogonadism, suggesting that DAX1 may actually play a role in testis differentiation (74).

Mutations of the SOX9 gene result in campomelic dysplasia. Some affected individuals also have 46,XY gonadal dysgenesis (75). Various missense mutations have been reported. Rearrangements of the locus encoding the SOX9 gene have also been described. Patients may present with either 46,XY complete or 46,XY partial gonadal dysgenesis.

Mosaicism and Chimerism Involving the Y Chromosome

Mosaicism and chimerism occur when cells with two or more karyotypes are found in the same individual. In the case of mosaicism, cells are all derived from the same zygote, and the various karyotypes usually result from nondisjunction. By contrast, chimerism occurs when cells are derived from two zygotes. The etiology may be double fertilization or fusion of two embryos.

The most common form of mosaicism involving the Y chromosome is that in which there is a 45,X/46,XY karyotype, a condition often referred to as mixed gonadal dysgenesis. It was originally thought that most subjects with 45,X/46,XY mixed gonadal dysgenesis had ambiguous genitalia. It is now recognized that 90% to 95% of subjects with 45,X/46,XY karyotypes have normal male external genitalia. However, many subjects have abnormal testicular histology. Affected subjects with abnormal sex differentiation usually present in the newborn period, and many of them have stigmata of Turner syndrome. Gonadal tumors occur in approximately 10% to 20% of subjects who have 45,X/46,XY karyotypes and abnormal sex differentiation. The management of these patients is similar to that of subjects with 46,XY partial gonadal dysgenesis. Complications associated with the Turner phenotype need to be addressed. In particular, therapy with growth hormone should be considered for patients with subnormal growth. Other forms of mosaicism involving the Y chromosome, such as 45,X/47,XYY and 45,X/46,XY/47,XYY, are extremely uncommon (62,76).

Chimerism may be the cause of a 46,XX/46,XY karyotype in some individuals. The most common presentation of these subjects is true hermaphroditism, although some present a clinical picture similar to 46,XY partial gonadal dysgenesis (62,77).

46,XX Sex Reversal

The term 46,XX sex reversal comprises two conditions: 46,XX maleness and 46,XX true hermaphroditism. In addition, 45,X sex reversal has been reported.

The 46,XX maleness is a condition in which individuals have testis determination despite a nonmosaic 46,XX karyotype. The phenotype of 46,XX males is similar to that of Klinefelter syndrome. In particular, most affected subjects have a normal male phenotype, although about 15% have hypospadias or ambiguous genitalia. Like subjects with Klinefelter syndrome, the majority of individuals present for evaluation of delayed puberty and gynecomastia. Hormonal evaluation indicates abnormally elevated serum levels of LH and FSH. Plasma testosterone concentration may be normal or somewhat low. Management may involve supplementation with testosterone and surgical correction of gynecomastia (78).

The 46,XX true hermaphroditism is characterized by the presence of both ovarian and testicular tissue in an individual with a nonmosaic 46,XX karyotype. Although the clinical phenotype is variable, most affected subjects have ambiguous genitalia and a mix of Müllerian and Wolffian structures. Surgical correction of external genitalia and subsequent hormonal supplementation depend upon sex of rearing. Only about 4% of subjects with 46,XX true hermaphroditism develop gonadal tumors.

Subjects with 45,X maleness have normal testicular determination, but frequently have micropenis, cryptorchidism, and azoospermia. In addition, there may be other associated problems such as developmental delay, short stature, and congenital anomalies. This condition is extremely rare. Some individuals with apparent 45,X sex reversal may have hidden mosaicism with the Y bearing cell line.

More than two-thirds of 46,XX males have a translocation of SRY from the paternal Y to the paternal X chromosome and inheritance of the translocated X. By contrast, most subjects with 46,XX true hermaphroditism and one-third of 46,XX males lack SRY. In these patients, it is necessary to exclude mosaicism. An explanation for 46,XX sex reversal in the absence of SRY has been proposed (79). It is based on the hypothesis that male sex determination is repressed and that testes determination involves derepression. This theory invokes the existence of a repressor of testis determination. In this model a mutation of the putative repressor could permit male sex determination in the absence of SRY. However, such a repressor has not been identified.

Studies of an individual with SRY-negative 46,XX maleness and duplication of the locus containing the SOX9 gene implicated overexpression of SOX9 as a cause of the 46,XX sex reversal (20). This possibility was supported by XX sex reversal in transgenic mice with overexpression of SOX9 (21). Other reports also described duplication of chromosome 22 in 46,XX in true hermaphroditism (80,81).

Abnormalities of Sex Differentiation in Individuals with 46,XY Karyotype

These abnormalities include conditions in which individuals have a 46,XY karyotype, normal testis determination, but incomplete masculinization of the external genitalia. These conditions have been referred to as male pseudohermaphroditism. However this term is confusing, and should be replaced by a classification system based on etiology. In 46,XY individuals with abnormal sex differentiation, the appearance of the genitalia ranges from completely female to slightly feminized external genitalia with only hypospadias and cryptorchidism (Table 3). These conditions include Leydig cell aplasia/hypoplasia, defects of testosterone biosynthesis, steroid 5α-reductase type II deficiency, and androgen insensitivity. Defects in synthesis or function of the AMH can also be considered part of this group.

Leydig Cell Aplasia/Hypoplasia: Abnormalities of hCG/LH Receptor

Subjects with Leydig cell aplasia have normal female appearing genitalia. By contrast, subjects with Leydig cell hypoplasia have a wide range of clinical presentation from micropenis to severe hypospadias. In both the severe and mild form, there is absence of Müllerian structures due to the secretion of AMH. Testes may be located in the abdomen, inguinal canals, or in the labia majora. Testosterone production is in the normal to low range depending on the severity of the condition. Sertoli cells are normal, but there is hyalinization of the seminiferous tubules and incomplete spermatogenesis.

Leydig cell aplasia and hypoplasia are typically caused by mutations of the hCG/LH receptor. Deletions, nonsense mutations, or frame shift mutations diminish binding of hCG/LH or reduce signal transduction. This is an autosomal recessive condition and most patients are homozygotes for the mutation or compound heterozygotes (82,83).

Table 3 Abnormalities of Sex Differentiation in Individuals with 46,XY Karyotype

Human chorionic gonadotropin/luteinizing hormone receptor dysfunction (Leydig cell aplasia/hypoplasia)
Testosterone biosynthesis defects
Steroidogenic acute regulatory protein deficiency (congenital lipoid adrenal hyperplasia)
3β-HSD/Δ^5 isomerase type II deficiency
CYP17 (17α-hydroxylase/17,20 lyase) deficiency
CYP17 (17,20 lyase) deficiency
P450 oxidoreductase deficiency
17β-HSD type III deficiency
Steroid 5α-reductase type II deficiency
Androgen insensitivity syndrome
Multiple congenital anomalies
Timing defects
Persistent Müllerian duct syndrome

Abbreviation: HSD, hydroxysteroid dehydrogenase.

Defects of Testosterone Biosynthesis

Mutations in six genes encoding seven enzymes have been associated with defects in testosterone biosynthesis. Deficiency of StAR, P450scc, deficiency of 3β-HSD are associated with decreased secretion of cortisol, testosterone, and aldosterone. In 17-hydroxylase and P450 oxidoreductase deficiency, cortisol secretion and testosterone biosyntheses are low. The two other enzymes, 17,20-desmolase and 17-keto-reductase, are not required for glucocorticoid or mineralocorticoid production. The pattern of inheritance in each of these defects is autosomal recessive.

The phenotype of affected individuals depends on the extent of testosterone secretion and ranges from completely female to almost completely male. Hence, it is impossible to distinguish these conditions on the basis of the external genitalia. The testis may be situated within the labioscrotal folds, inguinal canals, and abdomen. The Wolffian derivatives may be normally developed or hypoplastic, depending on the severity of the testosterone biosynthetic block. Müllerian structures are absent because the secretion of AMH by Sertoli cells is unaffected.

Subjects raised as males may require surgical correction of the external genitalia and surgery for cryptorchidism. Individuals raised as females will require removal of gonads and Wolffian structures, and may require revision of external genitalia.

Steroidogenic Acute Regulatory Protein Deficiency (Congenital Lipoid Adrenal Hyperplasia)

Marked enlargement of the adrenal glands occurs in StAR deficiency because of the abnormal accumulation of cholesterol and cholesterol esters. The StAR mediates the transfer of cholesterol from the outer to inner mitochondria membrane, and is the first rate-limiting step in steroid biosynthesis. StAR deficiency is a rare disorder, but more prevalent among people of Japanese, Korean, and Palestinian descent (60). Subjects with severe deficiency have female genitalia and early salt-losing crisis. Some subjects have late onset adrenal crisis. In these individuals there is sufficient StAR activity to permit cholesterol uptake early in life. However, lipotoxicity from marked cholesterol accumulation eventually precipitates an adrenal crisis. Late onset StAR deficiency has been implicated in some cases of Sudden Death syndrome (84).

Early studies suggested that congenital lipoid adrenal hyperplasia (CLAH) was related to defects in CYP11A, the gene for P450scc (CYP450scc). However, genetic analysis of this gene was normal in affected patients. Later work indicated that homozygous mutations of P450scc caused spontaneous abortion through lack of progesterone synthesis. Nonetheless haploinsufficiency of P450scc may present like late onset CLAH (85). In one unusual case, a homozygous deletion in CYP11A caused complete sex reversal and adrenal insufficiency at birth in a 46,XY subject (86).

3β-Hydroxysteroid Dehydrogenase Deficiency

3β-HSD catalyzes the conversion of 3β-hydroxy-, Δ^5-steroids to Δ^4-3-keto-steroids [pregnenolone to progesterone, 17-hydroxypregnenolone to 17-hydroxyprogesterone, and dehydroepiandrosterone (DHEA) to androstenedione]. Hormonal abnormalities are characterized by increased plasma levels of pregnenolone, 17-hydroxypregnenolone, and DHEA. Two genes encode 3β-HSD: type I in placenta, skin and breast tissues, and type II gene in adrenal and gonads. Types I and II are 93.5% homologous. These genes belong to the aldo-keto-reductase family rather than the cytochrome P450 family. Deficiency of 3β-HSD II impairs both adrenal and gonadal steroidogenesis. Genotypic males with the complete form have female genitalia and salt-losing crisis shortly after birth. Subjects with the partial form have ambiguous genitalia and no salt wasting.

Various mutations of 3β-HSD type II have been identified, including splicing abnormalities, deletions, and nonsense, frameshift and missense mutations. The salt-losing form generally results from severe mutations and is associated with absence of functional 3β-HSD. By contrast, the non salt-losing form usually results from a missense mutation (87). As deficiency of 3β-HSD results in subnormal conversion of 17-hydroxypregnelonone to 17-hydroxyprogesterone, the hallmark in the diagnosis of 3β-HSD deficiency is an abnormally high level of 17-hydroxypregnelonone. Ironically, some patients with 3β-HSD deficiency may be identified at birth because of abnormally high levels of 17-hydroxyprogesterone on the state screening. This apparent paradox may be explained by conversion of some of the 17-hydroxypregnelonone to 17-hydroxyprogesterone by activity of type I 3β-HSD in peripheral tissue. Hence it is advised to measure 17-hydroxypregnelonone in females with normal external genitalia or in undermasculinized males with high levels of 17-hydroxyprogesterone on state screening (88).

CYP17 (17α-Hydroxylase/17,20-Lyase) Deficiency

Affected individuals who have a 46,XY karyotype have phenotypes ranging from normal appearing female external genitalia with short vagina in the complete form to ambiguous genitalia in the partial form. The testes are located intraabdominally, in the inguinal canal, and in the labioscrotal folds. An abnormally high level of deoxycorticosterone (DOC) results in hypertension. Although cortisol production is subnormal, high levels of corticosterone accumulate and provide glucocorticoid effect. In the partial form there is subnormal virilization at puberty and gynecomastia occurs in some subjects. Serum levels of FSH and LH, 11-DOC, corticosterone, and progesterone are elevated. By contrast, plasma renin activity, aldosterone, and cortisol are decreased. Glucocorticoid supplementation is indicated to reduce the abnormally elevated level of DOC. Hypertension,

hypokalemia and hyporeninemia normalize with glucocorticoid treatment. At puberty gonadal steroid hormone replacement may be necessary (89).

Although 17α-hydroxylase and 17,20-lyase are encoded by the same gene, different sites on a single protein are responsible for the two activities. Hence, there are situations in which 17α-hydroxylase activity is preserved, but 17,20-lyase is defective. Enzymatic activity of the mutant protein correlates with phenotype. If the 17α-hydroxylase activity is less than 25% of normal, affected 46,XY subjects have feminized external genitalia. but enzymatic activity greater than 25% results in normal fetal masculinization (89). Cases of CYP17 deficiency have been related to mutations resulting from deletions, premature truncation, frameshift, and splicing errors. CYP17 deficiency is relatively more common in Brazil than in other countries. The majority of cases are related to one of the two mutations, suggesting a founder effect in that country (90).

P-450c17 (17,20 Lyase) Deficiency

Affected 46,XY subjects present a range in the appearance of external genitalia depending on severity of the defect. Plasma levels of 17-hydroxysteroids are normal, but plasma levels of DHEA, androgens, and 24-hour urinary 17-ketosteroids are all low. The ratio of 17-hydroxyprogesterone/androstenedione after hCG stimulation is abnormally elevated (>10). At puberty, administration of testosterone is required for development of secondary sexual characteristics. CYP17 mutations that cause isolated 17,20 lyase deficiency in 46,XY subjects have been reported. Mutations in the redox partner binding site of CYP17 can also cause selective loss of 17, 20 lyase activity (91).

P450 Oxidoreductase Deficiency

P450 is a flavoprotein that transfers electrons from the reduced NADPH to all microsomal type II P450 enzymes including 17-hydroxylase (P450 C17), 21-hydroxylase (P450C21A2), and aromatase (P450arom). Mutations of P450 cause disordered gonadal and adrenal steroidogenesis with a phenotype resulting from mixed partial 17α-hydroxylase and 21-hydroxylase deficiencies and occasionally from fetoplacental aromatase deficiency. 46,XY individuals have phenotypes ranging from normal appearing female external genitalia with blind vagina to individuals with micropenis, hypospadias, and undescended testes. Other dysmorphic features include craniosynostosis, radioulnar or radiohumeral synostosis, and femoral bowing. Although adrenal crisis is unusual, patients often need glucocorticoid supplementation for medical and surgical stress (92).

17β-Hydroxysteroid Dehydrogenase Deficiency

This condition is characterized by failure to convert androstendione to testosterone. Subjects usually present with female external genitalia, but some have ambiguous genitalia with a minimal degree of masculinization. Marked virilization occurs at puberty with muscular development, enlargement of the phallus, and development of pubic hair. However, gynecomastia is almost always present. Other genes are likely to modify 17β-HSD expression as the phenotype can vary even among members of the same kindred (93,94). Subjects have an increased ratio of androstenedione to testosterone in baseline samples, or following hCG stimulation. Baseline serum levels of LH and FSH are markedly elevated at puberty.

Five isoenzymes of 17β-HSD catalyze the reduction of androstenedione to testosterone, androstanediol to dihydrotestosterone, and estrone to estradiol. The type III isoenzyme is expressed in the testis, where it catalyzes the reduction of androstenedione to testosterone. Its gene is located on chromosome 9q22. Mutations in the 17β-HSD type III gene include missense, so-called splice junction abnormal-like, and frame-shift mutations.

Steroid 5α-Reductase Deficiency

Deficiency of the enzyme steroid 5α-reductase is a rare disorder that results in low plasma levels of DHT and abnormal differentiation of male external genitalia. In the newborn period, affected subjects present with feminized external genitalia including a small phallus, chordee, bifid scrotum, and a urogenital sinus that opens onto the perineum. Testes are found in the inguinal canal or labioscrotal folds. Müllerian ducts are absent. However, Wolffian ducts are well differentiated except for the ejaculatory ducts which end in a blind vaginal pouch or in the perineum, close to the urethra.

At puberty, spontaneous virilization occurs, and the body habitus is muscular. The phallus enlarges to 8 cm in length, and labioscrotal folds become rugated and pigmented. Testes enlarge and descend into the labioscrotal folds. Development of acne, facial hair, and enlargement of the prostate do not occur, indicating that these changes are dependent on DHT. Subjects do not develop gynecomastia. In a study of affected individuals from the Dominican Republic, almost all individuals initially raised as females adopted male gender identity during puberty. Some cases of paternity have been reported although the majority have oligospermia or azospermia. Normal sperm production may occur in subjects with descended testes (95,96).

In adult males, the plasma concentration of DHT is low despite normal or slightly elevated levels of testosterone. The T/DHT ratio is >36 (normal 8–16). Serum plasma LH levels are elevated, suggesting a role for DHT in negative feedback at the level of the hypothalamus. FSH levels are normal or high, reflecting damage to seminiferous tubules. In the newborn period, the T/DHT ratio is high. During infancy and childhood, hCG stimulation also results in normal serum levels of testosterone, but subnormal serum

levels of DHT. In the first few months of life, when plasma levels of testosterone and DHT are detectable, the normal T/DHT ratio is less than 12. Following hCG stimulation, normal boys from 17 days to 6 months have a T/DHT ratio of 5.2 ± 1.5. A T/DHT ratio of 11 ± 4.4 is considered normal in boys from 6 months to 14 years. Abnormally high T/DHT ratios indicate 5α-reductase deficiency (97).

46,XX subjects with 5α-reductase deficiency have a normal female phenotype and normal puberty, but decreased axillary and pubic hair. Menarche is usually delayed; fertility is normal (95).

The pattern of inheritance in 5α-reductase deficiency is autosomal recessive involving homozygous or compound heterozygous mutations. However, paternal uniparental disomy was found in one patient (98). All cases of steroid 5α-reductase deficiency are related to mutations in the coding region of the 5α-reductase II gene. Mutations are found in all five exons of the gene. The majority of subjects have missense mutations (99). Other individuals have deletions, splice-junction, and nonsense mutations. The end product is a nonfunctional or subfunctional protein with decreased affinity of the enzyme to NADPH or decreased binding to testosterone.

If affected babies are raised as males, surgical correction of the external genitalia and cryptorchidism should be performed. Prior to surgery, administration of androgens is recommended to increase phallic length and facilitate hypospadias repair. Normal levels of DHT have been achieved in adults following pharmacological doses of testosterone, presumably though activity of 5α-reductase 1. If the child is raised as a female, surgical correction of external genitalia should be performed and gonadal tissue should be removed before puberty. Cyclic hormonal therapy at puberty for development of secondary sexual characteristics is required (95).

Androgen Insensitivity Syndrome

Androgen Insensitivity Syndrome (AIS) is comprised of four conditions with distinct phenotypes. These conditions include complete AIS (CAIS), partial AIS (PAIS), Androgen insensitivity associated with the Infertile Man syndrome, and Kennedy syndrome.

Clinical Phenotype

Complete Androgen Insensitivity. CAIS is an X-linked trait with an incidence of 1:20,000 to 1:64,000 male births (100). This condition has also been referred to as the Testicular Feminization syndrome (101). CAIS is characterized by a normal 46,XY karyotype, female external genitalia with a short vagina, absence of Müllerian structures and absent or vestigial Wolffian structures. Gonads may be located in the inguinal canal, or may be intraabdominal. CAIS is diagnosed during infancy or childhood when surgery done for inguinal hernia reveals a testis in the hernia sack. If the diagnosis is not made early, and testes remain in place, subjects have spontaneous breast development at puberty but develop little or no axillary hair. The clitoris or labia majora are normal but the labia minora may be underdeveloped. These subjects are usually ascertained in the late teens or early twenties because of amenorrhea. At that time, serum levels of LH and testosterone are abnormally elevated. Estradiol, which is derived from peripheral conversion of testosterone and from testicular secretion, tends to be in the normal female range. Serum AMH tends to be abnormally high in women with CAIS because the physiologic suppression of AMH by testosterone does not take place (102). Women with CAIS have a greater incidence of testicular tumor, with risk increasing significantly after puberty. Management of adult women with CAIS involves gonadectomy and sex hormone supplementation. In some women vaginoplasty may be necessary to improve the length of the vagina.

Wisniewski et al. (103) examined the long-term outcome of women with CAIS. Fourteen women were studied using questionnaires and follow up physical examination. These women considered their development of secondary sexual characteristics to be satisfactory. Most patients were satisfied with their sexual function. All of the women studied were satisfied with sex of rearing. However, the study did indicate that more than two-thirds of the women had an incomplete understanding of their condition.

Partial Androgen Insensitivity. Subjects with PAIS usually present in the newborn period with ambiguous genitalia. Müllerian structures are absent and development of Wolffian ducts is typically abnormal. The diagnosis is suspected if serum testosterone levels and the testosterone/DHT ratio are normal. Several approaches have been suggested to distinguish between PAIS and other conditions that may have a similar hormonal profile. Some authors have suggested that the extent of penile growth after administration of testosterone or hCG provides an indicator of responsiveness to androgens and that an adequate response excludes AIS. As androgen secretion typically lowers sex hormone-binding globulin (SHBG) levels, detecting higher than normal levels of SHBG might also indicate androgen insensitivity (104). As blood levels of AMH are normally suppressed by androgens, some investigators have suggested that normal male levels of testosterone and DHT but unsuppressed AMH could indicate PAIS (105).

At puberty, serum levels of LH and testosterone are abnormally elevated, but in contrast to 5α-reductase deficiency the T/DTH ratio remains normal. There is enlargement of the penis at puberty, but it usually remains small. Serum levels of estradiol are abnormally elevated and gynecomastia is almost always present. Testes are usually small and there is azoospermia.

The Androgen Insensitivity Associated with Infertile Men. Subjects with this syndrome are ascertained during evaluation for infertility. Some subjects have mild hypospadias, but many have normal male external genitalia. The characteristic hormonal profile is similar to that of other forms of AIS, namely elevated levels of serum LH, testosterone, and estradiol (106).

Kennedy Syndrome. Kennedy syndrome is an X-linked from of spinal and bulbar muscular atrophy. Although most cases appear in mid to late adulthood some patients may present in adolescence (107). The association of gynecomastia and the variable presence of impotence and infertility suggest a form of androgen insensitivity (108).

Genetic Abnormalities Related with AIS

Most cases of CAIS and PAIS are related to mutations in the AR gene. The majority of them are point mutations. Relatively few mutations have been detected in the amino terminal domain. Mutations within this region that result in stop codons are typically associated with CAIS. Deletions of polyglutamine repeats are associated with PAIS. Kennedy syndrome results when the number of polyglutamine repeats in the amino terminal is over 42. Nonetheless, there does not appear to be a correlation between phenotype and the number of excess repeats. Abnormal expansion of the repeats appears to create problems by inducing an abnormal aggregation of ARs in cytoplasm and nucleus (51,109).

Mutations that result in stop codons within the DNA-binding domain and deletions of the second zinc finger are associated with CAIS (110). Mutations in the phosphorylation site are associated with both CAIS and PAIS.

Mutations in the hormone-binding domain can result in both complete and PAIS. Frame shift or point mutations that result in premature termination are associated with CAIS. By contrast, mutations causing less severe defects in hormone binding are more likely to be found in patients with PAIS. Splicing mutations in the AR gene have also been reported in PAIS. They result in transcription of mRNA encoding mutant AR as well as lower levels of mRNA encoding normal AR (111).

In one case of CAIS where no mutation of the AR gene was found, Adachi and colleagues presented evidence for an abnormality of an AF1-binding protein, suggesting that AIS may also be caused by mutations of coactivators (112).

Correlations have been established between phenotype and genotype in the AIS. However, these relationships do not always apply. For example, most mutations that result in absence of AR binding result in CAIS. However, some of these mutations are also associated with PAIS. Similarly most mutations that are associated with intact AR binding in cultured cells are associated to PAIS. Nonetheless, some of them are also associated with CAIS. More surprising is the observation that CAIS and PAIS have been associated with the same mutation in the AR, suggesting that other factors play a role in AR function (113).

Evidence from various studies provides a possible explanation for the lack of phenotype–genotype correlation in some patients. Holterhus described a patient with PAIS who had a mutation in the AR that resulted in a premature stop codon. Such mutations are usually associated with CAIS. The unexpected mild phenotype in this report was the result of somatic mosaicism (114). Other studies suggest that differences among affected family members could result from variable levels of DHT in each of the individuals in utero (115,116).

Multiple Congenital Anomalies

Some subjects have well described syndromes in which there is abnormal sex differentiation in association with other congenital anomalies, but no obvious defect in gonadal differentiation, testosterone biosynthesis, or androgen effect (117). Subjects frequently have other defects in the genitourinary system. Many of these cases are sporadic. In addition, maternal exposure to certain drugs, such as dilantin, trimethadione, and progesterone have been implicated in abnormal sex differentiation.

Timing Defects

Several patients have been reported who had a 46,XY karyotype and ambiguous genitalia, but normal production of testosterone and DHT at puberty and normal responsiveness to androgens. It was suggested that these subjects had delayed differentiation of Leydig cells and the term Timing Defect was applied to this condition. It is possible that subjects with so-called idiopathic male pseudohermaphroditism have such a timing defect.

Persistent Müllerian Duct Syndrome

This is a rare condition characterized by the presence of Müllerian derivatives (uterus, fallopian tubes, and superior two-thirds of the vagina) in an otherwise normal 46,XY individual (118). There are two forms. In the first form which comprises 85% of reported cases, there is unilateral cryptorchidism and contralateral inguinal hernia. The undescended testis is usually in the inguinal canal, but may also be found in the hernia sac (transverse testicular ectopia). In the second form both testes are intraabdominal and are embedded in the round ligament. In this form the uterus is fixed in the pelvis. The round ligament may be distended, resulting in an abnormal mobility of Müllerian derivatives. Testes are also abnormally mobile because they are not connected to the base of the scrotum. They contain germ cells, but are not

properly connected to male excretory ducts. Aplasia of the epididymis and aplasia of the upper part of the vas deferens have been reported. The Müllerian portion of the vagina contacts the posterior urethra at the veru montanum, but communication is usually not present. Although the urethra appears normal, there is usually infertility (118).

Affected subjects are usually ascertained because of undescended testes or inguinal hernia. Serum levels of testosterone are typically normal in these patients. The level of AMH helps determine the etiology. AMH levels are low if there is an abnormality in AMH production, but high if there is a defect in the AMH receptor (48). Although a pelvic ultrasonography may be useful to detect Müllerian structures, false negative results can occur. Congenital adrenal hyperplasia (CAH) in a female must be excluded in all subjects with bilateral cryptorchidism.

Persistent Müllerian Duct Syndrome (PMDS) is inherited either as a sex-limited autosomal recessive trait (the most common) or as a sex-linked recessive trait. It is caused by mutations in either the AMH gene or in the gene for the AMH receptor. The most common genetic anomaly causing PMDS is a 27 base pair deletion in exon 10 of the antiMüllerian type II receptor gene (119).

Optimal surgical management includes orchiopexy, but the uterus and fallopian tubes are left in place. Orchiectomy is indicated if testes cannot be brought into the scrotum. Careful identification of the vas deferens with meticulous dissection of the Müllerian structures facilitates intrascrotal placement of the testes. However, surgical management of patients with PMDS is controversial because there is potential morbidity with either retention or removal of Müllerian structures. Surgical excision of persistent Müllerian duct structures may result in ischemic and/or traumatic damage to the vas deferens and testes. However, the case of a boy with PMDS and uterine adenosarcoma raises question about the advisability of leaving Müllerian structures in place (120).

Abnormalities of Sex Differentiation in Individuals with 46,XX Karyotype

This group represents one-third to one-half of patients with abnormalities of sex differentiation. The condition has been referred to as female pseudohermaphroditism because of the discordance between the masculinization of the genitalia and the presence of normal ovaries (Table 4). In this group of conditions, Wolffian ducts (epididymis, vas deferens, and seminal vesicles) are absent. The degree of masculinization of external genitalia is determined by the extent of androgen secretion and by the timing of androgen production. For example, after 16 weeks female sex differentiation is complete. Androgen exposure after that time causes clitoral hypertrophy but no additional masculinization of the genitalia.

Table 4 Abnormalities of Sex Differentiation in Individuals with 46,XX Karyotype

Congenital adrenal hyperplasia
CYP21 (21-hydroxylase) deficiency
CYP11 (11β-hydroxylase) deficiency
3β-hydroxysteroid dehydrogenase II deficiency
P450 oxidoreductase deficiency
Exposure to maternal androgens excess
Iatrogenic: androgens and progestins
Virilizing ovarian or adrenal tumor
Luteoma of pregnancy
CYP19 (aromatase) deficiency
Congenital abnormalities

Congenital Adrenal Hyperplasia

The most common cause of abnormal sex differentiation in an XX individual is CAH. This is a group of conditions characterized by abnormal biosynthesis of cortisol and aldosterone. The diminished secretion of cortisol results in marked elevation of plasma ACTH and subsequent stimulation of excessive secretion of adrenal androgen. CYP21 (21-hydroxylase) deficiency is the most common cause of CAH. Two other virilizing forms of CAH are 11β-hydroxylase and 3β-HSD deficiencies. These conditions are discussed at greater length in the chapter on CAH. A brief summary is presented below.

CYP21 (21-Hydroxylase) Deficiency

CYP21 is required for conversion of 17-hydroxyprogesterone to 11-deoxycortisol and progesterone to DOC. As a consequence, deficiency of CYP21 results in decreased production of mineralocorticoid and glucocorticoid. Subjects with the severe form present with ambiguous genitalia, whereas patients with a mild form may present with signs of virilization later in life. Salt-losing crisis is a potentially life-threatening event in the severe form of this disorder. At presentation serum levels of 17-hydroxyprogesterone are markedly elevated (3000–40,000 ng/dL), the level depending upon age and severity of the enzyme defect. Androstenedione levels are also abnormally high (121,122).

CYP11 (11-Hydroxylase) Deficiency

This condition is characterized by lack of conversion of 11-deoxycortisol to cortisol and conversion of DOC to corticosterone. High blood pressure occurs due to increased levels of DOC (121,122).

3β-HSD/Δ^{4-5} Isomerase Deficiency

This condition is characterized by lack of conversion of Δ^5 3β-hydroxysteroids to 3-ketosteroids. The synthesis of both aldosterone and cortisol is impaired. Salt wasting and adrenal crisis can occur in severe forms of this condition. Serum levels of 17-hydroxypregnenolone may be increased due to impaired conversion of 17-hydroxypregnenolone to 17-hydroxyprogesterone.

In addition, the ratio of 17-hydroxypregnenolone to 17-hydroxyprogesterone is elevated in these patients (121,122).

P450 Oxidoreductase Deficiency

This condition is discussed in detail in section ''Defects of testosterone biosynthesis.'' The external genitalia of female subjects range from completely normal to severely masculinized. The dysmorphic features described in XY individuals with this condition are also present in affected women.

Maternal Androgens

Iatrogenic

Several drugs such as the progestins, norethindrone, and ethisterone have been associated with masculinization of the female external genitalia. Stilbestrol and its metabolites cause masculinization of female external genitalia through inhibition of 3β-HSD (36). Danazol has also been associated with abnormal masculinization of female external genitalia.

Androgen Secreting Tumors

Androgen secreting tumors in the mother can also cause masculinization of the female fetus. Luteoma of pregnancy is a rare tumor-like mass that emerges during pregnancy and regresses spontaneously after delivery. Hence, absence of maternal virilization after pregnancy does not exclude the condition. Other androgen secreting tumors of both the ovary and adrenal have also been reported. Ovarian tumors include Brener tumor and thecoma, among others. Adrenal tumors causing masculinization of a female in utero are extremely rare (123).

Placental Aromatase Deficiency

Human CYP450 aromatase is expressed in placental syncytiothrophoblast and in many other fetal tissues. After the ninth week of gestation, the placenta provides the primary source of circulating estrogens. Lack of placental aromatase exposes the fetus to androgen excess and can result in ambiguous genitalia in females. Diagnosis should be considered in a 46,XX infant with ambiguous genitalia in whom CAH has been excluded. The diagnosis is suggested by maternal virilization during pregnancy, associated with abnormally high serum levels of androstenedione, testosterone, and DHT. Low plasma levels of estriol, as well as low urinary estriol concentration, are present. Amniotic fluid concentrations of androstenedione and testosterone are high, while estrone, estradiol, and estriol levels are low (124).

Syndromes of Multiple Congenital Abnormalities

Cloacal anomalies, agenesis of kidneys, abnormalities of the urinary tract, and the gastrointestinal tract, have all been associated with ambiguous genitalia in a XX individual. The Müllerian structures in these patients may be poorly developed and dysplastic. The etiology of this defect is uncertain. There is often disorganized differentiation of the caudal structures including perineum, genital tubercle, and genital folds (125). In addition, maternal alcohol use during pregnancy has been associated with multiple anomalies including clitoral hypertrophy.

DIAGNOSTIC EVALUATION FOR AMBIGUOUS GENITALIA

When a child is born with ambiguous genitalia, a specialized care team should be convened. A rapid and organized evaluation should be initiated to develop information about karyotype, gonadal function, androgen biosynthesis, and internal anatomy.

Diagnostic Evaluation

History

A thorough family history is important with respect to perinatal or neonatal deaths, infertility, consanguinity, or history of infants with ambiguous genitalia. The patterns of inheritance in the various intersex disorders must be considered. A maternal history should focus on complications of pregnancy, especially during the first trimester, and should include information regarding drug and alcohol use, as well as hormone administration. Birth weight should be ascertained to exclude intrauterine growth retardation and its association with chromosomal anomalies.

Physical Examination

The physical examination should determine whether the infant has dysmorphic features, associated with a syndrome of multiple congenital anomalies. Abnormal body proportions suggest a syndrome of bone dysplasia. Stigmata, such as webbed neck and edematous hands and feet, may be present in mixed gonadal dysgenesis (45,X/46,XY). Table 5 includes some of the conditions associated with genital ambiguity.

The stretched phallic length should be measured along the dorsum from the pubic ramus to the tip of the glans. The degree of development of the corpora may be assessed by palpation of the shaft. Normal values for stretched penile length in neonates and preterm infants are available, as are normal values for clitoral length. It is important to note that premature female infants may appear to have clitoromegaly because they have a larger clitoral breadth compared to body size.

The urethral opening is assessed by careful examination of the ventral area of the phallus for grooves and chordee. The urethral meatus may be anywhere from the tip of the phallus to the perineum. A single

Table 5 Ambiguous Genitalia Associated Syndromes

	Clinical findings
Chromosomal abnormalities	
Trisomy 13	Holoprosencephaly, polydactyly, cleft lip, hypospadias, cryptorchidism
Trisomy 18	Clenched hand, short sternum, malformed auricles, cryptorchidism, virilized females
Triploidy syndrome	Prenatal growth failure, microphtalmia, congenital heart defects, hypospadias, micropenis
$4p^{-}$	Ocular hypertelorism, broad nose, microcephaly, low set ears, hypospadias, cryptorchidism
$13q^{-}$	Microcephaly, colobomata, thumb hypoplasia, hypospadias, cryptorchidism
Syndromes	
Aaskog	Hypertelorism, brachydactyly, shawl scrotum, cryptorchidism
Antley–Bixler syndrome	Craniosynostosis, radiohumeral synostosis, ambiguous genitalia, impaired steroidogenesis (defect in P450 oxidoreductase)
Campomelic dysplasia	Flat facies, bowed tibiae, hypoplastic scapulae, 46,XY partial gonadal dysgenesis
Carpenter	Acrocephaly, polydactyly, and syndactyly of the feet, lateral displacement of inner canthi, mental retardation, hypogonadism
CHARGE	Colobomata, heart defect, choanal atresia, retarded growth, genital hypoplasia, ear anomalies
Curradino syndrome	Partial sacral agenesis with intact first sacral vertebra (sickle-shaped sacrum), a presacral mass, and anorectal malformation (Currarino triad)
Ellis–Van Crevel	Mesomelic dwarfism, polydactyly, cardiac anomalies, cryptorchidism
Fraser	Cryptophtalmos (eye hidden, fused lids: absence of palpebral fissure), defect of auricle, males with cryptorchidism and hypospadias, females with vaginal atresia
Lissencephaly X-linked	Lissencephaly with ambiguous genitalia
Mecke–Gruber	Encephalocele, polydactyly, renal cystic dysplasia, ambiguous genitalia
Oral–facial–digital syndrome	Polydactyly, campomelia, ambiguous genitalia, cystic dysplastic kidneys, and cerebral malformation
Rieger	Iris dysplasia, maxillar hypoplasia, hypospadias
Robinow syndrome	Short stature, mesomelic and acromelic brachymelia, hypertelorism, wide palpebral fissures, midface hypoplasia and large mouth, and hypogenatilism
SCARF syndrome	Lax skin, joint hyperextensibility, umbilical and inguinal hernias, craniosynostosis, pectus carinatum, abnormally shaped vertebrae, enamel hypoplasia with hypocalcification of the teeth, facial abnormalities, wide webbed neck, ambiguous genitalia, multiple nodular liver tumors, and mild psychomotor retardation
Short rib polydactilia	Cleft lip, malformed larynx with hypoplastic epiglottis, pulmonary hypoplasia, renal cysts, ambiguous genitalia, pachygyria, and small cerebellar vermis
Smith–Lemli–Opitz syndrome type II	Mutations in the Δ^{7}-dehydrocholesterol reductase gene in chromosome 11q12–13, failure to thrive, facial dysmorphism, ambiguous genitalia, syndactyly, postaxial polydactyly, and internal developmental anomalies (Hirschsprung disease and cardiac and renal malformations)
Smith–Lemli–Opitz syndrome type I	Failure of masculinization, intraabdominal testes, a normally shaped uterus and vagina, polydactyly, cleft palate, blepharoptosis, and abnormalities of the kidneys, liver, and lungs
VATER	Vertebral anomalies, anal atresia, tracheo-esophageal fistula, radial and renal dysplasia, bifid scrotum
vonVass–Cherstvoy syndrome	Phocomelia of upper limbs, encephalocele, brain anomalies, ambiguous genitalia, thrombocytopenia
WAGR	Contiguous gene syndrome: Wilms tumor, aniridia, cataracts, genitourinary abnormalities, ambiguous genitalia, mental retardation

opening on the perineum indicates the presence of a urogenital sinus. The labioscrotal folds are examined for degree of fusion, development of rugae, and pigmentation. The presence of gonadal tissue must be carefully assessed. Each gonad is evaluated for size, texture, and the presence of an epididymis.

Minimal enlargement of the phallus and only mild posterior fusion may be associated with a mild form of masculinization in an XX individual, or a severe form of undermasculinization in an individual with an XY karyotype. Similarly, phallic enlargement with nearly complete fusion of the labioscrotal folds may be associated with a minimal defect of masculinization in an individual with a XY karyotype or a very severe abnormality of sexual differentiation in an individual with an XX karyotype. Hence, the extent of masculinization of the external genitalia does not provide information about the underlying diagnosis but merely provides information about the extent of the abnormality once the karyotype is known.

Etiologic Evaluation

Adrenal hyperplasia is always a possibility in an infant who presents with ambiguous genitalia. Therefore, throughout the period in which diagnostic tests are performed, careful attention must be paid to blood chemistry and vital signs to ensure that early and appropriate treatment is started if clinical presentation suggests CAH.

A karyotype is obtained on the first day of life and is usually done using peripheral lymphocytes. Occasionally chromosome analysis from other tissues may be necessary to exclude mosaicism. With respect to production of sex steroids, plasma levels of testosterone and dihydrotestosterone are determined on days 1 and 2 as there is a physiological peak of

testosterone secretion in normal boys at this time. Plasma levels of 17-hydroxyprogesterone and 17-hydroxypregnelonone should be determined on days 3 and 4. It is necessary to wait until days 3 and 4, because contaminating plasma steroids can cause spurious results (62). Assays of both testosterone and 17-hydroxyprogesterone require a chromatographic step prior to assay to prevent additional artifacts (126). Occasionally, assay of other plasma precursors of testosterone may be indicated. Determination of AMH level is also important as a marker of Sertoli cell function. In the newborn period AMH levels are elevated in boys (119). Other molecular genetic testing should also be considered.

If the baby is clinically stable, imaging studies should be performed on day 5 of life. A genitogram with retrograde injection of contrast media into the urogenital sinus should be performed to detect the presence of Müllerian structures, as well as to outline the anatomy of the urethra. Sonography may also be performed to detect Müllerian structures. In some instances sonography may be useful to identify abdominal or inguinal gonads. In addition, MRI of the pelvis may also be needed.

Diagnosis of Ambiguous Genitalia

If the karyotype indicates mosaicism or possible chimerism involving a Y chromosome, the abnormality of sex differentiation is considered to be a function of the cloning of the cell types in the gonad. If the karyotype is 46,XX or 46,XY the diagnosis will be established by the hormonal profile, the presence of specific internal duct structures, and, in some cases, by gonadal histology and genetic testing (62).

In patients with a 46,XY karyotype and a subnormal plasma level of testosterone, abnormally low levels of steroid precursors of testosterone indicate the possibility of 46,XY gonadal dysgenesis, 46,XY true hermaphroditism, and Leydig cell aplasia or hypoplasia. However, AMH levels are normal in Leydig cell aplasia but low in conditions with abnormal testis determination (119). Subnormal levels of plasma testosterone, but abnormal elevation of plasma precursors of testosterone, indicate a defect in the biosynthesis of testosterone. If levels of plasma testosterone and DHT are normal or elevated, but the ratio of T to DHT is abnormally elevated, a diagnosis of 5α-reductase deficiency is made. If plasma concentration of T and DHT are both normal the differential diagnosis includes androgen insensitivity, syndromes of multiple congenital anomalies, and timing defects.

In subjects with a 46,XX karyotype, diagnostic considerations include abnormalities of gonadal differentiation and abnormalities that result from exposure of the fetus to excess androgen. The former group comprises 46,XX true hermaphroditism and the XX maleness. In such cases serum AMH may be detected (119). When normal ovarian differentiation has occurred, masculinization may have resulted from CAH (deficiency of 21-hydroxylase, 3β-HSD, and 11-hydroxylase), androgen secreting tumors in the mother, placental aromatase deficiency, various drugs, and syndromes of multiple congenital anomalies.

MANAGEMENT OF INTERSEX

Introduction

The management of children born with ambiguous genitalia should include assessment of the anatomy of sex organs, on likely cosmetic appearance of the reconstructed genitalia, on the potential for normal sex steroid secretion at puberty, on the potential for normal sexual intercourse, and on the potential for fertility.

Gender Assignment at Birth

Gender assignment of infants with ambiguous genitalia at birth requires open and intense communication between the medical team and the family. Counseling requires avoidance of oversimplification. However, complete information must be provided and must be presented in a way that can be assimilated by the family. It is useful for health care professionals to develop a sex-assignment team where a multidisciplinary approach can be provided (127).

Long-Term Follow-Up of Patients with Abnormalities of Sexual Differentiation

Congenital Adrenal Hyperplasia

Almost all female CAH patients, when appropriately diagnosed, are raised as girls. However, some patients who are born with markedly masculinized external genitalia have been inadvertently assigned to the male gender. In cases in which the diagnosis was made later, many physicians have recommended sex reassignment to the female gender after appropriate parental consent. Most 46,XX patients with CAH who have been assigned or reassigned to the female gender at an early age remain female. If CAH is not diagnosed until later in life, those children raised male usually elect to remain male.

Various sexually dimorphic behaviors are masculinized in girls with CAH including preferences, rough-and-tumble play, aggressiveness, interest in sports, maternal behavior, and vocational preferences. Some investigators have suggested that the most important factor influencing the change in these behaviors is the extent of prenatal androgen exposure. Indeed there appears to be a correlation between the extent of masculinization of external genitalia and masculinization of behavior. However, this greater chance for atypical behavior is not associated with increased risk for gender dysphoria (128,129).

Children with CAH should be monitored regularly and assessed unobtrusively for the degree of gender atypical behavior. In addition, family milieu should be evaluated for acceptance of such behavior. In this regard, appropriate psychological/psychiatric

counseling promoting the acceptance of the behavior is advised. Girls with CAH with markedly gender-atypical behavior persisting into late childhood and early to midadolescence may become alienated from other girls.

In 1987, Mulaikal et al. published the results of a long-term follow-up study of 80 adult female subjects with CAH (130). Half of the subjects had the salt-losing form of CAH, whereas the other 40 patients had the simple virilizing form. Significant differences were found among the women with the two forms of CAH. Whereas only 17% of subjects with the simple virilizing form reported an inadequate vaginal introitus, 52% of subjects with the salt-losing form considered the vaginal introitus to be inadequate. Approximately 45% of women with salt-losing CAH and 30% of subjects with the simple virilizing form reported no sexual activity. Approximately 88% of women with the severe form and 60% of women with simple virilizing form never married, in comparison to 10% of women in the general population. A study by Stikkelbroeck et al. in 2003 examined surgical outcome in women with CAH. Among the five subjects who had gynecologic exams, two had mild vaginal stricture and one had severe stricture (131).

Abnormal Sex Differentiation in Subjects with a 46,XY Karyotype

These studies will ultimately provide information on the adequacy of recommendations for gender assignment. Data are available from studies of 39 46,XY individuals with perinoscrotal hypospadias followed at the Johns Hopkins Pediatric Endocrinology Clinic (132). The group included 21 individuals raised male and 18 raised female. Subjects were evaluated by physical examination, questionnaire, and semistructured interviews. Physical examination indicated significantly better appearance of genitalia among subjects raised female than among those raised male. However, the majority of both the men and the women were satisfied with body image. Most subjects whether male or female, had sexual partners and most were heterosexual. Most of the subjects in the study (16 of 21 males, and 14 of 18 females) were satisfied with gender assignment. Among the nine patients who voiced dissatisfaction, only one male and one female chose to reassign gender. A recent review summarized outcome in nearly 100 46,XY individuals with ambiguous genitalia and found that only nine subjects requested gender reassignment (133). Taken together these studies indicate that either gender assignment can result in a successful outcome and that gender dysphoria is an unusual finding among 46,XY subjects born with ambiguous genitalia when gender was assigned early in life by parents and physicians. However, it remains important to identify the cause of the gender dysphoria in the subjects who developed it.

Surgery During Infancy and Childhood

The timing of genital surgery in the course of a child's development is likely to have important psychological implications. In infancy, the decision must be made by the parents in consultation with physicians. Parents must have unequivocal commitment to the decision. Surgical techniques have advanced dramatically and skillful surgeons have achieved satisfactory outcomes in the majority of patients with intersex. In particular, advances in techniques for reconstruction of male external genitalia have allowed definitive repair in infancy and early childhood. Genital reconstruction in female subjects is typically done at an early age. However there are no data indicating the ideal time for surgery.

Examination of the Genitalia in Childhood and Adolescence

Examination of the genitalia is crucial for evaluating the outcome of surgery, and the impact of hormonal treatment. However, the physician must be aware of the potentially adverse psychological consequences of such examinations, even if the child seems overtly compliant. Genital examinations have more significant psychological implications than examinations of other body parts. Many patients become oversensitized by frequent examination. Hence, such examinations must be performed with psychological sensitivity. Their repetition by multiple trainees should be avoided. Alternative training strategies should be developed that do not adversely affect the patient.

Psychological Counseling

Most authors recommend an annual visit, particularly during adolescence, with a mental health professional who is familiar with the psychosocial and sexual problems of intersex patients. In addition, meeting one or more patients with the same condition can be extremely helpful. The development of clinic-affiliated support groups is recommended. Patient-support groups based on the Internet can also be useful. When recommending support-group affiliation, one should always make the patient aware of the risks, including biases of these groups. The patient should be encouraged to discuss novel information acquired from support groups with his or her physician or mental health specialist. Patients with specific sexual dysfunction may need to be evaluated by a specialist in the field. Given the scarcity of mental health personnel familiar with intersex problems, physicians and patient organizations should press for appropriate training of mental health liaison personnel and for third-party coverage of the respective services.

MICROPENIS

Micropenis is a condition characterized by a normally formed penis with a stretched penile length of more

than 2.5 standard deviations below the mean for age. The mean stretched penile length in newborns is 3.5 cm (−2.5 standard deviation is 1.8 cm).

Measurement of penile length should be made on the fully stretched rather than flaccid penis. A ruler should be pressed against the pubic ramus, depressing the suprapubic fat pad as completely as possible. The penis should be stretched by grasping the glans between the thumb and forefinger. The measurement is made along the dorsum to the tip of the glans without including the foreskin, if present. Accurate examination and measurement are essential in determining the presence of micropenis. Micropenis must be differentiated from ''hidden penis'' in which a normal penis is obscured by excessive suprapubic fat and from a penis held down by marked chordee, in which the penis has a downward bowing as a result of a congenital anomaly (134).

Patients with micropenis may be classified in four major groups:

1. ''Hypogonadotropic hypogonadism'' is characterized by an abnormality in the hypothalamic–pituitary axis, resulting in an inadequate androgen production. Syndromes in this category include Kallmann's syndrome, Prader Willie syndrome, Laurence–Moon syndrome, Rud's syndrome, and conditions with multiple pituitary hormone deficiency.
2. ''Hypergonadotropic hypogonadism'' is characterized by primary gonadal failure. Conditions included in this category are Klinefelter syndrome and other X polysomies, Robinow syndrome, trisomy 21, Noonan's syndrome, and Laurence–Moon syndrome.
3. ''Failure of androgens action'' includes subjects with mild partial androgen insensitivity.
4. ''Idiopathic micropenis.'' Subjects in this category have normal hypothalamic–pituitary–gonadal function. Very rarely the entire penis is absent, a condition named aphallia (135).

The evaluation of patients with micropenis should be directed toward early diagnosis and therapy. Infants should be carefully monitored. Potentially dangerous conditions such as hypothyroidism, hypocortisolemia, growth hormone deficiency, and diabetes insipidus, should be excluded and treated. Plasma levels of FSH, LH, and testosterone should be determined. A gonadotropin releasing hormone stimulation test and/or an hCG stimulation test may be helpful in establishing the etiology of the micropenis.

The ability of the penis to respond to androgens can be assessed following administration of testosterone or hCG in the newborn period. Treatment with intramuscular testosterone in infancy and childhood has been recommended to improve the appearance of the penis and to facilitate toilet training. This may be given after the second or third month of age, 25 mg of testosterone enanthate per month for two to four months. Accurate measurements of the penis should be done, once penile response is determined treatment need be discontinued. If no response is elicited gender reassignment may be considered. The side effects of this treatment are minimal, and include temporary acceleration in growth, and advancement of bone age, in addition to other side effects attributed to testosterone therapy. Replacement therapy at puberty may be necessary in some individuals.

Wisniewski et al. examined long-term outcome among subjects with congenital micropenis (13 raised as males and five raised as females). Penile length in individuals raised male was below the mean in all subjects and many men reported dissatisfaction with appearance of genitalia. However, both males and females were satisfied with their sex of rearing. Subjects raised female required several surgeries for reconstruction of genitalia (136). These results suggest that male gender assignment is appropriate in boys born with micropenis.

HYPOSPADIAS

Hypospadias is a condition in which there is abnormal placement of the urethral meatus. Isolated hypospadias is the most common congenital malformation in males with an estimated incidence of 1 in 300 live male births. In the United States, the incidence of hypospadias has increased over the past years (137). There is familial tendency and an increased risk among certain ethnic groups. Specifically, 21% of subjects with isolated hypospadias had another family member with hypospadias, 14% having an affected brother and 7% having an affected father (138).

The formation of the ventral foreskin of the penis is related to normal urethral development. Failure of the urethra to reach the tip of the glans is accompanied by absence of the ventral foreskin, which in turn causes ventral curvature of the penis known as chordee. If normal development of the urethra is arrested and the urethral folds fail to fuse, the meatus may be found anywhere from the perineum to the glans.

Hypospadias is classified based on the location of the urethral meatus after ventral curve has been surgically corrected. Glandular or coronal type hypospadias account for 50% of all cases. Distal midshaft and proximal penile forms comprise 30%. The remaining 20% are penoscrotal and perineal (139).

In most cases, isolated hypospadias has no identifiable etiology. However, severe isolated hypospadias should be investigated. Hypospadias may be part of many syndromes of human malformations. Hypospadias is also associated with intrauterine exposure to antiandrogens and to environmental estrogens. An etiology for hypospadias in xenoestrogen exposure has been suggested by the observation that estrogen induces overexpression of activating transcription factor 3 (AFT3) and that AFT3 levels are abnormally high in penile tissue of subjects with isolated hypospadias (140).

Surgical reconstruction of hypospadias requires correction of chordee when present. It is recommended that boys with hypospadias not be circumcised, because the foreskin may be used in the urethroplasty. If hypospadias is associated with micropenis, treatment with testosterone is usually performed before surgery.

CRYPTORCHIDISM

Epidemiology

Cryptorchidism is also a relatively common condition in boys, having a prevalence of 4% to 5% in full-term boys and 9% to 30% in prematures (42). Spontaneous testicular descent usually occurs by the end of the first year of life, at which time the prevalence of cryptorchidism has declined to 1%. Cryptorchidism is usually unilateral (90%), and most often right-sided. The undescended testis can be located in the abdomen (8%), in the inguinal canal (72%), and just distal to the external ring (20%). In rare instances, the testis migrates along an abnormal pathway. This so-called ectopic testis may be located in the superficial inguinal pouch, perineum, femoral canal, prepenile scrotum, or contralateral scrotum.

An undescended testis must be differentiated from a retractile testis. The retractile testis is a normal testis that has an active cremasteric reflex that pulls the testis back into the groin. In this condition, the testis can be brought into the scrotum easily without putting the testis under tension. A retractile testis can often be more easily brought into the scrotum when the child assumes a squatting position. Alternatively, it may be seen in the scrotum when the child is in a warm bath.

Etiology

Undescended testis results from a disruption in the physiologic testicular descent. Decreased androgen production as a consequence of abnormalities of the hypothalamic–pituitary axis, such as anencephaly, pituitary aplasia, and Kallmann syndrome, is associated with the occurrence of undescended testis. Defects of androgen synthesis and AR insensitivity are associated with the presence of undescended testis (141,142). Mechanical anomalies related to urogenital obstruction including Prune Belly syndrome, posterior urethral valves, and defects of the abdominal wall such as gastrochisis and omphalocele cause undescended testis (143). Various chromosomal abnormalities can also be identified. A genetic etiology is suggested in some patients by the occurrence of undescended testis in 1.5% to 4% of fathers and 6.2% of brothers of patients with cryptorchidism (42).

Abnormalities of the gene encoding INSL3, also known as Leydig insulin-like protein, have been implicated in the etiology of cryptorchidism. Mice with mutations of INSL3 have bilateral undescended testis with abnormal development of the gubernaculum (144). Tomboc et al. reported the presence of two mutations in the INSL3 gene in 2 of 145 patients with undescended testis (145).

Evaluation of Undescended Testes

In newborns with male external genitalia and bilateral undescended testis, salt-losing CAH in a female infant must be excluded. If the karyotype is 46,XY the presence of dysmorphic features may indicate a specific syndrome. Specific studies may be warranted in the diagnosis of undescended testis. Testes located in the external inguinal canal or just adjacent to the external inguinal ring are easily detected by a high-resolution ultrasound (146). In a 46,XY subject with unilateral undescended testis evaluation is advised at one year of age. In older children with undescended testis, hormonal evaluation, including determination of LH, FSH, and AMH is recommended. In children with hypogonadotropic hypogonadism, a complete hormonal evaluation is indicated.

Consequences of Cryptorchidism

Testicular neoplasm, infertility, testicular torsion, and inguinal hernia are the most common complications of undescended testis. Although the risk of testicular malignancy in the general population is one in approximately 45,000 males, 10% of testicular tumors in adults occur in men with a history of undescended testis. Furthermore, the risk of testicular tumor in subjects with unilateral and bilateral undescended testis is 15 and 33 times greater, respectively, than that of subjects with normal testes. Intraabdominal testes are five times more likely to develop a tumor. Seminomas are the most common type of malignancy followed by embryonal cell carcinoma. Hence, germ cell degeneration and dysplasia are considered the etiology of malignancy. Most studies indicate that surgical correction of cryptorchidism does not reduce the risk of testicular malignancy. The relationship between development of malignancy and an intrinsic abnormality of the testis is suggested by the observation that the contralateral, normally descended testis may also develop malignancy (42).

Infertility associated with cryptorchidism is related to lack or decrease in the number of germ cells. This occurs from the relatively high intraabdominal temperature. Decreased number of germ cells has been reported as early as three months of age (147). By two years of age the germ cells have decreased to 40% of normal. There is an increase in interstitial fibrosis and collagenization in peritubular connective tissue. The abnormality observed in the contralateral descended testis may also occur at an early age and may be related to production of autoantibodies. Paternity rates are lower in men with a history of bilateral cryptorchidism compared to men with a history of unilateral cryptorchidism. Sudden painful inguinal swelling in association with cryptorchidism may signal

testicular torsion, or hernia with incarceration, both of which indicate the need for urgent intervention.

Treatment

The therapeutic goals in treatment of cryptorchidism are designed to (i) prevent infertility, (ii) avoid malignancy, (iii) correct, if present, an associated hernia, and (iv) alleviate psychological stress caused by the empty scrotum.

Hormonal Therapy

Protocols with hCG and/or pharmacologic preparation of GnRH have been used with various success. The World Health Organization recommends hCG 250 IU twice a week for five weeks in boys up to one year of age. From one to five years of age, 500 IU is recommended twice weekly for five weeks. In older boys, 1000 IU twice weekly is suggested for five weeks. Nonetheless, published dosages and treatment schedules have varied from 100 to 4000 IU per injection given two to three days per week for one to five weeks. Combined treatment using hCG and GnRH analogs has been reported to improve response in nonpalpable testis (148). Successful treatment of the true undescended testis with hCG has been reported to be as low as 6% and as high as to 65%. Hormonal treatment is more effective in treatment of testis located immediately prescrotal.

Surgical Therapy

The optimal time to operate is unknown, considering the evidence that histological changes in the testis occur as early as two to three months of age. However, the recommendation, as proposed by the Action Committee on Surgery of the Genitalia, is to perform orchiopexy at 12 months of age. Numerous studies have reported that the cryptorchid testis that have not descended spontaneously by this age are not likely to descent thereafter. Most orchiopexies are performed as outpatient procedures. There is a 90% incidence of inguinal hernia with undescended testis, which is usually repaired simultaneously. The success rate is over 95%. Bilateral inguinal testes can be operated upon at the same time. The nonpalpable undescended testis can now be located by laparoscopy to inspect the peritoneal cavity. Of nonpalpable testes, 20% are atrophic, and blind-ending spermatic vessels and vas deferens are noted intraabdominally. Numerous methods have been used to investigate cryptorchidism, including sonogram, CT scan, venography of the spermatic vessels, and, recently, MRI. Laparoscopy can also be used to identify the testis before orchiopexy.

Most testes can be placed within the scrotal sac by one procedure. However, when the spermatic vessels are extremely short, a two-stage orchiopexy can be performed. The spermatic vessels are ligated or divided during the first stage, allowing collateral blood supply via the vasal artery to develop. Then, four to five months later, the testis can be brought into the scrotal sac and nourished with the vasal blood supply, with a success rate of 90%. In bilateral nonpalpable testis, hCG stimulation tests should be performed before laparoscopy to rule out testicular agenesis. Laparoscopy in children has been shown to be safe by the age of one year and can be done as an outpatient procedure (43).

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16

Thyroid Disorders in Infancy

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INTRODUCTION

Around the time of birth and during the first few years of life, thyroid hormone economy undergoes major changes that need to be understood for proper investigation and treatment of thyroid dysfunction. The dramatic consequences of congenital hypothyroidism (CH) that is not diagnosed during the neonatal period for later brain development underline the importance of prompt recognition of abnormal thyroid function test results (1). While congenital hyperthyroidism is a much less common and usually self-limited entity, it may also lead to dramatic consequences; indeed, heart failure and even death from this condition have been reported in hyperthyroid fetuses and newborns, and later developmental problems may occur as well (2).

The most striking changes in plasma thyroid-stimulating hormone (TSH) and thyroid hormone levels occur immediately after birth; thus, it is important to know about the neonatal TSH surge, which reaches its maximum in the first few hours of life, and is followed by a peak in plasma T_4 approximately 24 hours later. An isolated TSH measurement in the first 24 hours of life may therefore lead to an erroneous diagnosis of primary hypothyroidism, while an isolated measurement of T_4 on the second day of life may lead to an incorrect diagnosis of hyperthyroidism.

However, it is also important to recognize that several parameters of thyroid function, such as the normal ranges of free T_4 and of total T_3, extend to much higher levels in infants than in older children or adults. The mechanisms underlying these age-related changes in thyroid hormone levels are briefly reviewed in the following section, and the clinical problems are discussed next in order of frequency and/or importance. The vast majority of these problems present as abnormalities of thyroid function, but some structural thyroid problems may also present in infancy.

CHANGES IN THYROID HORMONE ECONOMY FROM CONCEPTION TO THREE YEARS OF AGE

Embryonic Period

The median anlage of the thyroid migrates from the lingual area to its normal location in the neck between the fifth and seventh week of embryonic life. Once migration is complete, the median anlage connects with the lateral lobes that are derived from the fourth and fifth pharyngeal pouches. However, from the functional standpoint, the capacity to concentrate iodine only appears at about 12 weeks, and control of thyroid function by the hypothalamopituitary axis is only established at 18 weeks. Because of this, the low amount of T_4 that can be measured in amniotic cavities in the first trimester must be of maternal origin, suggesting that some transplacental passage of T_4 already occurs at that stage (3). This may explain the deleterious effects of *maternal* hypothyroidism (which has been most dramatically illustrated in severe iodine deficiency) on the intellectual development of the offspring and may justify biochemical screening of women who have a personal or family history of thyroid disease and who are contemplating pregnancy (4).

Fetal Period

In fetal blood, T_3 is low because of the presence of the placenta, with its very rich content in type 3 deiodinase (which transforms the prohormone T_4 in the inactive hormone reverse T_3 and T_3 itself into the inactive T_2); the low T_3 milieu may be responsible for the maintenance of a low level of in utero thermogenesis; TSH is high, probably because of extrahypothalamic sources of thyrotropin-releasing hormone (TRH), such as the pancreas and the placenta. Between 20 weeks and term, fetal plasma free T_4 increases progressively because of increased secretion by the fetal thyroid (5). However, even in the complete absence of fetal

thyroid function, cord blood T_4 is 20% to 50% of the mean value of euthyroid neonates (6). This suggests that the transplacental passage of T_4 alluded to above may become substantial in the third trimester and allow some protection of the fetal brain against defective function in the fetal thyroid. The fetal brain is also protected against hypothyroidism by its rich content of type 2 deiodinase, the enzyme that converts T_4 into the active hormone T_3, and that is upregulated in hypothyroidism. These two protective mechanisms provide the framework supporting the concept that complete salvage of intellectual potential is possible even in severe CH provided effective treatment is administered soon after birth (see below).

Research into antenatal screening of all pregnant women for *fetal* hypothyroidism may lead to the development of noninvasive methods but is at present not feasible because it requires cordocentesis, and the risks of this procedure outweigh the possible benefits of diagnosing hypothyroidism in the fetus. However, in women at risk of fetal thyroid dysfunction because of Graves' disease, in whom antenatal ultrasound reveals the presence of a fetal goiter, the in utero diagnosis of hypothyroidism by cordocentesis and treatment of the fetus by intra-amniotic injections of levothyroxine have been reported (2). The major indication of cordocentesis is to determine the etiology of a fetal goiter discovered by ultrasonography (if it cannot be reasonably guessed from the clinical context), so as to choose the most appropriate treatment to decrease its size. Indeed, a large goiter in the fetus entails potential risks during labor (face presentation) or after birth (respiratory distress).

Neonatal Period

Presumably as a consequence of the precipitous drop in ambient temperature, plasma TSH increases markedly in normal newborns, with a peak in the first 24 hours of life. This is followed by a more shallow increase in plasma T_4, peaking during the second day of life. Thus, screening for CH using TSH as the primary method should be delayed until after 24 hours of life, otherwise the number of false positive tests would become unacceptably high. In premature newborns, the postnatal peaks of TSH and of T_4 occur within the same time frame, but their amplitude is somewhat lower than that observed in term newborns.

Infancy

The relative dose (in μg/kg/day) of thyroxine needed to maintain euthyroidism in hypothyroid subjects decreases exponentially during the first two years of life from about 10 to about 5 μg/kg/day (7). In absolute terms, the 50 μg of thyroxine needed by a newborn correspond to about 15% of the neonatal intrathyroidal iodine pool, whereas the 150 μg needed by an adult correspond to only 1% of the mature intrathyroidal iodine pool. A higher iodine turnover is in general associated with higher plasma T_3 (8) and, accordingly, the normal range of plasma T_3 extends to higher values in infancy than in adulthood (Table 1) (9,10).

CONGENITAL HYPOTHYROIDISM

Nomenclature

As is the case at later ages, hypothyroidism in infancy can be congenital or acquired, peripheral (primary) or central (secondary or tertiary), and permanent or transient in nature (Table 2).

Because the most common and most potentially deleterious for long-term intellectual outcome is permanent primary CH (PPCH), PPCH will be the focus of this section. On the other hand, transient hypothyroxinemia without elevation of plasma TSH is very common in premature infants, yet there is still controversy as to whether this is a disease entity requiring treatment or whether this is one of the many mechanisms whereby the premature infant adjusts to extrauterine life; transient hypothyroxinemia is therefore the subject of a section separate from CH. Likewise, newborns are very sensitive to iodine deficiency and this remains a major cause of CH worldwide; indeed, the percentage of newborns with TSH >5 mIU/L on neonatal blood spot samples has been proposed as a means to evaluate the extent of iodine deficiency in a population (15), although this recommendation needs to be validated (16). This major public health problem is beyond the scope of the present chapter.

The prevalence of PPCH is not increased in preterm infants (17). However, an increased prevalence of *transient* primary CH has been reported in some studies of premature newborns: the best characterized is due to the Wolff-Chaikoff effect, i.e., the induction of hypothyroidism by acute iodine overload (most often from iodine-containing antiseptic agents) (18); this condition has been mostly reported from areas of Europe where there is mild iodine deficiency and does not appear to occur frequently in North America (19), presumably because the iodine intake of pregnant women in this area remains, on average, above a critical threshold.

Finally, the hypothyroidism resulting from dominantly inherited mutations that inactivate the T_3 receptor can sometimes be severe enough to be recognized in the neonatal period (20). Interestingly, studies of this condition have illustrated that the excess thyroid hormone in the circulation of affected women, if transferred to an unaffected fetus, has deleterious consequences (miscarriage and low birth weight) (21). Molecular confirmation of this diagnosis has now been obtained in over 200 pedigrees, but this condition will not be discussed further in this chapter and the reader is referred elsewhere (22). Likewise, the discussion of other rare causes of peripheral hypothyroidism, such as the recently described "consumptive hypothyroidism" [from overexpression of deiodinase type 3 in large hemangiomas (23,24)] and

Table 1 Pediatric Reference Intervals for T_4, T_3, Thyroid-Stimulating Hormone, and Free T_4

		Females			Males		
Analyte	Age	Mean	Reference interval	*N*	Mean	Reference interval	*n*
T_4, nmol/L[a]	1–11 mo	122	82–162	116	120	79–161	135
	1–5 yr	120	79–160	471	116	75–158	589
	6–10 yr	115	75–154	462	111	69–152	600
	11–15 yr	109	69–149	799	106	63–147	614
	16–20 yr	104	64–144	565	99	58–142	200
	Total			2413			2138
T_3, nmol/L[b]	1–11 months	2.46	1.52–3.39	70	2.46	1.58–3.35	93
	1–5 years	2.37	1.43–3.30	262	2.38	1.54–3.27	340
	6–10 years	2.20	1.62–3.12	255	2.26	1.37–3.13	362
	11–15 years	2.03	1.09–2.95	483	2.12	1.24–3.00	341
	16–20 years	1.84	0.92–2.78	346	1.98	1.11–2.86	131
	Total			1416			1267
Thyroid-stimulating hormone, mIU/L	1–11 mo	2.2	0.8–6.3	131	2.2	0.8–6.3	158
	1–5 yr	2.0	0.7–5.9	523	2.1	0.7–6.0	659
	6–10 yr	1.8	0.6–5.1	562	1.9	0.7–5.4	698
	11–15 yr	1.5	0.5–4.4	1057	1.7	0.6–4.9	738
	16–20 yr	1.3	0.5–3.9	809	1.6	0.5–4.4	223
	Total			3082			2476
			Females and Males				
Free T_4, pmol/L[c]	1–11 mo	19.5	9.5–39.5	47			
	1–5 yr	18.4	9.0–37.2	91			
	6–10 yr	16.9	8.3–34.1	57			
	11–15 yr	15.5	7.6–31.5	88			
	16–20 yr	14.1	7.0–28.7	70			
	Total			353			

[a]To convert nmol/L to μg/dL, divide by 12.87.
[b]To convert nmol/L to ng/dL, multiply by 65.1.
[c]To convert pmol/L to ng/dL, divide by 12.87.
Source: From Ref. 9; these reference values were obtained with the AutoDelfia analyzer (Wallac, Finland); different values may be expected with other methods.

defects in transmembrane transport of T_4 into cells (25,26) or in its intracellular conversion to T_3 (27) are reviewed in Chapter 17.

Epidemiology and Etiologies of Permanent Primary Congenital Hypothyroidism

PPCH affects one in 2500 to 4000 newborns. On the basis of newborn screening programs, a study from the United States reporting a lower prevalence among Blacks is often quoted but it was based on small numbers of patients in whom the etiology of hypothyroidism had not been studied by nuclear medicine scanning (28); another small study from the United Kingdom reported a higher prevalence among Asian families (29). Overall, the worldwide incidence of PPCH is relatively similar over a wide range of ethnic groups and geographical areas.

About 80% to 90% of PPCH cases are due to developmental defects of the thyroid gland (thyroid dysgenesis), such as arrested migration of the embryonic thyroid in the sublingual area (ectopic thyroid) or an apparently complete absence of thyroid tissue on scan with sodium pertechnetate (athyreosis). Renewed interest in thyroid development has stemmed from the identification of transcription factors that are relatively thyroid-specific and from the generation of knock-out mice for these transcription factors (30). In humans, a few single gene defects have been shown to account for some cases of familial thyroid dysgenesis, but the vast majority are sporadic and result from as yet unknown mechanisms (31). The remaining 10% to 20% of PPCH cases have functional defects in one of the steps involved in thyroid hormone biosynthesis (thyroid dyshormonogenesis), defects that follow an autosomal recessive mode of inheritance.

Recent studies have reevaluated whether genetic factors are involved in thyroid dysgenesis. Thus, a nation-wide study of cases diagnosed by neonatal screening in France identified 48 familial cases out of 2472 (2%), 15-fold more than expected by chance. Analysis of the most typical pedigrees suggested autosomal dominant inheritance with incomplete penetrance, although there was also evidence for genetic heterogeneity (32).

Ectopy and athyreosis are generally considered as part of a spectrum. Recent arguments in favor of this view are that athyreosis and ectopy can coexist in the same pedigree (32) and that *Ttf2*-/-mouse embryos can have either ectopy or athyreosis, with a 50/50 distribution between the two phenotypes (33). On the other hand, the female preponderance classically described for thyroid dysgenesis as a whole is

Table 2 Etiological Classification of Congenital Hypothyroidism

Central (hypothalamic and pituitary causes): Rare (mutations of the TRH receptor, of beta-TSH, of (PROP) PIT-1), "idiopathic" (hypopituitarism usually with "classical triad" on MRI, see text)
Thyroidal: frequent (1 in 2500–4000 newborns)
Ectopic thyroid (most often sublingual): ~70% of cases, mechanisms? (possible role of mechanisms extrinsic to the thyroid (11)?
Athyreosis: ~15% of cases
True (undetectable plasma thyroglobulin): mutations inactivating *TTF-2* or other mechanisms
Apparent
Permanent: mutations inactivating *TSHR*, *PAX-8* or *NIS* or other mechanisms
Transient: maternal TSH-receptor blocking antibodies
Dyshormonogenesis (leading to goiter): ~10–20% of cases
Permanent: mutations inactivating *TPO, Tg, NIS, THOX-2*
Transient: heterozygous mutations in *THOX-2* (12)
Orthotopic thyroid of normal or small size (rare): mutations inactivating *PAX-8* or *TSHR* or other mechanisms
Thyroid hemiagenesis (frequent in euthyroid subjects, but rarely the cause of CH): mechanisms unknown (role of vasculature?) (13,14)
Peripheral (rare): "consumptive hypothyroidism," mutations in monocarboxylase transporter 8, in Selenocysteine incorporation sequence binding protein 2, T_3 receptor

Abbreviations: TSH, thyroid-stimulating hormone; TSHR, thyroid-stimulating hormone receptor; CH, congenital hypothyroidism; TRH, thyrotropin-releasing hormone.

in fact often more pronounced for ectopy in most recent surveys: this may suggest distinct molecular mechanisms for the two forms of thyroid dysgenesis or sex-specific modifiers of a common initial event (34).

It should also be kept in mind that athyreosis itself may be heterogeneous (Table 2). Undetectable uptake on scan may represent "true athyreosis" (a diagnosis that should be validated by an undetectable plasma thyroglobulin) or "apparent athyreosis" from (i) transplacental transfer of TSH receptor–blocking antibodies; (ii) ectopic tissue too small for the limit of detection of nuclear medicine scans (35); (iii) Na/I symporter mutations, in which case a goiter may be identifiable clinically or by ultrasound (36).

The distinction between athyreosis and ectopy is of more than academic interest. Apparent athyreosis from transplacental transfer of TSH receptor–blocking antibodies leads to transient CH but has a very high risk of recurrence in subsequent pregnancies (37,38). On the other hand, several pedigrees have been described with PPCH and apparent athyreosis due to either compound heterozygosity (39) or homozygosity (40) for mutations that result in complete inactivation of the TSH receptor. Anatomically, the absent uptake was due to the fact that the gland was severely hypofunctional, but careful ultrasonography demonstrated that a hypoplastic gland was present and was of normal shape and position. This is consistent with the concept that TSH and its receptor are necessary for growth and function of the thyroid, but are not involved in the initial differentiation of thyroid cells or in the migration of the thyroid anlage.

A milder form of TSH resistance can be seen in the following situations: (i) pseudohypoparathyroidism, in which an increased level of TSH at neonatal screening or during infancy may be the presenting sign (41), before obvious phenotypic features are recognized and (ii) non-TSH receptor–related TSH resistance, with a consistently normal plasma T_4, a normal orthotopic gland usually with low uptake on ^{99m}Tc imaging and a dominant pattern of inheritance (42). Permanent or transient hyperthyrotropinemia of infancy, with normal plasma T_4 and normal thyroid anatomy, can also be seen without a family history and its mechanisms remain to be elucidated; it may occur in otherwise normal children or in children with other phenotypic abnormalities, such as respiratory distress and developmental delay [as in patients with mutations in TTF-1 (Table 3)]. It can also be seen in Down syndrome (see below).

A search for mutations in the genes coding for the TSH receptor, for thyroid transcription factor (TTF)-1, for TTF-2, or for the paired domain factor PAX-8 has so far yielded only a handful of positive results, and it is important to note that, to date, no case of thyroid ectopy documented by scintigraphy has been found to be associated with a germline mutation in a candidate gene. The discordance of more than 90% of reported monozygotic twin pairs for thyroid dysgenesis suggests that non-Mendelian mechanisms need to be

Table 3 A Guide to Searching for Germline Mutations in Permanent Primary Congenital Hypothyroidism

Thyroid phenotype	Other features	Gene	Transmission	References
From apparent athyreosis to normally appearing gland	None	*TSHR*	AR	(39,40)
From apparent athyreosis to normal gland, usually mild ↑ thyroid-stimulating hormone	Respiratory distress syndrome, developmental delay/hypotonia ataxia/choreoathetosis	*TTF-1*	De novo or AD	(43–45)
True athyreosis	Cleft palate Choanal atresia Kinky hair Bifid epiglottis	*TTF-2*	AR	(46,47)
From apparent athyreosis to normally appearing gland	Cysts within thyroid remnants	*PAX-8*	AD or de novo	(48–53)

Abbreviations: TTF, thyroid transcription factor; AR, autosomal recessive; AD, autosomal dominant; TSHR, thyroid-stimulating hormone receptor.

studied as well (54). However, the careful description of the phenotypes of the rare naturally occurring mutation in humans and of the corresponding knock-out experiments in mice is of great importance for our understanding of thyroid gland development, but is beyond the scope of this chapter. The reader is referred elsewhere for this aspect (30,31). For clinical purposes, Table 3 proposes guidelines for when a specific gene should be examined.

Clinical Aspects and Rationale for Biochemical Screening

Signs and symptoms of hypothyroidism in the newborn period are almost always overlooked (Table 4), yet this is when irreversible brain damage occurs.

For this reason, systematic biochemical screening of newborns was developed in the 1970s and is becoming the standard of care in an ever-increasing number of countries. The screening strategy is based on primary TSH measurements in most countries, sometimes followed by T_4 measurement if TSH is raised above a certain cutoff. Alternatively, the "primary T_4 strategy" is still used in many states of the United States. The time at which the sample is taken may also vary between centers, with some taking cord blood, but the majority taking blood from a heel prick after 24 hours of age, to avoid an unacceptable percentage of recall due to the neonatal TSH surge alluded to above (55).

Knowledge of the specific technique and cutoff levels used by the screening program is not as important as the clinician's awareness that (i) a positive screening result should prompt immediate action and (ii) continuous auditing of the turnaround time of the screening program is essential. Many individuals are involved between the time the sample is taken and sent to the screening laboratory and when the laboratory technician reports an abnormal result to the clinician. Human errors are the single most important factor in delayed or missed diagnoses of CH. The current guidelines used by the Quebec and French screening programs are given in Figures 1 and 2, respectively. Because the Quebec program was based on a primary T_4 screening strategy until 1987, it still uses T_4 as a secondary measure on which to base referral decisions, whereas the French program is based on TSH measurements alone. Regardless of these technical differences, experience shows that delays in sending the blood spots to the screening laboratory are the commonest cause of delayed diagnosis.

Aside from human errors, truly normal screening TSH and T_4 values have been found in infants who developed severe hypothyroidism during infancy. Thus, for unknown reasons, children with thyroid dyshormonogenesis can have normal TSH and T_4 as newborns and yet present in the first two years of life with clinical and biochemical evidence of severe hypothyroidism (17,56). The second situation arises from the fact that monozygotic twins are generally discordant for thyroid dysgenesis, with the affected twin having normal screening values because of subtle blood mixing between the euthyroid and the hypothyroid fetus (54). Lastly, dopamine infusions may lead to a falsely normal plasma TSH in spite of primary hypothyroidism (57). Thus, in spite of normal neonatal screening results, the appearance of signs and symptoms suggestive of hypothyroidism (Table 4) in an infant justifies repeating the determination of TSH and T_4.

Table 4 Signs and Symptoms of Hypothyroidism in Neonates and Infants

At birth
Postmaturity
Macrosomia
Open posterior fontanel, large head circumference
Generalized delay in skeletal maturation (but normal length)
During early infancy: should prompt plasma TSH and free T_4 determinations even if screening has been performed
Decreased muscle tone, lethargy, poor feeding
Hypothermia
Constipation
Prolonged jaundice
Abdominal distension, umbilical hernia
Dry and mottled skin
Macroglossia
Hoarse cry
Myxedematous appearance

Diagnostic Evaluation of the Hypothyroid Newborn

As stated above, a clinical diagnosis is almost never made in the newborn period. However, large fontanels at birth (58) in a neonate with unexplained postmaturity and macrosomia (59) may lead to immediate recognition and treatment, which is likely important for intellectual development in these few severely affected cases (60) in whom the protective mechanisms described above appear to have been overridden. In addition, when a baby is referred for a positive test, it is sometimes possible to elicit a history of increased

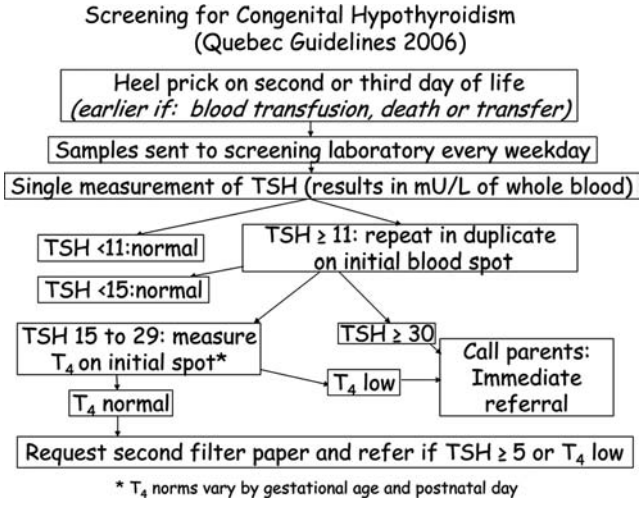

Figure 1

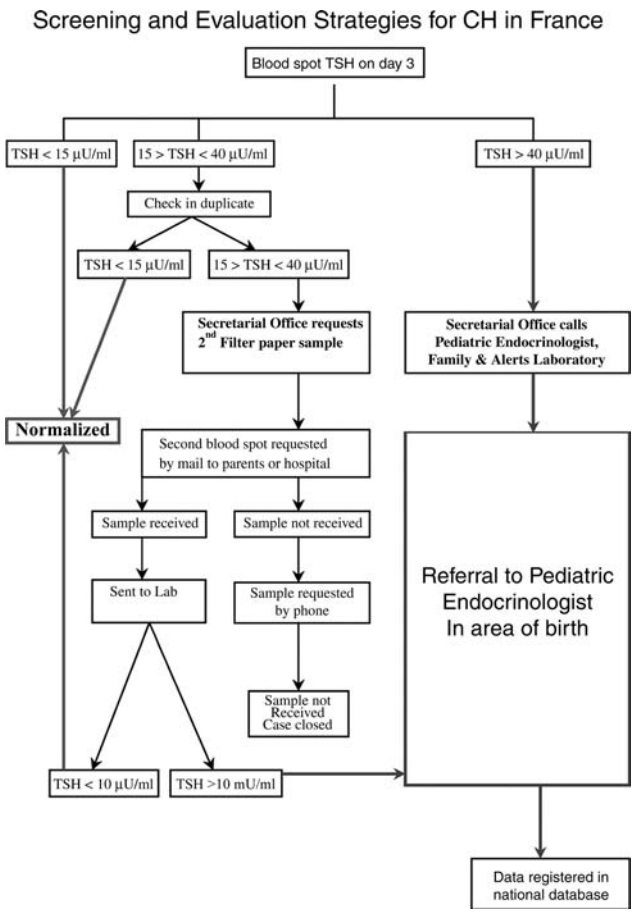

Figure 2 TSH 40 mU/L or more: the clinician is called to ask the parents and child to come for a workup. TSH between 15 and 39 mU/L: the secretarial office of the screening program will send a letter asking for a new blood filter paper sample (at that stage the clinician will not hear about this child). If this second TSH sample is 10 or more: the clinician will be notified and will ask the parents to bring the child for workup. If the second TSH sample is below 10: child is considered normal. If the second blood sample cannot be obtained, despite letter and phone call, the secretarial office will close the case (this may be a problem, though the idea is that it is not a severe thyroid production deficiency). In France, there are three partners involved in the newborn screening program: secretarial office, screening lab, and the referral clinician. The secretarial office is responsible to notify the screening laboratory and the pediatric endocrinologist and is in charge of handling the request for the second filter paper sample. The physician is in charge of calling the family and of the newborn workup. *Abbreviations*: TSH, thyroid-stimulating hormone; CH, congenital hypothyroidism.

sleepiness, poor feeding, or constipation. The family history should focus on whether there are other cases of CH or whether the mother is known to have autoimmune thyroid disease. On physical examination, prolonged icterus and large fontanelles are the most frequently encountered signs. A careful inspection of the cervical area, with the neck hyperextended, is important to detect a goiter. However, even obvious goiters on nuclear medicine scanning (Fig. 3) are often missed by experienced clinicians and imaging studies are almost always necessary. Extrathyroid abnormalities should be noted: CH can be part of a polymalformative syndrome (Table 3) and a fivefold increase in the prevalence of generally minor heart defects (mostly in septation) has been reported in children with thyroid dysgenesis (32,61,62).

Blood is taken to confirm the positive screening results. In addition to TSH, we routinely measure on this sample free T_4, total T_3, and thyroglobulin. Although the prevalence of transient CH from transplacental transfer of TSH receptor–blocking antibodies is low (63), this possibility should be investigated in cases with apparent athyreosis or with a gland of normal shape and size but decreased uptake.

Because of the importance of a precise etiological diagnosis in establishing that CH is permanent (as in true athyreosis and ectopy) or that it has a 25% recurrence risk in subsequent siblings (as in dyshormonogenesis), a nuclear medicine scan should be obtained. This should ideally be carried out on the day of the initial diagnostic evaluation. The isotope of choice is sodium pertechnetate (^{99m}Tc), which is available daily in most nuclear medicine services and with which the etiological diagnosis can be made in 20 minutes. Some laboratories use ^{123}I, but the neonate then has to return to the nuclear medicine service several hours after administration of the isotope. Difficulties in arranging for imaging studies should never be taken as an excuse for not initiating treatment at the first visit. A nuclear medicine scan can always be obtained after withdrawing treatment for a month at three years of age (when hypothyroidism no longer has permanent consequences for brain development) (64); however, obtaining good imaging is easier in a newborn than in a toddler. Lastly, one should *not* wait for the results of confirmatory blood tests before starting treatment.

The technical quality of nuclear medicine scans is important (65): the unequivocal demonstration of ectopic sublingual tissue requires that the salivary

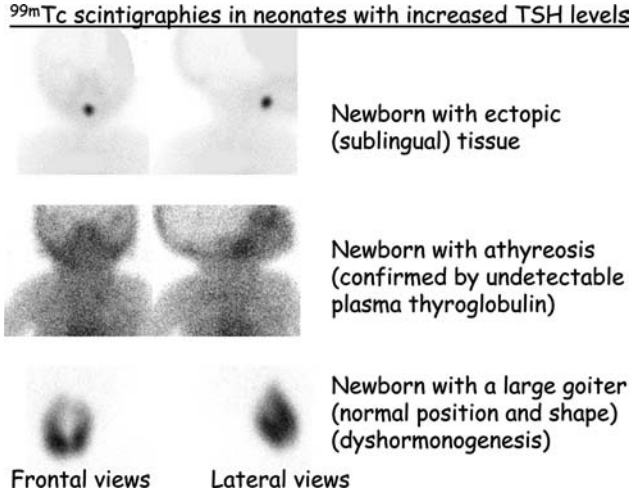

Figure 3

glands be empty (which can easily be achieved in newborns by feeding them between the intravenous injection of ^{99m}Tc and scanning, and in toddlers by giving them a candy). Ensuring that the ^{99m}Tc has been injected in the vein is also essential. Because of these pitfalls of nuclear medicine imaging (and the radiation involved, although the dose is minimal), ultrasound scanning has been evaluated for the etiological diagnosis of CH; however, its sensitivity in identifying ectopic tissue is low (66–68) and differentiating between normal thyroid lobes and the hyperechogenic structures (likely the ultimobranchial bodies) that are in the same location when there is no orthotopic thyroid is difficult and requires a highly skilled pediatric radiologist (69). At present, ultrasound scanning cannot replace nuclear medicine imaging for the identification of the commonest cause of PPCH, i.e., thyroid ectopy.

Treatment and Outcome of Congenital Hypothyroidism

Before systematic biochemical screening of newborns, the mean intelligence quotient (IQ) of CH children was 76 (70) and 40% required special education (71). Even in those with normal IQs, specific cognitive deficits were common (72). These numbers provide the historical background against which to gauge the success of biochemical screening and of different treatment regimens (Fig. 4).

In the first generation of screened CH newborns, treatment was started at a mean age of 23 to 30 days and the starting dose of levothyroxine was 5 to 6 μg/kg/day. While it was recognized that such a dose did not normalize plasma TSH for weeks or even months, this was thought to reflect resistance to the normal feedback control mechanisms. However, such a resistance occurs only in a minority of CH newborns (74). The first report of developmental outcome of screened CH children suggested that intellectual impairment had been completely eliminated (60). This first report also found no impact of the initial severity of hypothyroidism at diagnosis. However, a meta-analysis published 15 years later and including 675 children with CH and 570 controls from seven studies clearly showed that initial disease severity was an important determinant of outcome. Specifically, the subgroup of children with severe CH had a mean loss of six IQ points compared to controls, and in some studies, the difference was in the range of 10 to 20 IQ points, a difference that is not only statistically, but also clinically significant (75).

Severity of CH at diagnosis can be evaluated in a number of different ways. Most commonly, this has been done on the basis of the plasma level of T_4, on the bone maturation or on the etiology (e.g., athyreosis vs. ectopy). An important concept is that the impact of severity of CH on developmental outcome does not appear linear: rather, there seems to be a threshold below which an infant with CH was at greater risk of developmental problems. This has been most convincingly demonstrated by Tillotson et al. (71), who defined a plasma total T_4 at diagnosis of 43 nmol/L as the critical point below which the IQ became affected by CH.

A more detailed description of these studies mostly carried out in the 1980s does not seem necessary, because age at starting treatment and initial dose of levothyroxine have changed substantially since then. In the last 15 years, through regular audits, most centers have been able to start treatment at a mean age

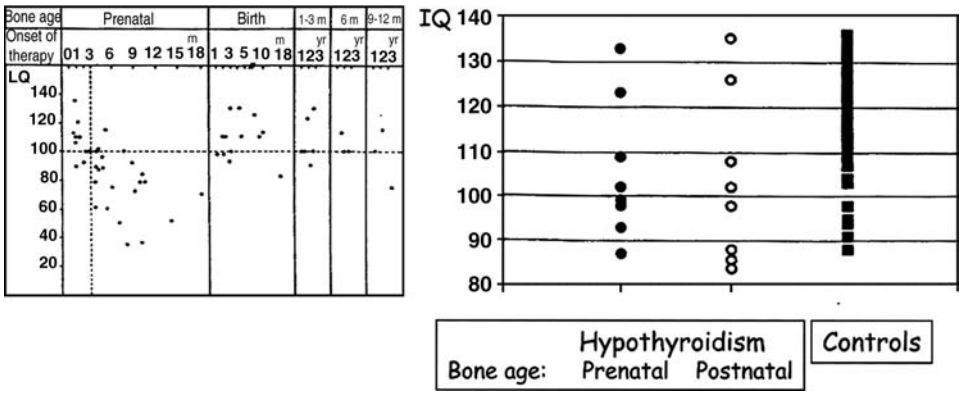

Figure 4 IQ values in children with congenital hypothyroidism in the past (prescreening era) and present (screening era). Left panel: individual IQ values of children aged 3 to 17 with congenital hypothyroidism diagnosed clinically in the prescreening era, as a function of bone age at diagnosis and age at onset of therapy. There was no control group in that study; the theoretical population mean of 100 is indicated by the dotted line. IQ values below 80 were most common in children with a prenatal bone age at diagnosis *was noted*. Right panel: individual IQ values of children aged *five* years *nine* months with congenital hypothyroidism identified by newborn biochemical screening, subdivided by bone age at diagnosis and compared with a control group matched for socioeducational level of the family. Note that, even in the subgroup with a prenatal bone age, the mean and the distribution of IQ values are indistinguishable from those of the control group. *Abbreviation*: IQ, intelligence quotient. *Source:* From Ref. 1.

of 9 to 14 days (76). At the same time, because several lines of evidence suggested that the dose used was suboptimal, the starting dose of levothyroxine has been increased at many centers to 10 to 15 μg/kg/day. A recent randomized prospective study showed that a dose of 50 μg/day for neonates weighing 3 to 4 kg promptly normalized plasma TSH and also resulted in the best developmental outcome. This regimen was associated with plasma-free T_4 levels that are above the reference range of most laboratories (77) but, as reviewed above, the normal range of plasma free T_4 extends to much higher levels in infants than in older children or adults (Table 1). Beyond the neonatal period, the practice regarding frequency of blood sampling, biochemical endpoints of treatment, and criteria for change in dosage vary widely. Table 5 summarizes some practical aspects of the treatment of CH.

The most important aspect of outcome is developmental and several recent studies have shown that the developmental gap that existed between severe CH children and controls has now been closed; both early and high-dose treatment appear necessary (78). Detailed studies of neurophysiological functions that may be more sensitive to the effects of both over- and undertreatment than the measurement of IQ may lead to greater individualization of dosage recommendations. In the meantime, starting as early as possible with 50 μg for neonates weighing between 3 and 4 kg appears safe and effective in achieving the major goal of neonatal screening, i.e., to allow all CH children, including those with a severe form of the disease, to achieve their full intellectual potential.

Table 5 Practical Guidelines for the Evaluation and Treatment of Congenital Hypothyroidism

Upon referral
History and physical
Anteroposterior X-ray of knee
^{99m}Tc scintigraphy (or ^{123}I scan) of cervical, lingual and mediastinal area (thyroid ultrasound if experienced pediatric ultrasonographer)
Family History, palpation of mother's thyroid
Blood for TSH, Free T_4, total T_3
Antithyroperoxidase and TSHR antibodies in infant and mother, except if ectopic thyroid
Plasma thyroglobulin in infant if no uptake scan
Medication
Start treatment without waiting for test results
Treat as early as possible with a dose of 50 μg/day for neonates weighing 3 to 4 kg (bioavailability of liquid T_4 may be higher than T_4 in pills)
Infants with athyreosis require higher doses than those with thyroid ectopy, dyshormonogenesis or central hypothyroidism
Levothyroxine in tablets to be crushed or in solution (available in some parts of Europe, 1 drop = 5 μg)
Do not put in the bottle, apply directly in the mouth, on the tongue
There is decreased absorption in the presence of soy formula
Daily dosing best, if a dose is missed give a double dose the next day
Monitoring
Growth and developmental milestones
Plasma TSH, T_4, and T_3 determination: day 15 after the start of treatment (TSH should have returned to normal levels, may require fT4 levels as high as 65 pmol/L, 5 ng/dL)
First year: Assess thyroid function every 2–3 mo

Abbreviations: TSH, thyroid-stimulating hormone; TSHR, thyroid-stimulating hormone receptor.

HYPOTHYROXINEMIA OF THE NEWBORN

Here, it is essential to define whether one discusses decreased-total or decreased-free T_4. The first condition that needs to be ruled out in a newborn with low total T_4 concentrations associated with normal plasma TSH is thyroxine-binding globulin (TBG) deficiency. This X-linked condition is discovered only by screening programs using a primary T_4 approach (for technical reasons, it is total and not free T_4 that is measured on the neonatal blood spot). It does not require treatment, since the plasma levels of free thyroid hormones are normal and the subjects are euthyroid. Loss of protein from nephrotic syndrome may also lead to low total T_4. With the generalization of free T_4 assays, unnecessary investigation and "treatment" of TBG deficiency has become rare.

In a term neonate with a low free T_4 but normal TSH, true central hypothyroidism needs to be ruled out. Isolated central hypothyroidism is exceedingly rare but may also have profound long-term deleterious effects on later development: it can be caused by mutations that inactivate the gene coding for the beta-subunit of TSH (79) or the gene coding for the TRH receptor (80). More commonly, central hypothyroidism occurs in association with other anterior pituitary hormone deficiencies: hypoglycemia, prolonged conjugated hyperbilirubinemia, microphallus, and/or cryptorchidism will suggest associated deficiencies in growth hormone, adrenocorticotropic hormone, and luteinizing hormone, respectively. Clinical clues that increase the likelihood of hypopituitarism in this context include cleft lip and palate and optic nerve hypoplasia (Vol. 2; Chap. 3). Magnetic resonance imaging reveals the "classical triad" of ectopic posterior pituitary, thin, interrupted or absent stalk, and hypoplastic anterior pituitary in most cases of congenital hypopituitarism. An intact stalk, an orthotopic posterior pituitary, and an anterior pituitary of normal or small size should lead to consideration of mutations in *PIT-1* (81) or in *PROP-1*, in which case the size of the anterior pituitary may be increased early in life (82).

On the other hand, hypothyroxinemia relative to term values, but with normal TSH, is a very common finding in premature newborns (17). It does not only reflect low TBG levels because free T_4 is low as well (83). This should probably be considered as a situation akin to that seen at later ages in the presence of severe nonthyroidal illness (Vol. 2; Chap. 25). Indeed, numerous studies have shown that there is a correlation between the degree of lowering of T_4 and negative outcomes, both short term (mortality) and long term (developmental problems). However, correlation does not imply causation: indeed, randomized, double blind, placebo-controlled studies of thyroxine

supplementation have not shown an overall benefit in terms of morbidity, mortality, or developmental outcome. In fact, lower IQs were observed in levo thyroxine-treated infants born after 27 weeks of gestation. Further research is ongoing to determine if the apparent benefit from thyroxine supplementation in children born before 26 weeks is confirmed (84). A Cochrane review of the evidence did not support systematic supplementation of all low birth-weight babies (85).

A special situation of true transient central hypothyroidism may occur in infants born to mothers with untreated or insufficiently treated Graves' disease, because TSH secretion from the fetal pituitary has been suppressed and may take a few months to come back to normal (86).

CONGENITAL HYPERTHYROIDISM

As is the case later in life, high plasma T_4 does not necessarily indicate hyperthyroidism. As noted above, there is a physiological, transient peak of plasma T_4 on the second day of life. Aside from the precise postnatal age, the next most important thing to know is whether the laboratory reports total or free T_4. A high T_4 in the face of a normal free T_4 suggests TBG excess, a condition that, like TBG deficiency, should not be treated. TBG excess may be genetically determined or may be due to liver disease. In the syndrome of generalized resistance to thyroid hormone mentioned above, high circulating levels of thyroid hormones are in fact associated with euthyroidism or hypothyroidism at the tissue level and plasma TSH is either inappropriately normal or high (20).

On the other hand, and as is also true in later life, a low or undetectable plasma TSH, even with ultrasensitive third generation assays, is not sufficient to establish a diagnosis of hyperthyroidism: a low TSH can be seen in central hypothyroidism or in severe nonthyroidal illness. To make a diagnosis of true hyperthyroidism, the combination of a low or undetectable TSH with high T_4 and/or T_3 is required.

Transient Graves' Disease

Most commonly, hyperthyroidism in infancy occurs during the early neonatal period and is due to the transplacental transfer of TSH receptor–stimulating antibodies from a mother with either a current or a past history of Graves' disease. Given the high prevalence of Graves' disease in the population of women of childbearing age, it is surprising that clinically significant hyperthyroidism occurs so seldom in newborns: estimates of 1 in 25,000 births, or of 1% to 3% of the offspring of mothers with Graves' disease are quoted in the literature. The measurement of TSH receptor–stimulating antibodies in the plasma of pregnant women with past or current Graves' disease has been advocated as a means to detect the fetuses more at risk (2), but is not universally practiced. In spite of its rarity, the serious nature of the condition justifies careful clinical screening: the mother should be warned of the potential risk, fetal thyroid size, heart rate, and growth and should be monitored, the newborn should be examined for the presence of a goiter, of exophthalmia, or of tachycardia without underlying heart disease, and weight gain during the first three months of life should be meticulously followed.

The recommendations about antithyroid drug treatment in pregnancy are that it should be aimed at maintaining the mother slightly hyperthyroid so as to avoid fetal hypothyroidism from transplacental transfer of the drug. However, a woman may occasionally be treated with doses of antithyroid drugs that are high enough to make the fetus hypothyroid. This may even lead to the development of a large goiter in the fetus and newborn, which may become apparent on antenatal ultrasound (2) or because of the development of respiratory distress from tracheal compression in the immediate neonatal period. This transient drug-induced hypothyroidism may be followed after a few days (i.e., after the drug is cleared from the newborn's circulation) by a period of a few weeks of hyperthyroidism from the maternally derived TSH receptor–stimulating antibodies.

It is important to be aware that TSH receptor–stimulating antibodies may remain present long after definitive cure of hyperthyroidism has been achieved by the therapeutic administration of radioactive iodine to an adolescent or adult with Graves' disease. Therefore, it is important to enquire from the great many women on thyroxine replacement for hypothyroidism who become pregnant whether their hypothyroidism results from radioiodine treatment, even if this has occurred years earlier.

Once this transient type of hyperthyroidism is recognized clinically in a newborn, it should be treated vigorously to prevent the development of heart failure. Admission is usually required initially, and tachycardia may be severe enough to justify continuous electrocardiogram monitoring. Beta-blockers such as propranolol should be administered at a dose of 2 mg/kg/day in four divided doses. Lugol's solution, at a dose of one drop every eight hours, will block the release of the thyroid hormone that is stored in the gland and can be given for a few days. The short-acting propylthiouracil is the preferred antithyroid drug because, compared to methimazole, it has the extra benefit of decreasing the conversion of T_4 into T_3. The starting dose of propylthiouracil is 5 to 10 mg/kg/day, divided in three doses, and should be carefully tapered over the first few weeks of life so as to avoid the development of drug-induced hypothyroidism once the TSH receptor–stimulating antibodies are cleared. Pharmacological doses of glucocorticoids have also been used, because they block the conversion of T_4 to T_3. More heroic treatments such as exchange blood transfusions (87) or less traditional ones such as the administration of sodium ipodate (88) have been proposed. Although their use is logical on the basis of the pathophysiology of neonatal Graves'

disease, they have not gained wide acceptance and, in our experience, have not been necessary.

With prompt recognition and early, vigorous treatment, the classically reported high death rate of neonates with Graves' disease should be a thing of the past. Likewise, the classically described intellectual impairment and development of craniosynostosis (89) has no longer been reported in recent series (90).

Thyroid-Stimulating Hormone Receptor Activating Mutations

A familial form of nonautoimmune, persistent hyperthyroidism with dominant inheritance, had been described 25 years ago in Nancy, France. The authors had hypothesized that this phenotype resulted from intrinsic activation of TSH receptor function (91). The cloning of the human TSH receptor has allowed to confirm this hypothesis (92). Affected neonates typically experience an unrelenting course, in contrast to those with congenital hyperthyroidism from maternal antibodies. Thus, definitive treatment is required in these patients. Given the fact that there is no experience with radioiodine in the very young, therapeutic options include thyroidectomy or long-term antithyroid treatment.

A handful of cases with de novo germline mutations activating the TSH receptor have also been described (93,94). Clinically, their phenotype has been uniformly severe and early thyroidectomy is therefore advised. From the molecular standpoint, it is interesting to note that these de novo mutations are ''private,'' i.e., they are different from the ones that have so far been found in the pedigrees with the dominantly inherited form (and are, in fact, only seen also as somatic mutations in ''hot'' nodules): it has been suggested that the phenotypic consequences of these de novo mutations are so severe that they became ''extinguished'' because the affected individuals could not reach the age of reproduction before modern medicine led to recognition and definitive treatment of this condition (95).

Lastly, somatic mutations activating the TSH receptor have now been recognized as the most common mechanism underlying the development of "hot" nodules (96). This entity is predominantly a disease of adults and older children, but a single case of a somatic mutation leading to the formation of a ''hot'' nodule and to hyperthyroidism of fetal onset has been reported (97).

ACQUIRED HYPO- AND HYPERTHYROIDISM IN INFANCY

The most common causes of acquired hypo- and hyperthyroidism are Hashimoto's thyroiditis and Graves' disease, respectively (Chapters 18 and 19). These two entities are exceptional in infancy. Specifically, Hashimoto's thyroiditis has been reported in only a handful of cases below the age of three (98) and the youngest patient we have observed with Graves' disease was three years and eight months. The principles of diagnostic evaluation are the same as in older children. Prompt treatment of hypothyroidism is important, since infancy is still a period during which the brain may undergo irreversible alterations from hypothyroidism (99). Treatment of hyperthyroidism is usually restricted to the use of antithyroid drugs (with thyroidectomy as a second line), although one three-year-old has been treated with radioiodine (100).

Beyond infancy, patients with Down syndrome are known to have a higher prevalence of autoimmune thyroid disease (101). However, even in the absence of antibodies, infants with Down syndrome often present with mild hyperthyrotropinemia. In fact, a study of CH screening in all newborns with Down syndrome born in the Netherlands over a two-year period showed that, compared to normal newborns, the distribution of T_4 was shifted downwards and that of TSH was shifted upwards (102). Furthermore, the elevated TSH is biologically active, suggesting that there is a mild primary thyroid defect in all children with Down syndrome: indeed, a recent randomized trial of thyroxine versus placebo for the first two years has shown a small but statistically significant difference between the thyroxine and placebo groups in some motor development subscales (103).

STRUCTURAL THYROID PROBLEMS

Thyroglossal Duct Cysts

During its migration, the median thyroid anlage described above leaves a track of degenerated thyroid follicular cells (104). This ''thyroglossal tract,'' usually disappears after migration is complete. However, fluid may occasionally collect at its more caudal end and form a cyst. These cysts typically present as a midline, soft, regular mass in the lower neck of an asymptomatic infant (or older child), who has a normal orthotopic thyroid. However, they may also present with midline neck swelling, pain, and redness secondary to an acute infection.

Elective surgical removal of thyroglossal duct cysts is therefore advised, because surgery after an acute infection has occurred is more difficult. Before elective surgery, we still recommended to perform a ^{99m}Tc scan (rather than an echogram, for the technical reasons discussed above), to ascertain that the midline neck mass does not represent ectopic thyroid tissue. Because this tissue is usually the only thyroid tissue present in the patient, inadvertently removing it will lead to severe and irreversible hypothyroidism.

Suppurative Thyroiditis

Because of its rich content in iodine, a potent disinfectant, the thyroid gland is seldom the site of bacterial infections. In infancy (and even later), a bacterial infection of the thyroid in a host with normal immune function is often associated with a fistula arising from the pyriform sinus. Although thyroid abscesses can

present at any age, the fact that they are causally linked to a congenital malformation (which is usually present on the left side) explains their relatively high frequency in young children. Thus, in an infant presenting with a rapidly growing mass in the left thyroid lobe, thyroid abscess is the first diagnosis. The mass is very tender, but redness of the overlying skin may be absent. Increased leucocytosis and sedimentation rate are further clues to the diagnosis. Fine needle aspiration has been used and allows identification of the germs causing the abscess (which are most often from the flora of the oropharynx). The aspiration, coupled to antibiotic treatment, may also contribute to curing the abscess and to obviate the need for hemithyroidectomy (105). A careful search for a fistula from the pyriform sinus should be carried out through barium swallow studies and/or laryngoscopy. If such a fistula is found, definitive surgical treatment is required to prevent recurrent abscess formation.

Subacute Thyroiditis

In contrast to bacterial thyroiditis, subacute thyroiditis (also known as de Quervain's thyroiditis) occurs very rarely in the pediatric age group (Vol. 2; Chap. 19). However, cases have been reported as early as two years (106).

Thyroid Cancer

This entity is described in great detail in another section (Vol. 2; Chap. 20). In infants, it is important to remember that medullary thyroid cancer can occur very early in life in the familial syndrome of multiple endocrine neoplasia type IIB: in such a family, we have observed a boy in whom we performed thyroidectomy at 1.5 years of age because of high plasma calcitonin concentrations, and in whom pathological examination revealed definite medullary thyroid cancer (107).

CONCLUSION

Thyroid disorders during infancy are as varied, if not more so, than any other period of the lifespan. Their importance is that, if not promptly recognized and treated, they can lead to irreversible short- or long-term sequelae, as exemplified by congenital hyper- and hypothyroidism, respectively. Hence the importance for all clinicians to always bear these diagnoses in mind whenever suggestive clinical clues are present.

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17

Hypothyroidism

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INTRODUCTION

Hypothyroidism or thyroid insufficiefncy is the most common endocrinopathy, affecting 2% of women and 0.2% of men (1–3). In children, its population prevalence is about 0.15%, with a 2.8:1 female to male ratio (4). Optimal levels of thyroid hormone are required for normal neurodevelopment and growth. By maintaining an appropriate index of suspicion, the clinician can often recognize hypothyroidism in its early stages.

CLASSIFICATION AND TERMINOLOGY

The production and secretion of thyroid hormone is regulated by the hypothalamic–pituitary–thyroid axis. The hypothalamus secretes the tripeptide thyrotropin-releasing hormone (TRH), which stimulates the thyrotrophs of the pituitary to secrete thyrotropin. Thyrotropin, also called thyroid-stimulating hormone (TSH), stimulates thyroid growth and the synthesis and secretion of thyroid hormone, primarily as thyroxine and to a lesser extent triiodothyronine. Thyroxine (T4) is converted into the biologically more active 3,5,3′-triiodothyronine (T3) by outer ring deiodinases (ORDs) expressed in the peripheral tissues (5,6). Both thyroid hormones, thyroxine and triiodothyronine, exert negative feedback on the hypothalamus and pituitary (Fig. 1).

Thyroid hormones are poorly soluble in water and most circulating T4 and T3 is reversibly bound to plasma proteins, predominantly thyroxine binding globulin but also transthyretin (previously termed T4-binding prealbumin) and albumin. In normal serum, only the free fraction (approximately 0.02% for T4 and 0.30% for T3) is immediately available to tissues and the bound hormone serves as a reservoir of inactive ligand (7). Several abnormalities in the serum proteins that transport thyroid hormone have been characterized but, as serum free hormone concentrations are normal, these conditions do not cause thyroid disease (8). In the peripheral tissues, thyroid hormone acts by binding specific nuclear DNA-bound thyroid hormone receptors (TRα1, TRβ1, TRβ2, and TRβ3) that are encoded by two separate genes, *TR*α and *TR*β (7).

Hypothyroidism can result from defective secretion at any level of the hypothalamic–pituitary–thyroid axis, i.e., from decreased secretion of TRH, TSH, or T4 and T3, and also from tissue resistance to thyroid hormone action, and accelerated thyroid hormone degradation. This chapter will review the major etiologies of hypothyroidism in childhood, divided into the following categories: primary hypothyroidism; central hypothyroidism; resistance to thyroid hormone; and consumptive hypothyroidism. Congenital hypothyroidism is discussed in detail in Vol. 2, Chap. 17 (Thyroid Disorders of Infancy).

PRIMARY HYPOTHYROIDISM

Primary hypothyroidism refers to decreased thyroidal production of T4 and T3. As T4 and T3 secretion falls, inhibitory feedback of the pituitary decreases and serum TSH rises (Table 1). Primary hypothyroidism is the most common form of hypothyroidism. It is subdivided into overt hypothyroidism, defined as a high serum TSH concentration and low serum free T4 concentration, and subclinical hypothyroidism, defined as a high serum TSH concentration and a normal serum free T4 concentration.

Chronic Autoimmune Thyroiditis

Chronic autoimmune thyroiditis is the most common cause of acquired hypothyroidism (1,9). As reviewed in Vol. 2; Chap. 19, there are two types of autoimmune thyroiditis, type 2A (goitrous, classic Hashimoto's disease) and type 2B (nongoitrous, atrophic thyroiditis), that cause persistent hypothyroidism. Both are characterized by lymphocytic infiltration and the presence of lymphoid germinal centers in the thyroid, destruction of thyroid epithelial cells, and high serum antithyroid antibody concentrations; they differ only in the presence or absence of goiter (1). In patients with hypothyroidism, the finding of a high serum antithyroid antibody concentration is sufficient to diagnose chronic autoimmune thyroiditis. Serum concentrations of antithyroid peroxidase or antithyroglobulin antibodies are high in more than 95% of

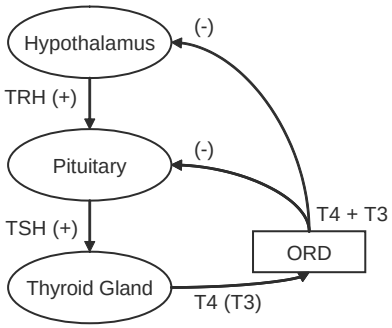

Figure 1 The hypothalamic pituitary thyroid axis. *Abbreviations*: ORD, outer ring deiodinase; +, stimulation; −, inhibition.

affected patients; of the two antibodies the serum concentrations of antithyroid peroxidase antibodies are high slightly more often than are the concentrations of antithyroglobulin antibodies, making measurements of the former the most sensitive test for chronic autoimmune thyroiditis (10,11). While the pathophysiology of chronic autoimmune thyroiditis is poorly understood, there is clearly genetic susceptibility, and associations with several genes whose products regulate immunologic reactions have been demonstrated in affected kindreds (1). In addition, factors such as pregnancy, advanced age, infection, thyroid irradiation, and high iodine intake have been proposed as risk factors for chronic autoimmune thyroiditis (12).

Other Causes of Acquired Primary Hypothyroidism

Other causes of primary hypothyroidism are thyroid surgery and irradiation (either radioiodine or external-beam radiation). Hypothyroidism is particularly common among children with Hodgkin's disease who are treated with external-beam radiation (13). Other, rare causes of primary hypothyroidism include hemachromatosis, environmental goitrogens such as resorcinol, and medications such as lithium, iodine, amniodarone, and aminoglutethimide (1,14,15). The thionamide antithyroid drugs, methimazole and propylthiouracil, given deliberately to decrease thyroid hormone production in patients with hyperthyroidism can cause hypothyroidism when dosing is excessive.

Inherited Hypothyroidism

Inborn errors of thyroid hormone biosynthesis are usually considered as causes of congenital hypothyroidism (chap. 16; Thyroid Disorders of Infancy), but it is important to recognize that in some patients these disorders do not become clinically evident until later in childhood or even adolescence (16,17). For any single biochemical defect, the degree of enzymatic deficiency as well as environmental factors such as iodine status results in variable compensation and phenotypic heterogeneity. Complete inborn errors of thyroid hormone biosynthesis produce congenital hypothyroidism, but patients with partial biochemical function can develop hypothyroidism in later childhood or even maintain full biochemical compensation with euthyroid goiter (Vol. 2; Chap. 19) (8). In addition to these goitrous disorders, familial subclinical hypothyroidism owing to germline loss-of-function mutations of the TSH receptor gene has also been reported (18).

Transient (Post-Thyroiditis) Hypothyroidism

Transient hypothyroidism can occur after transient thyroiditis (painless sporadic thyroiditis or painful subacute thyroiditis) and should be considered in patients who had transient thyrotoxicosis or thyroid pain and tenderness in the recent past, hypothyroidism of recent onset, and normal serum antithyroid antibody concentrations. The hypothyroidism is transient in about 85% of these patients (1). Most recover without treatment, but some have sufficient symptoms to warrant T4 treatment, which can usually be withdrawn later.

Subclinical Hypothyroidism

Subclinical hypothyroidism is defined, as noted above, as a high serum TSH concentration and normal serum free T4 concentration. The log-linear relationship between serum TSH and free T4 explains how small reductions in serum free T4 lead to large increases; for example, a 10% to 15% decrease in serum free T4 concentrations results in a 100% to 200% increase in serum TSH concentrations. The causes of subclinical hypothyroidism are the same as for overt hypothyroidism, chronic autoimmune thyroiditis being the most common cause. Most patients with subclinical hypothyroidism are asymptomatic, and it may be transient (19). Studies of indirect calorimetry in infants and glycemic control in children with type 1 diabetes indicate that subclinical hypothyroidism can have metabolic effects (20,21). Two published reports describe improved linear growth in subclinical hypothyroidism after levothyroxine therapy, but their specific inclusion criteria prevent the generalization of this finding to all

Table 1 Thyroid Function Tests in Patients with Hypothyroidism

	Serum TSH	Serum FT4	Serum FT3	Thyroid radioiodine uptake
Primary hypothyroidism	High	Normal or low	Normal or low	Low
Central hypothyroidism	Low/med/high	Low	Normal or low	Low
Resistance to thyroid hormone	Normal/high	High	High	High
Consumptive hypothyroidism	High	Normal or low	Normal or low	High

children with subclinical hypothyroidism (22,23). Given the critical importance of thyroid hormone in neurodevelopment, persistent hyperthyrotropinemia in infancy should be empirically treated and a trial of reduced therapy considered after the age of two to three years. In older children and adults, the role of treatment is more controversial but, in practice, levothyroxine can be offered to growing children and then discontinued after the achievement of final height (24). Risk factors (in adults) for progression to overt hypothyroidism are serum TSH concentrations $>10\,\mu U/mL$ and high serum antithyroid antibody concentrations. We recommend T4 therapy for patients with serum TSH values $\geq 10\,\mu U/mL$ and those with serum TSH values greater than $5\,\mu U/mL$ in combination with goiter or a high serum antithyroid antibody concentration (2).

CENTRAL HYPOTHYROIDISM

Central hypothyroidism is caused by deficiency of TSH (secondary or pituitary hypothyroidism) or TRH (tertiary or hypothalamic hypothyroidism). The frequency of central hypothyroidism is estimated to be about 0.1% of that of primary hypothyroidism (1). The most common causes of central hypothyroidism in children are pituitary adenomas or craniopharyngiomas, or more often, the surgical or radiation treatment for pituitary adenomas or craniopharyngiomas. Central hypothyroidism can also occur in children with germinomas, gliomas, meningiomas, and chordomas. Sarcoidosis, hemachromatosis, and Langerhans-cell histiocytosis can cause hypothalamic hypothyroidism (1). As described in Vol. 2, Chap. 16 (Thyroid Disorders in Infancy), isolated central hypothyroidism can occur in children with mutations of the TRH receptor gene or with homozygous mutations of the *TSH*β-subunit gene (25–30). More often, congenital central hypothyroidism occurs in combination with other hormone deficiencies, secondary to developmental abnormalities that are idiopathic or because of a mutation of pituitary transcription factors such as PIT1, PROP1, HESX1, LHX3, and LHX4 (31).

Patients with central hypothyroidism typically have low serum free T4 concentrations and normal or low serum TSH concentrations (Table 1). Other causes of these biochemical findings are severe nonthyroidal illness, especially in patients also receiving dopamine and glucocorticoids (15). These potential confounders must be excluded before the diagnosis of central hypothyroidism is made. Once a diagnosis of central hypothyroidism is confirmed, all patients should have a pituitary imaging [magnetic resonance imaging (MRI)] study and biochemical screening for adrenocorticotropin, gonadotropin, and growth hormone deficiency.

RESISTANCE TO THYROID HORMONE

Resistance to thyroid hormone is a dominantly inherited syndrome of reduced tissue responsiveness to thyroid hormone. The actions of thyroid hormone are mediated by binding of T3 to one of three thyroid receptor (TR) isoforms (TRα1, TRβ1, and TRβ2). In patients with loss of receptor function, peripheral tissues are resistant to thyroid hormone action and are therefore functionally hypothyroid. Over 600 known cases have been reported, nearly all caused by dominant negative mutations of the *TR*β gene (32). Affected patients have high serum concentrations of free T4 and free T3 combined with normal or slightly high serum TSH concentrations, reflecting their reduced peripheral and central responsiveness to thyroid hormone (Table 1). Resistance to thyroid hormone is thus mechanistically distinct from the secretory defects described in the previous sections of this chapter. Unlike patients with primary or central hypothyroidism, thyroid function in patients with resistance to thyroid hormone is increased. Women with generalized resistance to thyroid hormone are fertile. Unaffected fetuses of mothers with the syndrome are often aborted or have hyperthyroidism, with poor intrauterine growth and low serum TSH concentrations (affected fetuses are more normal) (33).

Most infants and children with resistance to thyroid hormone have goiter, but clinical presentation is otherwise variable. Some have hearing impairment and poor growth in infancy, suggestive of hypothyroidism, but when older may have tachycardia, probably due to activation of TRα receptors by the high serum T4 and T3 concentrations. Approximately, half have some learning disability, although frank metal retardation (IQ $<$ 60) is rare (3%) (32,34,35). Clinical thyroid status is variable, which has led clinicians to designate patients as having generalized resistance to thyroid hormone if they are clinically euthyroid or hypothyroid, and as having pituitary resistance to thyroid hormone if they are clinically hyperthyroid. These terms may be helpful in describing the clinical manifestations of resistance to thyroid hormone, but there is no consistent pathophysiologic correlation between these manifestations and either genotype (specific TRβ mutation) or other objective measures of peripheral thyroid hormone action (36). Once serum thyroid hormone-binding abnormalities and laboratory artifacts have been excluded, a TSH-secreting pituitary tumor must be excluded before resistance to thyroid hormone is diagnosed. Only 15% of cases of resistance to thyroid hormone are sporadic and therefore measuring serum TSH and free T4 in first-degree relatives is a useful way to screen for the disorder. Other tests that have been proposed to differentiate TSH-secreting adenomas from resistance to thyroid hormone are measurements of the serum alpha subunit of the pituitary glycoprotein hormones (TSH, luteinizing hormone, and follicular stimulating hormone) and the serum TSH response to TRH. Characteristically, but by no means invariably, patients with resistance to thyroid hormone have normal serum alpha subunit concentrations and an increase in serum TSH in response to TRH, whereas

the former are high and there is no response to TRH in patients with a TSH-secreting adenoma. Pituitary imaging with MRI is required for definitive diagnosis.

Patients with resistance to thyroid hormone produce large amounts of thyroid hormone to compensate for the receptor defect, and therefore no attempt should be made to reduce thyroid secretion. Also, spontaneous improvement of clinical symptoms with age has been reported (32). A trial of levothyroxine is sometimes offered to very young children for the relative indications of (i) high serum TSH concentrations; (ii) failure to thrive that cannot be explained on the basis of another illness or defect; (iii) unexplained seizures; (iv) developmental delay; or (v) a history of growth or mental retardation in affected members of the family. In these cases, reduction of the serum TSH concentration to normal is used to guide replacement (32). The benefit of this intervention is uncertain.

CONSUMPTIVE HYPOTHYROIDISM

The main pathway of thyroid hormone degradation in humans is sequential monodeiodination, catalyzed by a family of selenoenzymes termed the iodothyronine deiodinases. Type 3 deiodinase (D3) catalyzes the conversion of T4 and T3 to inactive metabolites, respectively, reverse T3 and 3,3′-diiodothyronine, preventing activation of the prohormone T4 and terminating the action of T3. D3 is normally expressed in the uteroplacental unit and in the developing embryo, and it is also expressed in some tumors including astrocytomas, oligodendrogliomas, and glioblastoma multiforme (37–39). Tumor D3 activity can be robust, and the highest D3 activity reported in any human tissue thus far has been in vascular tumors, including infantile hemangiomas and a hemangioendothelioma (40,41). Patients with large tumors rich in D3 activity can develop "consumptive hypothyroidism" because of the rapid degradation of T4 and T3 (42). When the rate of degradation exceeds the capacity of the pituitary and the thyroid to increase TSH and T4 and T3 production, respectively, hypothyroidism occurs.

The first example of consumptive hypothyroidism was in a three-month-old infant with massive hepatic hemangiomas and severe hypothyroidism who required up to nine times the daily dose of thyroid hormone needed by an athyreotic infant of the same size (40). Studies of the tumor revealed a very high-level of D3 activity. Analysis of other hemangiomas revealed many to contain D3 activity and, since the original report, more than 10 additional cases have been reported (40,43–46) The tumoral D3 expression in these vascular lesions together with the disappearance of hypothyroidism after hemangioma involution support the concept that this endocrinopathy is because of excessive degradation rates of T4 and T3 relative to the functional reserve of the infant thyroid. Two adults with histologically distinct D3-expressing tumors who had consumptive hypothyroidism have been reported, a 21-year-old woman with a hepatic hemangioendothelioma and a 55-year-old man with a large, malignant fibrous tumor (41,47).

Certain clinical and laboratory features should raise the suspicion for consumptive hypothyroidism. Hypothyroidism appears during a period of tumor growth and resolves after tumor involution or resection. Increased inner-ring deiodination results in a marked elevation in serum rT3 concentrations and/or supernormal requirements for exogenous thyroid hormone. Endogenous thyroid hormone secretion is increased, as determined by high serum thyroglobulin concentrations, high thyroid radioiodine uptake, and goiter. As the quantity of thyroid hormone degraded by a D3-expressing tumor is a result of cellular D3 activity and tumor mass, only patients with large tumors are at risk for hypothyroidism unless there is also independent pituitary–thyroid dysfunction. Thyroid function should be monitored in patients with large proliferating vascular lesions. Because hemangiomas proliferate through the first-year of life and neurodevelopment is critically dependent upon thyroid hormone during this period, serum TSH should be measured monthly in infants with large hemangiomas through 12 months of age. Because the half-life of T4 and T3 can be markedly shortened, higher than normal doses of levothyroxine may be required to normalize thyroid function until the tumor involutes or is resected, at which time the dose should be reduced.

CLINICAL PRESENTATION

Common symptoms and signs of hypothyroidism are listed in Table 2. None is entirely sensitive or specific, illustrated by one study of adults in which only 30% reported symptoms at presentation and 17% of normal subjects reported having the same symptoms (48). The symptoms may be subtle, even in patients with severe biochemical hypothyroidism. The initial history should include inquiries into energy level, sleep pattern, growth, pubertal development and menarche, preference for cold or warm temperature,

Table 2 Symptoms and Signs of Hypothyroidism

Goiter
Growth retardation
Delayed skeletal maturation
Pubertal disorders (delay or pseudoprecocity)
Slowed mentation (lethargy and impaired school performance)
Fatigue
Bradycardia (decreased cardiac output)
Constipation
Cold intolerance
Hypothermia
Fluid retention and weight gain (impaired renal free water clearance)
Dry, sallow skin
Delayed deep tendon reflexes
Muscle pseudohypertrophy

bowel function, and school performance. In addition to palpation of the thyroid, fluid status, muscle strength, and deep tendon reflexes should be carefully assessed. Chronic autoimmune thyroiditis may be the initial presentation of an autoimmune polyglandular syndrome, and therefore the possibility of coexisting autoimmune diseases such as type 1 diabetes, Addison's disease, and pernicious anemia should be considered.

Growth is often retarded, and pubertal development may be deranged. Similar to other endocrine causes of growth failure, linear growth is compromised to a greater degree than weight gain, and the bone age is delayed. Hypothyroidism typically causes pubertal delay, but may also induce a syndrome of pseudoprecocity manifested as testicular enlargement in boys and breast enlargement and vaginal bleeding in girls (9,24). This differs clinically from true precocity by the absence of accelerated bone maturation and linear growth. The importance of thyroid hormone in development is illustrated by the consequences of treatment delay, with hypothyroid infants being at a very high risk for permanent neurologic impairment (Fig. 2) (49). Older children have slowing of mental function, but not permanent impairment.

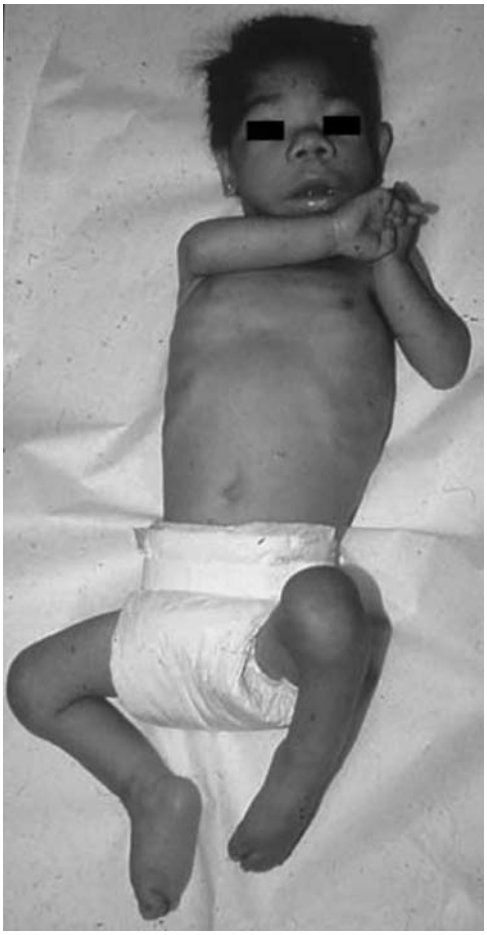

Figure 2 Severe neurodevelopmental and growth retardation in a 17-year-old girl with untreated congenital hypothyroidism. Her bone age was nine months at the time of this photograph. *Source*: From Ref. 12.

DIAGNOSIS

Laboratory Studies

When hypothyroidism is suspected, and indeed in any short child, serum TSH and free T4 (or free T4 index) should be measured (Table 1). In serum, 99.98% of the T4 and 99.7% of the T3 are bound to serum protein, primarily thyroxine-binding globulin. Therefore, serum total T4 and total T3 concentrations are abnormal not only in patients with thyroid dysfunction, but also in those with abnormalities in the production of thyroxine-binding globulin. This is obviated by measurement of serum free T4 (and T3), and these tests are now widely available and can be used routinely. The alternative is to measure serum total T4 and T3-resin uptake, and use the results to calculate what is known as the serum free T4 index. The direct measurements of serum free T4 are somewhat binding-protein dependent, and therefore may not be accurate in patients with large changes in serum thyroid hormone-binding proteins, for example, patients with severe nonthyroidal illness and pregnant women. These issues have been discussed comprehensively by the National Academy of Clinical Biochemistry (www.nacb.org) (50).

All patients with primary hypothyroidism have high serum TSH concentrations, and serum TSH alone may be measured as a screening test for hypothyroidism. If hypothyroidism is strongly suspected, serum free T4 (or free T4 index) should be measured at the same time. Serum T3 (or free T3) should not be measured; serum T3 concentrations are normal in many patients with hypothyroidism, owing to the increased conversion of T4 to T3 by type 2 deiodinase and preferential secretion of T3 by residual thyroid tissue stimulated by the high serum TSH concentrations.

Patients with central hypothyroidism have low or inappropriately normal serum TSH concentrations and low serum free T4 concentrations (or low serum free T4 index values). Rare patients with central hypothyroidism have slightly high ($<20\,\mu U/mL$) serum TSH concentrations due to the synthesis of TSH with reduced biologic activity (1,9,51,52). In patients with resistance to thyroid hormone, serum free T4 and free T3 concentrations are high and serum TSH concentrations are inappropriately normal or slightly high. In patients with consumptive hypothyroidism, the serum TSH and free T4 values are the same as those in patients with primary hypothyroidism, and serum thyroglobulin and rT3 concentrations are high.

Thyroid Imaging

Thyroid imaging is rarely needed in the evaluation of patients with hypothyroidism. In patients with autoimmune thyroid disease, ultrasonography usually reveals a heterogeneous pattern of echoes and an enlarged thyroid gland, but neither finding is specific. Where iodine intake is adequate, the lower limit of radioiodine uptake values in normal subjects is low, for example, 5% in 6 hours and 10 or 15% at 24 hours, so that the test is not useful in identifying patients with hypothyroidism (7). A perchlorate discharge test is occasionally done as part of the evaluation of patients with goiter who might have a block in intrathyroidal organification of iodine, because of Pendred's syndrome, other congenital organification defects, or the ingestion of some antithyroid agent. If radioiodine is given, followed by administration of perchlorate several hours later, the radioiodine content of the thyroid will fall if there is a block in organification. In patients with resistance to thyroid hormone and consumptive hypothyroidism, thyroid radioiodine uptake is high, but there is rarely a need for the test.

THERAPY

Medications

Levothyroxine (L-T4) is the treatment of choice for all patients with hypothyroidism. There are virtually no adverse reactions, and its long half-life of five to seven days allows the convenience of once-daily administration. A few children have had the onset of pseudotumor cerebri after the initiation of levothyroxine and allergy to the dyes used to color some T4 preparations has been reported (53). The main hazard of T4 therapy is excessive dosage, and therefore thyrotoxicosis. T4 therapy may be initiated using a graded approach (54). Alternatively, it may be initiated with a dose estimated to be a full replacement dose based upon the patient's age and ideal body weight (Table 3), with the assumption that the long half-life of T4 insures gradual equilibration over the course of five to six weeks (note the changes in dosage on a weight basis shown in Table 3, indicative of the marked slowing in T4 clearance that occurs with age) (24). Dosing is ultimately individualized on the basis of clinical and biochemical monitoring. With respect to serum TSH, the goal of therapy is a serum TSH concentration of 0.5–3.0 μU/mL. This will usually be associated with a serum free T4 concentration in the upper half of the normal range. Thyroid function should be assessed six weeks after the initiation or adjustment of the levothyroxine dosage. Growth and sexual development should be followed systematically, as in any child. Once biochemical euthyroidism has been achieved, serum TSH should be measured every four to six months in growing children and yearly after final height has been attained. When noncompliance is suspected as the cause of treatment failure, a serum TSH concentration and serum free T4 (or free T4 index) should be measured. A serum TSH concentration greater than twice normal in a patient who has a normal or even high serum free T4 concentration suggests intermittent ingestion of levothyroxine, in particular, ingestion of multiple tablets just before the tests were done.

Several conditions or drugs may alter the requirement for levothyroxine (Table 4). Levothyroxine prescriptions often carry a label stating that it should be taken at least 30 minutes before eating or any medication known to impair its absorption. However, from a practical viewpoint, it is more important that the patient take the levothyroxine at the same time every day.

Approximately 80% of the T3 in serum is produced by the (ORD) of T4 in peripheral tissues, thus explaining the normal serum T3 concentrations found in patients taking adequate doses of levothyroxine (5). Because some T3 is secreted from the thyroid gland, and also because some patients (adults) do not feel well while taking seemingly adequate doses of levothyroxine, the effects of combination levothyroxine and liothyronine (T3) therapy have been studied. In one crossover study of 33 adults with hypothyroidism, combined therapy was associated with improved mood and cognitive function, as compared with levothyroxine alone (55). However, several subsequent trials have failed to confirm this finding or

Table 3 Recommended Doses of Levothyroxine, Based on Body Weight

Recommended L-T4 treatment doses	
Age	L-T4 dose (mcg/kg)
0–3 mo	10–15
3–6 mo	8–10
6–12 mo	6–8
1–3 yr	4–6
3–10 yr	3–4
10–15 yr	2–4
> 15 yr	2–3
Adult	1.6

Source: From Ref. 24.

Table 4 Conditions that Alter Levothyroxine Requirements

Increased requirements for T4	
Pregnancy	
Gastrointestinal disease	Celiac disease, inflammatory bowel disease, jejunoileal bypass, and small bowel resection
Drugs that impair T4 absorption	Cholestyramine; sucralfate; aluminum hydroxide; calcium carbonate; ferrous sulfate
Drugs that enhance CYP3A4 and thereby accelerate T4 clearance	Rifampin; carbamazepine; phenytoin; sertraline (?)
Drugs that increase thyroxine-binding globulin production	Estrogen; oral contraceptives
Drugs which impair T4 to T3 conversion	Amiodarone
Conditions that may block type 1 deiodinase	Selenium deficiency

Source: From Ref. 12.

to identify other advantages to combined therapy. Thus, levothyroxine monotherapy remains the preferred treatment for patients with primary or central hypothyroidism (56,57). For infants with severe consumptive hypothyroidism caused by massive D3-expressing hemangiomas, combined therapy with high doses of both levothyroxine and liothyronine has been used to rapidly restore euthyroidism (42).

Therapeutic Guidelines

Specific guidelines for the monitoring and treatment of patients with congenital hypothyroidism have been provided by expert consensus and are detailed in Vol. 2; Chap. 16 (Thyroid Disorders of Infancy) as well as in publications by the American Academy of Pediatrics (58). For older children with primary hypothyroidism, serum TSH should be measured periodically and corrected to the middle of normal range (0.5–3.0 μU/mL), as noted above. In patients with central hypothyroidism, serum free T4 should be measured at similar intervals, and corrected to the upper half of the normal range to approximate the concentrations associated with adequate treatment in patients with primary hypothyroidism (2). Patients with resistance to thyroid hormone are often followed without therapy but, for those who are offered hormone replacement, levothyroxine is the most common medication and normalization of serum TSH is used to guide dosing. Thyroid hormone replacement in patients with consumptive hypothyroidism is guided by the same goals as primary hypothyroidism (normalization of serum TSH), but tumor status must be considered as, independent from glandular function, the growth or involution of D3-expressing tumors can increase or decease requirements for exogenous hormone.

Transfer to Adult Care

Parents of children with chronic autoimmune thyroiditis should be advised that the hypothyroidism will likely be permanent, although exceptions have been reported (59). Therefore, life-long monitoring of thyroid function will be needed. After the completion of linear growth, serum TSH should be measured annually once euthyroidism has been restored. Treatment with medications that can alter levothyroxine requirements (Table 4) warrants additional monitoring. Of particular importance is the need for more levothyroxine during pregnancy, because there is evidence that the intellectual development of fetuses may be impaired in mothers with hypothyroidism (2,60,61). In women with hypothyroidism, the requirement for levothyroxine increases by an average of 45% during gestation. Accordingly, serum TSH should be measured as soon as a woman knows she is pregnant, and at three to six week intervals thereafter, and the dose of levothyroxine adjusted to maintain serum TSH concentrations in the range of 0.5–3.0 μU/mL (61). Alternatively, women who are treated for hypothyroidism can be advised to increase their dose of levothyroxine empirically by taking two extra daily doses each week as soon as she knows she is pregnant, and to seek evaluation soon thereafter (61).

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18

Hyperthyroidism

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OVERVIEW

Thyrotoxicosis is an uncommon disorder of childhood and is characterized by accelerated metabolism of body tissues resulting from excessive levels of unbound circulating thyroid hormones. Graves' disease accounts for at least 95% of cases in children (1). Other causes are rare and are listed in Table 1.

Mechanisms that can produce thyrotoxicosis include thyroid follicular cell hyperfunction with increased synthesis and secretion of T_4 and T_3, thyroid follicular cell destruction with release of preformed T_4 and T_3, and ingestion or administration of thyroid hormone or iodide preparations.

Hyperfunction of thyroid follicular cells can either be autonomous or mediated through stimulation of thyrotropin (TSH) receptors by substances such as TSH or thyrotropin receptor antibodies (TSHrAb). Autonomous hyperfunction of thyroid follicular cells is rarely seen during childhood and is represented by toxic adenoma, familial nonautoimmune hyperthyroidism (FNH), hyperfunctioning thyroid carcinoma, and the hyperthyroidism of the McCune–Albright syndrome (MAS).

Stimulation of TSH receptors by TSHrAb produces the diffuse toxic goiter of Graves' disease and accounts for the majority of childhood thyrotoxicosis. Rarely, increased TSH secretion resulting either from a TSH-producing pituitary adenoma or from pituitary resistance to thyroid hormone can produce thyrotoxicosis. Another glycoprotein hormone, human chorionic gonadotropin (hCG), also binds to the TSH receptor and stimulates thyroid cell function (2,3). The thyrotropic potency of hCG is much less than that of TSH; but abnormally high serum levels of hCG such as those seen in many individuals with hyperemesis gravidarum or with trophoblastic or germ cell tumors, can lead to hyperthyroidism (4,5).

Inflammation of thyroid follicular cells can be associated with viral or autoimmune processes, and extensive destruction can release large amounts of preformed T_4 and T_3 into the circulation. The resultant thyrotoxicosis tends to be mild and transient, usually only lasting a few weeks to a few months. Examples include the toxic thyroiditis of Hashimoto's disease and subacute thyroiditis.

Acute or chronic ingestion of thyroid hormone preparations such as L-thyroxine or desiccated thyroid can produce excessive levels of circulating thyroid hormones. Ingestion may be surreptitious, iatrogenic, or accidental. Ingestion or parenteral administration of iodides may also result in thyrotoxicosis. This phenomenon, known as Jod-Basedow, most frequently occurs in iodine-deficient areas when supplemental iodides are added to the diet. Iodine-induced hyperthyroidism generally occurs in thyroid glands that are functioning independently of TSH stimulation (6,7). However, iodine-induced hyperthyroidism has also been described both in adults (6,7), and in a neonate (8) with apparently normal thyroid glands, who were exposed to high concentrations of iodine over prolonged periods. Iodine-induced hyperthyroidism has also been reported in children being treated with the iodine-rich antiarrhythmic drug, amiodarone (9,10). Amiodarone (~35% iodine content) can induce two types of thyrotoxicosis (11). Type 1 occurs in patients with an underlying thyroid condition such as Graves' disease, who develop hyperthyroidism after exposure to excessive amounts of iodine; and type 2 occurs as the result of inflammatory destruction of the thyroid with release of preformed thyroid hormones. Management of amiodarone-induced thyrotoxicosis (AIT) is difficult and withdrawal of amiodarone may not be possible, especially when it is being used for treatment of ventricular arrhythmias. Even if withdrawal of the drug is possible, thyrotoxicosis may persist for several months due to the prolonged half-life of amiodarone (12). Therapeutic interventions for type 1 AIT include thionamide drugs, perchlorate, or

Table 1 Etiology of Thyrotoxicosis in Childhood and Adolescence

Graves' disease
Autonomous functioning nodule(s)
Toxic adenoma
Hyperfunctioning papillary or follicular carcinoma
McCune-Albright syndrome
Familial nonautoimmune hyperthyroidism
Thyrotropin (TSH)-induced hyperthyroidism
TSH-producing pituitary adenoma
Pituitary resistance to thyroid hormone
Thyroiditis
Subacute thyroiditis
Toxic thyroiditis of Hashimoto's disease
Exogenous thyroid hormone
Iodine-induced hyperthyroidism (Jod-Basedow)
Human chorionic gonadotropin–induced hyperthyroidism
Gestational hyperthyroidism
Hydatidiform mole
Choriocarcinoma

surgery; prednisone therapy is the treatment of choice for type 2 AIT (11,12).

GRAVES' DISEASE

Introduction

Graves' disease is an immunogenetic disorder characterized clinically by thyromegaly, hyperthyroidism, and infiltrative ophthalmopathy. A family history of autoimmune thyroid disease is present in up to 60% of patients (13). Recent linkage and family-based association studies have identified several susceptibility loci at various chromosomal regions, providing evidence that Graves' disease, like most other autoimmune disorders, is inherited as a complex polygenic trait. Chromosomal regions containing susceptibility loci include 6p21 (major histocompatibility complex), 2q33 (cytotoxic T-lymphocyte antigen-4, CTLA-4), 14q31 (GD-1), 18q21, 20q13 (GD-2), and Xq21 (GD-3) (14–17). A number of HLA haplotypes have been associated with Graves' disease in children. HLA-DRB1∗03 and -DRB1∗08 are positively associated with Graves' disease in Caucasian children, whereas the -DRB1∗07 haplotype is protective (18,19). For the Japanese population, the frequency of HLA-DPB1∗0501, -DRB1∗0405, and -DQB1∗0401 is significantly increased in children with Graves' disease (20,21). Recently, an association between Graves' disease and a single-nucleotide polymorphism in the *PTPN22* gene on chromosome 1p13 has been demonstrated in British and Polish Caucasians (22,23); this gene encodes for lymphoid protein tyrosine phosphatase, which is a negative regulator of T-cell activation. In addition to these immune response–related genes, the gene encoding thyroglobulin has been identified as a major gene involved in TSH receptor–related autoimmunity; amino acid substitutions in this gene predispose individuals to Graves' disease and autoimmune thyroiditis (24). Interestingly, polymorphisms in the gene for the TSH receptor do not appear to play a significant role in the development of Graves' disease (24).

The concordance rate between monozygotic twins has been reported to range between 20% and 60% (15–17), thus implying that environmental factors play a significant role in the development of the disease. The extent to which chemicals, drugs, infections, and psychological stress can alter immunoregulatory genes is unknown. However, the existence of a two-way interaction between the immune and neuroendocrine systems may provide a mechanism by which biologic and psychologic stresses can affect lymphocyte subpopulations and immunoregulation (25).

The exact incidence of Graves' disease during childhood in North America is unknown, but it is uncommon and has been reported to account for less than 5% of the cases seen in most thyroid clinics (26). A recent study has estimated the incidence of childhood thyrotoxicosis in Denmark to be 0.1/100,000 in the very young increasing with age to 3/100,000 by 14 years of age (27). Just as in North America, the vast majority ($>95\%$) of childhood thyrotoxicosis in Denmark is due to Graves' disease (27). More than two-thirds of childhood cases in North America occur between the ages of 10 and 15 years (1), and it occurs more frequently in females in a ratio of 3:1 to 5:1 (13).

Pathogenesis: TSH Receptor and TSH Receptor Antibodies

The TSH receptor is a member of the large family of guanine-nucleotide-binding (G) protein-coupled receptors and represents the primary target antigen for autoantibodies that mediate the hyperthyroidism and thyromegaly of Graves' disease. As deduced from the cDNA sequence, the *TSHR* gene on chromosome 14q3 codes for a 764-amino acid protein; the first 21 amino acids represent a signal peptide, which is not present on the mature receptor. The mature receptor is a glycoprotein with a single polypeptide chain of 743 amino acids (24). Similar to other G-protein–coupled receptors, the TSH receptor has an extracellular domain (394 amino acid residues encoded by 9 exons), along with seven transmembrane domains and a cytoplasmic tail (349 amino acids encoded by exon 10). The TSH and other glycoprotein hormone [i.e., LH/CG and follicle-stimulating hormone (FSH)] receptors each have relatively large extracellular domains, and this characteristic differentiates them from the other G-protein–coupled receptors. The TSH, LH/CG, and FSH receptors are closely related structurally and share about 70% and 45% homology in their transmembrane and extracellular domains, respectively (28).

The extracellular domain of the TSH receptor represents the amino (N) -terminal end, and the transmembrane and cytoplasmic domains represent the carboxyl (C) -terminal end of the protein. The extracellular domain contains six potential *N*-glycosylation sites and nine leucine-rich repeats of a loosely conserved 25 amino acid residue motif. Proper glycosylation appears to be important both for normal

expression of the receptor on the thyroid cell membrane and for normal hormone-receptor interactions (29,30). The leucine-rich repeats, which have the potential to form amphipathic α-helices, are believed to be involved in protein-protein or protein-membrane interactions. Recent studies have confirmed that both TSH and autoantibodies to the TSH receptor (TSHrAb) bind to the extracellular domain (29,30). The transmembrane and cytoplasmic domains are involved in signal transduction, acting through G-protein to stimulate the production of cyclic adenosine monophosphate (cAMP) by adenylyl cyclase (29,30).

The hyperthyroidism and thyromegaly of Graves' disease are mediated through immunoglobulin G (IgG) that bind to the extracellular domain of the TSH receptor and stimulate follicular cell function and growth. In addition to the stimulating TSHrAbs, sera from patients with Graves' disease may also contain other IgG to the TSH receptor that inhibits thyroid cell function and growth. Stimulating TSHrAbs are primarily restricted to the IgG1 subclass, suggesting that they are either oligo- or monoclonal in origin (31). On the other hand, blocking TSHrAbs appear to be polyclonal in origin and may be of IgG1, IgG2, IgG3, or IgG4 subclass (32). Current evidence suggests that disease caused by the immune system (i.e., autoimmune disease) appears to result from a restricted immune response involving B- and/or T-lymphocytes against one or a few epitopes of the target antigen (33). These observations, therefore, support the importance of stimulating TSHrAbs in the cause of Graves' disease and imply that blocking TSHrAbs, much like antithyroglobulin and antithyroid peroxidase, arise as a result of thyroid tissue damage. Nevertheless, blocking TSHrAbs can still modulate the biological effects of stimulating TSHrAbs. Therefore, a patient's clinical presentation and course may be determined by the net biological effect of the simultaneous interaction of various stimulating and blocking TSHrAbs with the TSH receptor. In addition to stimulating and blocking TSHrAbs, neutral TSHrAbs have recently been identified (24). By definition, these TSHrAbs do not affect TSH binding to the TSH receptor, and they do not possess either stimulating or blocking activity. The clinical or pathophysiological relevance of neutral TSHrAbs remains unclear.

Several investigators have proposed that the functional effect(s) a particular TSHrAb exhibits is determined by the specific region to which the antibody binds on the TSH receptor (34,35). Following the successful cloning of the TSH receptor, numerous studies have attempted to identify binding sites for the various TSHrAbs. To date, the major experimental approaches to define TSHrAb epitopes have included the following: (i) transfecting mammalian cells with mutant cDNA of the TSH receptor; (ii) using synthetic peptides derived from the predicted amino acid sequence of the TSH receptor; and (iii) using monoclonal antibodies to the TSH receptor (24,30,36–38). While some of these studies have localized functional epitopes to a few relatively narrow regions of the extracellular domain (39), others have identified multiple regions throughout the entire extracellular domain, which appear to be involved in TSHrAb binding (40). Although controversy still exists regarding the specific sites that compose TSHrAb epitopes, current evidence supports the concept that the antibodies bind to conformational epitopes made up of discontinuous segments across the extracellular domain (15). Some studies support that epitopes of stimulating TSHrAbs are largely contained on the N-terminal region (aa 8–165) of the extracellular domain, while epitopes of blocking TSHrAbs are largely contained on the C-terminal portion (aa 270–395) of the extracellular domain (41). Other studies, in contrast, support that binding of stimulating TSHrAbs and blocking TSHrAbs to the TSH receptor is not restricted to distinct and distant epitopes; and TSH-receptor stimulating and blocking antibodies cannot be distinguished purely on the basis of their conformational epitope recognition (42). Despite these recent advances, the exact mechanisms by which stimulating and blocking TSHrAbs exert their biological effects remain unknown. It has been postulated that TSHrAbs exert their biological effects as the result of different structural changes in the TSH receptor that occur when the various types of autoantibodies interact with their specific epitopes (24).

The major source of TSHrAb production appears to be intrathyroidal lymphocytes (35), but lymphocytes in the spleen, lymph nodes, bone marrow, and peripheral blood may also produce these antibodies (43,44). The mechanisms and control of stimulating TSHrAb production are uncertain, but several hypotheses have been proposed. One hypothesis suggests that a deficiency of specific suppressor T-cell function accounts for TSHrAb production (45), whereas a second hypothesis suggests that a breakdown in the idiotype–anti-idiotype network of B-lymphocyte immunoregulation may be responsible (46). An increased frequency of antibodies to certain serotypes of *Yersinia enterocolitica* has been reported in patients with Graves' disease (47), and infection with this bacterium has been proposed as an important initiating event in the development of the disease (48). *Y. enterocolitica* has a specific, saturable binding site for TSH, and antibodies produced against this site may cross-react with the TSH receptor on the thyroid follicular cell membrane (47). A fourth hypothesis relies on the fact that thyroid follicular cells can express HLA-DR antigens and are, therefore, endowed with the capacity to present other antigenic material to primed T-lymphocytes. Through this mechanism, the follicular cell could present the TSH receptor as antigen and direct the synthesis of TSHrAb (49). Although experimental evidence exists for each of the above hypotheses, none can fully account for all aspects of TSHrAb production. Experimental animal models of Graves' disease have now been developed (50), and it is hoped that these models

will help to identify some of the mechanisms responsible for TSHrAb production.

Currently, there are two major types of assays used to measure TSHrAbs (51). Receptor assays assess the ability of Graves' IgG to inhibit labeled TSH from binding to the TSH receptor, and antibodies detected by this method have been designated thyrotropin-binding inhibitory immunoglobulins (TBII). It should be emphasized that receptor assays do not differentiate TSHrAb that stimulate thyroid cell function from TSHrAb that inhibit thyroid cell function; both types of TSHrAbs can be detected as long as they inhibit TSH from binding to its receptor. Receptor assays, which employ a combination of detergent-solubilized porcine TSH receptors and receptor-purified [^{125}I]-labeled bovine TSH (referred to as first-generation TSH receptor assays), are both sensitive and specific, and they provide a reproducible, inexpensive means of measuring TSHrAb in unextracted serum (35,52). Studies using these receptor assays have detected TSHrAb in 82% to 100% of adults (35) and 93% of children (53) with untreated, active Graves' disease. TSHrAb can also be detected by receptor assays in small numbers (10–20%) of patients with Hashimoto's thyroiditis (35,53,54). A second-generation TSH-receptor antibody assay is now commercially available (55). This assay utilizes purified labeled bovine TSH and an immobilized recombinant human TSH-receptor protein (55). Recent reports show that this assay retains very high specificity and is more sensitive than the first-generation receptor assay (55,56). In one report (56), the second-generation assay detected TSHrAb in 41 out of 46 patients with Graves' disease that had negative results with the standard first-generation assay. More recently, a third-generation assay for TSHrAbs has been developed (57). In this assay, serum TSHrAbs inhibit binding of a biotin-labeled human monoclonal thyroid stimulating antibody (M22) to immobilized porcine TSH-receptor protein; M22-biotin binding is detected by addition of streptavidin peroxidase. This assay method has been shown to have better sensitivity, specificity, and precision than a similar assay system that utilizes biotin-labeled TSH instead of the human monoclonal M22 (58). While these newer assays can detect TSHrAbs in over 95% of patients with active Graves' disease, the clinical value of these assays for the prognosis of Graves' disease with respect to remission or relapse is currently unknown.

Bioassay methods constitute the other major type of assay currently used to measure TSHrAb. Most commonly, these assays employ isolated thyroid cells in culture to assess the ability of Ig concentrates from patient sera to stimulate thyroid cell production of cAMP. Antibodies detected by these assays have been designated thyroid-stimulating immunoglobulins (TSI). These assays can be performed using cells taken from human or porcine thyroid tissue as well as from the immortal rat thyroid line, the FRTL-5 cells. More recently, transfected mammalian cells (e.g., Cos-7 and CHO cells) expressing the recombinant human TSH receptor have been used to detect stimulating TSHrAbs (29,59). Recently, a bioassay method utilizing CHO cells transfected with the human TSH receptor was found to detect TSI in 10 of 11 (91%) children with active Graves' disease, whereas TSI were not detected by this assay in 13 normal children, 2 children in remission from Graves' disease, and 11 children with chronic lymphocytic thyroiditis (60). Although the bioassays possess high sensitivity and specificity, they are less precise and are more expensive and time-consuming to perform than the receptor assays (34,35,51,60).

Although some reports have demonstrated highly positive correlations between TSHrAb levels detected by the receptor and bioassay methods (61), most have demonstrated no such correlation (34). Some investigators suggest the lack of correlation between TBII and TSI levels in patient sera is due to the presence of different populations of TSHrAbs that exhibit different degrees of TSH-agonist activity (62). Others suggest the poor correlation results from the coexistence of both stimulating and blocking TSHrAb in some patients' sera (63).

Clinical Manifestations

During childhood and adolescence, most patients with Graves' disease present with the classic symptoms and signs (13). Early during the course of the disease, the symptoms and signs specific to children (Table 2) may be minimal because the disease usually develops insidiously over several months (13). Often the initial awareness of any problem is in school where teachers notice changes in behavior and academic performance. Insomnia, restless sleep, and nocturia are common and often are associated with easy fatigability and lethargy during the day. Other clinical manifestations include palpitations, increased stool frequency, increased sweating, and proximal muscle weakness. Advanced linear growth and bone maturation, reduced bone mineral density, diminished left ventricular reserve, and mitral regurgitation also have been reported in thyrotoxic children and adolescents (64). Children who develop Graves' disease before the age of three to four years can

Table 2 Common Symptoms and Signs of Graves' Disease in 290 Children and Adolescents

Symptoms and signs	Percentage affected
Goiter	98
Tachycardia	82
Nervousness	82
Increased pulse pressure	80
Proptosis	65
Increased appetite	60
Tremor	52
Weight loss	50
Heat intolerance	30

Source: From Ref. 13.

experience chronic diarrhea, transitory speech and language delays, mental retardation, and craniosynostosis (65,66). The symptoms of hyperthyroidism in Graves' disease, although variable, tend to be more severe than in other causes of hyperthyroidism.

Ophthalmic abnormalities are present in over one half of the patients (refer to section 2-2-4), and thyromegaly is almost invariably present. In fact, the absence of goiter raises serious doubt about the diagnosis of Graves' disease, and other causes of hyperthyroidism should be sought. The thyroid gland usually is symmetrically enlarged, smooth, soft, and nontender. A palpable thrill or an audible bruit may be present and reflects increased blood flow through the gland. Less often, and usually in association with coexisting Hashimoto's thyroiditis, the gland may be firm, bosselated, and asymmetrically enlarged. Although pretibial myxedema is observed in about 1% to 2% of adults with Graves' disease (15), it rarely, if ever, occurs in children. Other diseases have been observed in association with Graves' disease and include Hashimoto's thyroiditis, vitiligo, systemic lupus erythematosus, rheumatoid arthritis, Addison's disease, insulin-dependent diabetes mellitus, myasthenia gravis, and pernicious anemia (Vol. 2; Chap. 26) (1).

Thyroid Storm

Although their occurrence is extremely rare, thyroid storm and thyrotoxic periodic paralysis (TPP) are two endocrine emergencies that have been reported in children/adolescents with hyperthyroidism. Although most reported patients have had Graves' disease, these situations can also occur with other causes of hyperthyroidism (67–70). Thyroid storm is a life-threatening manifestation of thyrotoxicosis characterized by fever (generally greater than 38.5°C), tachycardia out of proportion to the fever, high output cardiac failure, gastrointestinal dysfunction (such as vomiting, diarrhea, and jaundice), and neurologic changes (such as confusion, obtundation, seizures, and coma). The diagnosis of thyroid storm requires a high index of suspicion. The syndrome complex may occur either in previously undiagnosed patients or in patients with poorly controlled hyperthyroidism. If left untreated, mortality rates of up to 90% have been reported (71). The exact mechanisms underlying the clinical progression from uncomplicated thyrotoxicosis to storm have not been determined. A number of precipitating factors have been identified and include infection, trauma, surgery, concomitant ingestion of sympathomimetic agents (e.g., pseudoephedrine), withdrawal of antithyroid medication, and radioactive iodine therapy (67,72–76). Therapeutic intervention includes the following: (i) emergency and supportive care to maintain adequate respiratory and cardiovascular functions and to control body temperature; (ii) management of precipitating factors, if indicated; and (iii) limiting the amount of thyroid hormones available to the peripheral body tissues, by using propylthiouracil (PTU), iodide, β-adrenergic blockers, and glucocorticoids (75,76). PTU inhibits production of new thyroid hormone and blocks conversion of T_4 to T_3 in peripheral tissues. During the first 24 to 48 hours of management, PTU can be administered orally, rectally, or by nasogastric tube in doses ranging from 100 to 200 mg every four to six hours (77). Once initial control of thyrotoxicosis has been achieved, PTU doses can be reduced to 5 to 10 mg/kg/day in divided oral doses every six to eight hours. Iodides (SSKI, five to six drops orally every eight hours) rapidly inhibit release of preformed hormones from the gland and acutely impair thyroid peroxidase activity, thus slowing synthesis of new T_4 and T_3. Preferably, iodides should be used only after PTU has been administered to avoid a potential increase in new thyroid hormone production. Propranolol (2 mg/kg/day in divided oral doses every six to eight hours) and hydrocortisone [2 mg/kg as intravenous (IV) bolus, then 36 to 45 mg/m^2/day in divided IV doses every six hours] are used to treat the exaggerated adrenergic effects and possible relative glucocorticoid insufficiency, respectively, which accompany thyroid storm. Both also inhibit conversion of T_4 to T_3. If desired, dexamethasone or betamethasone (0.5–1.0 mg IV every 6–12 hours) may be substituted for hydrocortisone.

Thyrotoxic Periodic Paralysis

TPP is a reversible cause of sudden onset weakness that most commonly affects hyperthyroid patients of Asian descent. However, TPP has also been observed in susceptible individuals from Caucasian, African-American, Hispanic, and Native American populations (70). The disorder affects 1% to 2% of hyperthyroid patients in Asian populations, but only 0.1% to 0.2% of hyperthyroid patients in North America (78,79). A very strong male preponderance has been observed, but the mode of inheritance is unknown and the majority of affected individuals do not have a family history of periodic paralysis. Most patients present between the ages of 20 and 39 years, but older adolescents with TPP have been reported (80). At presentation, the clinical signs and symptoms of thyrotoxicosis are often subtle and may be overlooked. In the majority of patients, episodes of weakness usually occur precipitously and vary from mild weakness to total paralysis of affected muscle groups. Weakness usually involves the limbs, with proximal muscles being more severely affected than distal muscles. Mental function, sensory function, respiratory, ocular, and bulbar muscle groups are not affected. However, cardiac rhythm disturbances and electrocardiogram abnormalities (e.g., U waves, ST segment abnormalities, prolongation of QT interval) are common (81). Rhabdomyolysis has been reported in some patients with TPP, probably as a result of hypokalemia-induced vasoconstriction in the muscle arterioles followed by ischemic changes in the sarcolemma (82).

TPP typically occurs in the early morning hours following a day of strenuous exercise. Other apparent precipitating factors include high carbohydrate intake, trauma, infection, menses, emotional stress, and alcohol ingestion (70,83). The frequency of attacks is variable, and individual episodes typically last from 3 to 36 hours. Laboratory evaluation during episodes reveals biochemical evidence of thyrotoxicosis (e.g., elevated serum levels of total and free thyroid hormones with suppressed TSH), and, in the vast majority of cases, significant hypokalemia. Body stores of potassium are normal, and hypokalemia is the result of intracellular shifts of potassium. Neuromuscular symptoms appear to resolve as potassium moves back out of the cells and may be hastened with supplemental potassium administration. Although the severity of muscle weakness/paralysis tends to reflect the degree of hypokalemia, episodes have occurred in a few patients with normal potassium levels (79,83). Although the exact mechanisms responsible for this disorder remain unclear, patients experience TPP only while they are thyrotoxic. Thyrotoxicosis alters plasma membrane permeability to sodium and potassium, a function linked to Na^+-K^+ ATPase activity. Thyrotoxicosis also enhances tissue responsiveness to β-adrenergic stimulation, and this increases Na^+-K^+ ATPase activity further. Additionally, Na^+-K^+ ATPase is also activated by insulin, and this may explain the relationship between attacks and large carbohydrate loads. In addition, a defect in the muscles themselves has been proposed, as they fail to respond to direct electrical stimulation during the period of paralysis (84). Medical management includes hospital admission for the acute paralysis, cardiac monitoring, and close observation of serum potassium levels. Potassium supplements should be used to correct hypokalemia, and antithyroid therapy should be started. Caution should be exercised when using IV potassium supplementation because rebound hyperkalemia may occur during the recovery phase. Unless cardiac complications dictate otherwise, potassium supplementation during the paralytic phase should not exceed 10 mmol/hr (85). Episodes of TPP always cease once thyrotoxicosis is corrected, and permanent treatment for the overactive thyroid is imperative. While awaiting normalization of thyroid status, patients should avoid precipitating factors such as strenuous exercise and high carbohydrate intake. β-adrenergic blocking agents and pharmacologic glucocorticoid therapy can be useful adjunctive treatments for TPP (78,79).

AUTONOMOUS THYROID NODULE

The autonomously functioning thyroid nodule is a discrete thyroid nodule that functions independently of normal pituitary control. The pathogenesis has not yet been established in all cases, but recent evidence supports that autonomous thyroid nodules can result from somatic mutations in either the α-subunit of G-protein ($G_{s\alpha}$; see below) or the TSH receptor (86,87). Most of the somatic mutations of the TSH receptor have been localized to exon 10, which codes for the transmembrane and cytoplasmic regions (24). In either situation, the mutations result in constitutive activation of adenylyl cyclase and unregulated production of cAMP. The unregulated cAMP production is responsible for the subsequent tissue hyperplasia and hyperthyroidism.

This disorder predominantly occurs in adults, is rare during childhood, but has been reported in a child as young as 22 months (88). Most children with a thyroid nodule come to the attention of a physician because of a mass in the region of the thyroid gland. The majority of patients with autonomous thyroid nodules are clinically euthyroid, and in contrast to adults, clinical hyperthyroidism occurs very rarely in children. Autonomously functioning nodules that cause hyperthyroidism are almost invariably benign adenomas (toxic adenoma), but very rarely hyperthyroidism caused by hyperfunctioning papillary or follicular carcinoma has been reported (89). In these cases, the patients usually have extensive metastatic disease, and the diagnosis of carcinoma has been established prior to onset of hyperthyroidism.

The hyperthyroidism of the MAS is also associated with single or multiple hyperfunctioning adenomatous nodules. This syndrome is characterized by polyostotic fibrous dysplasia, multiple café-au-lait spots, and endocrine hyperfunction. The most common endocrinopathy is isosexual precocious puberty, but hyperthyroidism, acromegaly, Cushing's syndrome, and hyperparathyroidism have been reported (90). In contrast to polyostotic fibrous dysplasia and precocious puberty that occur more commonly in girls with the syndrome, hyperthyroidism occurs with equal frequency in boys and girls. The age of onset of hyperthyroidism tends to be between 3 and 12 years (91), and this is somewhat younger than the usual age of onset of hyperthyroidism caused by hyperfunctioning nodules in other individuals. The hyperthyroidism is clearly due to autonomous function of the thyroid gland; basal TSH levels are suppressed and the TSH response to TRH is blunted, thyroid-stimulating antibodies are undetectable, and T_3 treatment fails to suppress radioactive iodide uptake by the thyroid.

Current evidence indicates that the receptors for each of the hormones (i.e., LH, FSH, TSH, GHRH, adrenocorticotropic hormone, and PTH) that might otherwise be implicated in the observed endocrinopathies of MAS are all coupled to G-proteins. The G-proteins are hetereotrimers composed of α-subunit and a tightly coupled βγ-dimer (92). The α-subunit contains the guanine-nucleotide–binding site and has intrinsic GTPase activity. In the normal situation, the binding of one of these stimulatory hormones to its receptor facilitates the exchange of guanosine triphosphate (GTP) for guanosine diphosphate (GDP) in the

guanine-nucleotide–binding site of the α-subunit ($G_{s\alpha}$). This results in the release of the G-protein from the receptor and its dissociation into free $G_{s\alpha}$-GTP and free βγ-dimer. Free $G_{s\alpha}$-GTP stimulates adenylyl cyclase activity, with the subsequent production of intracellular cAMP. After a preset time, the intrinsic GTPase of $G_{s\alpha}$ hydrolyses GTP to GDP, and the $G_{s\alpha}$-GDP reassociates with the βγ-dimer. The G-protein is thus returned to its inactive state and can now reassociate with its receptor and participate in another cycle (92).

Recent studies have identified somatic mutations in the $G_{s\alpha}$ gene in endocrine organs, bone, and skin from patients with MAS (92–94). These mutations involve the amino acid residue Arg^{201} that is critical for the intrinsic GTPase activity of $G_{s\alpha}$. Certain substitution mutations involving this amino acid residue disrupt GTPase activity and result in constitutive activation of adenylyl cyclase with unregulated production of intracellular cAMP. In some cases, the mutation has been found in abnormal sections of tissue but not in histologically normal sections from the same tissue (92). This observation is in keeping with the postzygotic nature of the mutation, and, therefore its mosaic cellular distribution and tends to explain the development of hyperfunctioning nodules within the thyroid gland. Recent evidence supports that in MAS patients with hyperthyroidism, the $G_{s\alpha}$ gene mutation may be on either the maternal or the paternal allele (95).

The amount of thyroid hormone that an autonomously functioning nodule produces appears to be related to its size. In adults with single autonomous nodules, hyperthyroidism usually occurs only when the nodule measures greater than 2.5 to 3 cm in diameter (96). Both T_4 and T_3 can be produced in excess, but an elevated serum T_3 level is frequently the only biochemical abnormality. The T_3 level may be elevated enough to inhibit the TSH response to TRH, but not enough to cause clinical hyperthyroidism (97).

A radionuclide image, preferably using ^{123}I, should be included in the evaluation of the hyperthyroid child with a thyroid nodule. The radioiodine (RAI) image allows one to study both trapping and organification by the nodule. Technetium images only demonstrate trapping by the nodule, and images are not always identical to those obtained with iodine. The diagnosis of a hyperfunctioning or ''hot'' nodule is established when the image reveals increased accumulation of the radioisotope in the nodule and decreased or absent uptake in the surrounding thyroid tissues.

For the single toxic thyroid nodule, surgical removal is the preferred method of treatment and usually is accomplished by partial thyroidectomy. Significant surgical complications are not expected, and postoperative hypothyroidism seldom occurs. With complete surgical removal of the autonomous nodule, hyperthyroidism should not recur postoperatively. Because the hyperthyroidism produced by the autonomous nodule is usually mild, a long preoperative preparation with antithyroid drugs is seldom necessary. A β-adrenergic blocker may be used to decrease the symptoms of hyperthyroidism. The administration of iodides is not indicated in the preoperative treatment of the autonomous nodule. Percutaneous intranodular ethanol injection under ultrasound guidance has been employed for the ablation of autonomous thyroid nodules (98). This approach appears to be safe and effective in adults and may prove to be a practical alternative to surgical treatment in children. Total thyroid ablation, by either surgical or RAI therapy, is the indicated treatment for children with thyrotoxicosis due to multiple autonomous thyroid nodules.

FAMILIAL NONAUTOIMMUNE HYPERTHYROIDISM

FNH is a rare condition that clinically can be confused with Graves' disease. The disorder occurs because of a germline mutation in the TSH-receptor gene. These so-called ''gain of function'' mutations result in the constitutive activation of the TSH-receptor–G-protein–effector system complex that ultimately leads to increased thyroid follicular cell growth and function (99). The first family recognized to have this disorder was described in 1982 (100); as of this writing, several kindreds with FNH have now been identified (101,102). Except for amino acid 281 (Ser^{281}) in the extracellular domain, all the other identified mutation sites are located in transmembrane domains 1, 2, 3, 5, 6, and 7 of the TSH receptor (101). It is estimated that FNH (also referred to as nonautoimmune hereditary hyperthyroidism) may account for up to 2% to 5% of all cases of diffuse hyperthyroidism (99). In a recent nationwide study from Denmark, one case of FNH was identified out of 121 (0.8%; 95% CI: 0.02–4.6%) individuals with juvenile thyrotoxicosis (103).

The disease is transmitted in an autosomal-dominant fashion. Therefore, unlike in Graves' disease, males and females can be affected equally. In described families, hyperthyroid individuals are spread over three to four generations. The onset of clinical hyperthyroidism is highly variable, with some patients presenting before the age of one year and others presenting in adolescence or early adulthood. However, clinically asymptomatic individuals can exhibit suppressed serum TSH levels for years prior to the clinical appearance of goiter or thyrotoxicosis (99). A recent review of all reported cases of FNH due to activating TSH-receptor germline mutations indicates that preterm delivery and low birth-weight are consistent features in affected individuals (104).

In affected individuals, both thyroid gland size and structure tend to change over time. In the youngest patients, the gland tends to be normal to slightly enlarged. In older patients, the thyroid is symmetrically enlarged and bruits may be audible over the lobes. Eventually, the diffusely enlarged gland may

evolve into a multinodular goiter (99). Although eye signs of thyrotoxicosis (e.g., stare, lid lag, widened palpebral fissures, and mild proptosis) may be present, infiltrative ophthalmopathy has not been observed in FNH. Laboratory evaluation reveals elevated serum levels of both total and free thyroid hormones and suppressed TSH. TSH-receptor antibodies are not present. In general, serum antibodies to thyroid peroxidase and thyroglobulin also are not present, but these autoantibodies have been detected in a few patients with confirmed FNH (102). In the research setting, DNA from peripheral blood leukocytes can be used to sequence the TSH-receptor gene for identification of point mutations associated with FNH.

The recognition of FNH is of great importance for the management. The diagnosis should be considered in cases of apparent Graves' disease when extrathyroidal signs and thyroid antibodies are absent and in patients with an extensive family history of hyperthyroidism. As in Graves' disease, antithyroid drug therapy can control the hyperthyroidism; but, due to the persistent functional effects of the TSH-receptor mutation, remission does not occur. Similarly, while subtotal thyroidectomy may restore euthyroidism in some patients (101), significant regrowth of thyroid tissue may occur with subsequent recurrence of clinical hyperthyroidism (99). Therefore, total ablation of the gland, either surgically or with RAI, should be considered in patients with FNH. Regular systematic screening for either clinical symptoms or early biochemical evidence of hyperthyroidism (i.e., suppressed serum TSH levels) should be undertaken to identify other affected family members. In addition, genetic counseling should be offered to affected families.

TSH-INDUCED HYPERTHYROIDISM

Hyperthyroidism from increased TSH secretion can occur as the result of either a TSH-secreting pituitary adenoma or selective pituitary resistance to thyroid hormone. Although both are rare, each has been reported in childhood and adolescence (105). Unlike Graves' disease, the sex ratio in patients with TSH-induced hyperthyroidism is 1:1. Most cases of TSH-producing pituitary adenoma occur sporadically, but familial cases have been reported (106). Pituitary resistance to thyroid hormone appears to be familial, with an autosomal dominant pattern of inheritance (105). The etiology of pituitary resistance to thyroid hormone has not been established for all cases, but most represent forms of the syndrome of generalized resistance to thyroid hormone (GRTH; see below) (107,108). The syndrome of GRTH is caused by a mutation in *THRB*, one of the thyroid hormone-receptor genes (108). About 100 different mutations have been identified in patients from over 500 families (109). Although the reasons remain unclear, affected members within a family can exhibit different degrees of resistance to thyroid hormone, and various tissues (e.g., heart, liver, bone, and pituitary) can be affected to a greater or lesser degree (108,110). Therefore, the pituitary gland in such individuals would be relatively more resistant to thyroid hormones than other tissues in the body. Pituitary thyrotroph resistance in these individuals is selective for thyroid hormones because there is normal inhibition of pituitary TSH secretion by glucocorticoids and dopaminergic agents (105).

Criteria essential for the diagnosis of this disorder include the following: evidence of increased peripheral metabolism, diffuse thyromegaly, elevated free thyroid hormone levels, and inappropriately elevated serum levels of TSH (106). Although the TSH level may not be elevated above the normal range, it is always detectable, even in highly sensitive and specific immunoassays. In all other causes of hyperthyroidism, sensitive immunoassays will reveal very suppressed or undetectable serum levels of TSH.

The clinical presentation is often very similar to Graves' disease, and a high degree of suspicion is needed to make the diagnosis. The patient with pituitary adenoma, however, may present with visual complaints due to compression of optic nerve tracts by the adenoma. Increased pituitary secretion of growth hormone and prolactin has also been reported in patients with TSH-secreting tumors (106).

Once the diagnosis of TSH-induced hyperthyroidism has been established, the clinician needs to determine if the increased TSH secretion results from a pituitary tumor or from pituitary resistance to thyroid hormone in order to determine the proper course of therapy. The TRH and T_3 suppression tests may help differentiate these two disorders. In general, serum TSH levels do not increase in response to TRH when a pituitary tumor is the cause of hyperthyroidism. In contrast, the TSH response to TRH tends to be normal or exaggerated in pituitary resistance to thyroid hormone (105). Pharmacologic doses of T_3 cause significant TSH suppression in patients with pituitary resistance but fail to reduce TSH levels in patients with TSH-secreting pituitary adenomas.

Determination of serum levels of the free α-subunit of the glycoproteins, including TSH, also can aid in differentiating these conditions; patients with TSH-secreting pituitary tumors generally have elevated (>1) molar α-subunit/TSH ratios, whereas this ratio tends to be less than one in patients with pituitary resistance to thyroid hormone (106). The measured α-subunit is usually expressed in ng/mL, whereas TSH is usually expressed in μU/mL. In order to determine the molar α-subunit/TSH ratio, one assumes a molecular weight for TSH of 28,000 Da, a molecular weight for α-subunit of 13,600 Da, and a specific activity for human TSH of 5 μU/ng (111). This results in a conversion factor of (28,000/13,600)/0.2 or approximately 10 (112). Therefore, [α-subunit (ng/ml)/TSH (μU/mL)] × 10 = molar α-subunit/TSH ratio. Computed tomography scan and magnetic resonance imaging studies of the pituitary region also can help to establish the diagnosis and to guide treatment.

Treatment for TSH-secreting adenomas consists of selective adenonectomy or radiotherapy, or a combination of the two (110,113). In the past, a brief course of antithyroid drugs was used to render the patient euthyroid prior to surgery. More recently, the somatostatin analogue, octreotide, has proven useful in the management of TSH-producing pituitary tumors (110,113). This drug normalizes thyroid hormone levels in most patients and causes a decrease in tumor size in some. However, because of tachyphylaxis, octreotide cannot be considered definitive treatment. The current approach to managing the TSH-producing pituitary tumor consists of achieving a euthyroid state with octreotide, followed by surgical resection of the tumor.

Treatment of patients with pituitary resistance to thyroid hormone is more difficult. Ideally, treatment should be aimed at reducing TSH secretion by the pituitary. A number of agents including L-T_3, D-T_4, bromocryptine, and triiodothyroacetic acid (Triac) have been advocated (106,110,114–116). To date, each agent has been used in a limited number of patients, and the overall efficacy of each has not been determined. Octreotide has not been useful in the long-term treatment of these patients (110). Atenolol, a β-adrenergic blocking agent that does not impair the peripheral conversion of T_4 to T_3, can be used to decrease the symptoms of hyperthyroidism (117). Although antithyroid drugs will reduce serum thyroid hormone levels, they will also increase TSH secretion and goiter size. Because prolonged TSH hypersecretion may lead to thyrotroph hyperplasia and potentially to the development of a TSH-secreting pituitary adenoma (118), prolonged treatment with antithyroid drugs is discouraged. Likewise, subtotal thyroidectomy and RAI therapy should not be used in patients with pituitary resistance to thyroid hormone.

SUBACUTE AND HASHIMOTO'S THYROIDITIS

Subacute or granulomatous thyroiditis is a self-limited, presumably viral inflammation of the thyroid gland. This entity is rarely seen in children, occurring more frequently between the third and fifth decades of life. Mild symptoms of thyrotoxicosis may occur, but they are often overshadowed by malaise, fever, and tenderness of the thyroid gland. The erythrocyte sedimentation rate is consistently elevated. Thyroid antibodies are usually negative early in the disease, but titers may rise transiently to abnormal levels during recovery. The thyrotoxic phase of this disease probably results from destruction of thyroid follicular cells with release of large amounts of preformed thyroid hormones.

The toxic thyroiditis of Hashimoto's disease (aka Hashitoxicosis) usually occurs early in the course of chronic lymphocytic thyroiditis and probably results from extensive autoimmune destruction of thyroid follicular cells. The child may present with mild symptoms of thyrotoxicosis and a slightly enlarged, sometimes tender, thyroid gland. Thyroid antibodies are usually positive. A recent review of eight children with Hashitoxicosis showed that the thyrotoxic phase ranged from 31 to 168 days; three of these children became hypothyroid after an average of approximately 46 days, and the other five became euthyroid after an average of approximately 113 days (119).

Laboratory evaluation of both disorders reveals elevated serum T_4, free T_4, and T_3 levels and undetectable TSH levels. The TSH response to TRH is either blunted or absent. The RAI uptake (RAI-U) is typically low or absent during the thyrotoxic phase of these disorders and helps to differentiate toxic thyroiditis from Graves' disease. A recent study has shown that certain hematological parameters and serum thyroid hormone levels can be useful in differentiating Graves' disease from subacute thyroiditis: an eosinophil to monocyte ratio (Eo/Mo) below 0.2 and/or an Eo/Mo multiplied by serum free T_3 (pmol/l) below 4.5 tend to support a diagnosis of subacute thyroiditis (120).

Treatment of these disorders is symptomatic. Antithyroid drugs are not indicated in the treatment, but β-adrenergic blockers can be used to relieve the symptoms of thyrotoxicosis in both. Iopanoic acid (500 mg daily or every other day) has been used to control clinical symptoms of thyrotoxicosis due to thyroiditis (121). The pain and tenderness of the thyroid gland may be relieved by therapeutic doses of salicylates, but on occasion, glucocorticoids may be required. These disorders have been discussed in detail in the chapters on thyromegaly and hypothyroidism (Vol. 2; Chaps. 17 and 19).

EXOGENOUS THYROID HORMONE

Thyrotoxicosis may result from the ingestion, usually chronic, of excessive quantities of thyroid hormone preparations (122). The term thyrotoxicosis factitia has been used to describe this situation. In children and adolescents, this ingestion may be surreptitious, iatrogenic, or accidental. Although therapeutic thyroid hormone preparations are the most obvious source, the clinician should keep in mind that ground meats and diet pills have reportedly been contaminated with large amounts of thyroid hormones and implicated in some patients with thyrotoxicosis factitia (122).

Although acute accidental or intentional overdoses of thyroid hormones can produce marked elevations in serum T_4 levels, the majority of children who take as much as 5 to 10 mg of L-T_4 in a single dose have few or no symptoms of thyrotoxicosis (123). When symptoms of thyrotoxicosis develop in these cases, they are usually mild and consist of fever, tachycardia, irritability, vomiting, diarrhea, and "hyperactive" behavior. Although more serious reactions such as seizures have been reported, these occur

very infrequently and several hours to days after the acute overdose (124,125). When preparations containing significant levels of T_3 have been ingested, the onset of symptoms is within 6 to 12 hours. The onset of symptoms following acute ingestion of L-T_4 is generally within 12 to 48 hours, but may be as late as 7 to 10 days after ingestion. The delayed onset of symptoms may be explained by the conversion of T_4 to its biologically more active metabolite, T_3. Serum levels of T_4 and/or T_3 following acute ingestion correlate poorly with development of toxicity (126). Because the majority of these cases are relatively benign and symptoms are absent or delayed, initial therapy should be limited to gastric decontamination with syrup of ipecac followed by activated charcoal and/or a cathartic (127). Some authorities also recommend cholestyramine as an initial adjunctive therapy because this agent binds thyroid hormones and reduces their enterohepatic circulation (126). Patients who have ingested thyroid hormone accidentally can then be followed closely at home pending the onset of symptoms. As in other accidental poisonings, the parents should be counseled on child safety measures. Only when symptoms develop should hospitalization or further treatment be considered. β-adrenergic blockade is helpful in controlling tachycardia as well as improving symptoms of nervousness, diaphoresis, or tremor. Iopanoic acid can be used to rapidly ameliorate clinical and hormonal parameters (121). Acetaminophen may be useful for control of fever. In the rare situation when a massive ingestion results in a life-threatening situation, exchange transfusion has been shown to effectively reduce serum thyroid hormone concentrations (126). Psychiatric evaluation may be indicated for patients with acute intentional overdoses.

Chronic ingestion of thyroid hormone preparations can produce symptoms similar to hyperthyroidism of thyroid origin. However, thyromegaly is not present unless the patient also has a coincident thyroid disease such as Hashimoto's thyroiditis. Likewise, infiltrative ophthalmopathy is absent; however, as in other causes of thyrotoxicosis, lid lag, and stare may be present. The diagnosis of this disorder is not difficult if the clinician is able to obtain a history of thyroid hormone ingestion. However, this history may be difficult to obtain, especially in cases of surreptitious ingestion. Nevertheless, the clinician should still be able to diagnose this disorder using a limited number of tests. Thyroid function test results will depend on the type of preparation responsible for the thyrotoxicosis. If the preparation is composed mainly of T_4, the patient will have elevated serum T_4 and free T_4 levels. If the preparation is T_3 or has a high T_3:T_4 ratio, the patient will have a low to normal serum T_4 level. In both cases, the serum T_3 level is elevated. The RAI-U is low to reflect the suppression of thyroid gland activity induced by exogenous thyroid hormone. Unlike all other causes of thyrotoxicosis, the plasma thyroglobulin level in this disorder is undetectable or extremely low. Therefore, the plasma thyroglobulin level may be extremely helpful in differentiating this disorder from other causes of thyrotoxicosis.

Treatment of thyrotoxicosis resulting from chronic ingestion of thyroid-hormone preparations should be guided by the circumstances surrounding ingestion. For example, patients receiving excessive replacement for treatment of hypothyroidism should have their dose reduced. The patient who is taking thyroid hormone surreptitiously should be advised to discontinue the medication; in some cases, psychotherapy may be necessary.

HUMAN CHORIONIC GONADOTROPIN–INDUCED HYPERTHYROIDISM

Because of structural homology between chorionic gonadotropin (CG) and TSH, and also between their respective receptors, CG possesses weak thyrotropic activity. Therefore, in certain clinical conditions associated with increased serum hCG levels, hyperthyroidism can occur. Although hCG-induced hyperthyroidism is a rare cause of clinical thyrotoxicosis, it has been reported in both female and male children (128–131).

The most common clinical condition associated with hCG-induced hyperthyroidism is hyperemesis gravidarum. Hyperemesis gravidarum complicates 1% to 2% of all normal pregnancies and appears to be more prevalent in Asian females than in Caucasians (132). It is characterized by prolonged and severe nausea and vomiting in early pregnancy that results in significant weight loss, dehydration, and ketosis. Abnormalities of electrolytes (hyponatremia, hypokalemia, and hypochloremic alkalosis) and liver function are often observed. Although there is a correlation between the degree of vomiting and the serum hCG level, the etiology of hyperemesis gravidarum remains unknown. The vomiting may be because of hCG itself or various other hormones; progesterone, estrogen, leptin, placental growth hormone, prolactin, thyroid, and adrenocortical hormones have all been implicated in the etiology (133). Management includes hospitalization, IV fluid and electrolyte replacement, thiamine supplementation, conventional antiemetics, and psychological support.

The syndrome of transient hyperthyroidism of hyperemesis gravidarum (also referred to as gestational hyperthyroidism) has been attributed to the thyrotropic action of hCG. Many, but not all, pregnant females with gestational hyperthyroidism have abnormally elevated serum levels of hCG. This indicates that other factors such as glycosylation of hCG contribute to the hyperthyroidism in pregnant females with normal serum hCG levels. In this regard, deglycosylated and/or desialylated isoforms of hCG have been shown to possess enhanced thyrotropic bioactivity (132). Gestational hyperthyroidism should be

considered in any sexually mature adolescent female presenting with prolonged vomiting and biochemical evidence of thyrotoxicosis. It is important to distinguish gestational hyperthyroidism from Graves' disease. The following are characteristic of gestational hyperthyroidism: thyrotoxic symptoms in early pregnancy, marked increase in serum free T_3 and free T_4, suppressed serum TSH, association with hyperemesis gravidarum, spontaneous disappearance in the latter half of pregnancy, negative thyroid antibodies (including TPO and TSH receptor antibodies), absent or small goiter, and circulating hCG with high thyrotropic bioactivity (132). Because most patients exhibit either no or only very mild symptoms of hyperthyroidism, antithyroid drug therapy is usually unnecessary. However, some patients will have frank clinical hyperthyroidism; under these circumstances, treatment with PTU and/or a β-adrenergic blocker is indicated, but rarely required beyond 22 weeks of gestation.

Recently, a familial form of gestational hyperthyroidism has been described (134). The patients (the proband and her mother) were heterozygous for a mutation (Lys183Arg) in the extracellular domain of the TSH receptor. Both the proband and her mother experienced hyperemesis gravidarum and hyperthyroidism during pregnancy, but remained euthyroid when not pregnant. During pregnancy, the proband did not have abnormally elevated serum hCG levels. Functional studies using COS-7 cells transfected with the human TSH receptor demonstrated that the mutant receptor was more sensitive to hCG than the wild-type receptor.

CG-induced hyperthyroidism has also been reported in patients with hydatidiform mole and choriocarcinoma. In the United States, hydatidiform mole occurs in 0.5 to 2.5/1000 pregnancies; prevalence is greatest in women below 15 or above 50 years of age (132). Hydatidiform moles secrete large amounts of hCG; the resultant serum hCG level is proportional to the mass of the tumor. In addition, hCG extracted from molar tissue contains less sialic acid than normal and possesses enhanced thyrotropic bioactivity. The prevalence of increased thyroid function in patients with hydatidiform moles ranges between 25% and 64%, and about 5% have clinical hyperthyroididism (132). In general, the development of hyperthyroidism requires abnormally elevated serum hCG levels ($>$200,000 IU/L) that are sustained over several weeks (132). The diagnosis is made by ultrasonography of the uterus that shows a "snowstorm" appearance without a fetus. Therapy consists of evacuation of the mole by suction curettage or removal by hysterotomy. This results in prompt reduction in serum hCG and thyroid hormone levels. Serum hCG levels should be monitored afterwards to detect either persistence of molar tissue or the development of choriocarcinoma.

Although it is extremely rare in adolescent females, gestational choriocarcinoma has been reported in this age group (135). Further, clinical hyperthyroidism has been reported in adolescent males and females with metastatic choriocarcinoma arising from gonadal tumors (129–131). In these reports, hyperthyroidism was associated with markedly elevated circulating hCG levels ($>$200,000 IU/L); the degree of hyperthyroidism in these children ranged from very mild to severe. Antithyroid drugs and/or β-adrenergic blocking agents should be used to treat clinical hyperthyroidism, until chemotherapy achieves control of the primary tumor and serum hCG levels normalize.

EUTHYROID HYPERTHYROXINEMIA

The term "euthyroid hyperthyroxinemia" is used to describe the various conditions in which the serum T_4 level, either total or free, is elevated in the absence of thyrotoxicosis. The causes are listed in Table 3 and can be divided into four major categories: increased T_4 binding by serum proteins, GRTH, impaired peripheral conversion of T_4 to T_3, and changes in thyroid stimulation associated with psychiatric illness.

Alterations in any of the serum thyroid hormone-binding proteins can produce elevations of the total T_4 level, but the free T_4 level remains normal. Increased thyroxine-binding globulin (TBG) concentration results from a variety of causes (Table 4) and produces concurrent elevations of the serum total T_4 and T_3 levels. Familial dysalbuminemic hyperthyroxinemia (FDH) is due to the presence of significant amounts of serum albumin with an unusually high affinity for T_4. Because this albumin typically binds T_3 only weakly, the serum T_3 level remains normal. FDH is inherited in an autosomal dominant fashion and is expressed equally in males and females. Increased serum concentration or binding affinity of thyroxine-binding prealbumin (TBPA), or transthyretin, can produce elevated serum total T_4 levels, but as in FDH, the serum T_3 level remains normal. The presence of endogenous antibodies directed against T_4 can produce either true or spurious elevations in serum total T_4 levels.

The serum free T_4 is normal in the disorders of protein binding when it is determined by equilibrium

Table 3 Conditions Causing Hyperthyroxinemia in the Absence of Thyrotoxicosis

Increased T_4-binding by serum proteins
Increased concentration of thyroxine-binding globulin
Familial dysalbuminemic hyperthyroxinemia
Increased T_4-binding by transthyretin
Anti-T_4 antibodies
Generalized (pituitary and peripheral tissues) resistance to thyroid hormone
Impaired conversion of T_4 to T_3
Pathophysiologic conditions (e.g., type I deiodinase deficiency and certain nonthyroidal illnesses)
Pharmacologic agents (e.g., amiodarone, propranolol, heparin, iodine contrast agents, amphetamines, L-thyroxine)
Changes in thyroid stimulation associated with psychiatric illness

Table 4 Factors Associated with Increased Thyroxine-Binding Globulin Concentration

Pregnancy
Neonatal state
Estrogens
Oral contraceptives
Acute intermittent porphyria
Infectious and chronic active hepatitis
Perphenazine
Genetic determination

dialysis or the two-step coated-tube method. Determination of the free T_4 by an analog-based "free T_4" method gives falsely high results in patients with FDH and endogenous anti-T_4 antibodies. This occurs because the variant albumin or anti-T_4 antibody in the serum readily binds the analog tracer used in these competitive immunoassays, and, thereby decreases the amount of tracer available to compete for the assay antibody. The low binding of tracer by the assay antibody gives the false impression of a high free T_4 concentration.

The free thyroxine (FT_4) index, as usually calculated from the resin T_3 uptake test, accurately reflects the FT_4 level only when increased T_4 binding is due to TBG excess. T_4 and T_3 share the same binding site on TBG. When the concentration of TBG is increased, the available binding sites for both T_4 and T_3 are increased. The resin T_3 uptake is inversely proportional to the number of available binding sites for T_3; that is, when the available serum binding sites for T_3 are increased, the resin T_3 uptake is decreased. The FT_4 index, when calculated as the product of the T_4 and the resin T_3 uptake, is usually normal in TBG excess because the elevated T_4 is offset by the decreased resin T_3 uptake. However, when increased serum T_4 binding results because of FDH or increased T_4-binding by TBPA or anti-T_4 antibodies, the resin T_3 uptake remains normal because none of these proteins bind significant amounts of T_3. Consequently, the FT_4 index values are spuriously elevated. Therefore, one should always consider the possibility of an abnormal T_4-binding protein when serum T_3 and resin T_3 uptake results are normal in the face of an elevated serum T_4 level. It should be emphasized that patients with elevated serum T_4 levels resulting from abnormal serum binding proteins are euthyroid, and no antithyroid treatment is indicated.

Generalized (pituitary and peripheral tissues) resistance to thyroid hormone (GRTH) is a rare disorder characterized by thyromegaly, elevated serum total and free T_4 and T_3 levels, a preserved TSH response to TRH, and absence of the usual symptoms and signs of thyrotoxicosis. Although this syndrome is probably congenital, it is rarely diagnosed at birth and more often recognized during childhood and adult life (136). In the majority of affected individuals, it is inherited in an autosomal dominant fashion, but recessive transmission has also been reported (136). The male-to-female ratio in GRTH is close to one. The tissue resistance to thyroid hormones is selective, and studies have shown that the pituitary thyrotrophs and peripheral tissue fibroblasts respond normally to dopaminergic drugs and/or glucocorticoids (137,138). Pituitary secretion of TSH is responsible for thyromegaly, increased thyroid gland activity, and excessive thyroid hormone synthesis and secretion seen in this syndrome. Although the serum TSH level may not always be elevated, it is always detectable, and administration of TRH produces a further increase in TSH levels. On the other hand, administration of supraphysiologic doses of exogenous T_3 suppresses pituitary secretion of TSH in virtually all affected patients.

The syndrome of GRTH results from mutations in one of the thyroid hormone receptor genes (108). There are two thyroid hormone receptor genes, *TRHB* and *THRA*, which are located on chromosomes 3 and 17, respectively (108,109). By alternative splicing of primary transcripts, these two genes code for four thyroid hormone (T_3) receptors (α1, β1, β2, and β3), and two proteins that do not bind T_3 (α2 and α3). Like the steroid hormone and retinoic acid receptors, thyroid hormone receptors consist of modular structures with ligand-binding and DNA-binding domains. About 85% of patients with GRTH harbor mutations in the T_3-binding domain of the *THRB* gene (139). About 100 different mutations in *THRB* have now been identified in patients from over 500 families; the mutations consist of either single amino acid substitutions at a single codon, single amino acid deletions, frameshift mutations, or truncations due to premature termination of translation from a mutation-generated stop codon (108,109). These mutations result in thyroid hormone receptors with defective T_3 binding. In some cases, the same mutations have been described in different families. The clinical phenotype can vary among the families that have the same mutation and also vary within a family. This suggests that there may be other genetic modifiers that determine the clinical phenotype (108). Patients who inherit this disorder in an autosomal recessive fashion have mutations in both alleles of the *THRB* gene. On the other hand, those patients who inherit GRTH in an autosomal dominant fashion have a wild-type allele, as well as a mutant allele for the receptor. These mutations are dominant negative in that the mutant receptors inhibit the function of the normal β-receptor (from wild-type allele) and the normal α-receptor (108). Recently, families with GRTH have been identified that have neither *THRB*- nor *THRA*-gene mutations (140). It has been proposed that either abnormal intracellular thyroid hormone transport, mutations in thyroid hormone receptor cofactors, or dysregulation of cofactor expression may be responsible for the GRTH phenotype in these families. Thus far, no defects have been identified in any of the several thyroid hormone cofactor genes that have been studied in these patients (141). To date, no germline

THRA-gene mutations have been described in humans with GRTH (109). Recent studies involving animal models of *THRA*- and *THRB*-gene mutations may help explain the reason for this (109). While knock-in mice harboring a mutation in the *Thrb*-gene locus ($Thrb^{PV/+}$) exhibit resistance to thyroid hormone, the phenotype of knock-in mice with the same mutation in the *Thra*-gene locus ($Thra^{PV/+}$) is markedly different. The $Thra^{PV/+}$ mice exhibit impaired growth and reduced fertility and survival, but they possess no apparent abnormalities in thyroid function. At present, the molecular basis of the differential action of these thyroid hormone receptor mutant isoforms remains unclear.

Despite the elevated levels of circulating thyroid hormones, most patients with GRTH are clinically euthyroid. Although symptoms and signs of hypo- or hyperthyroidism are generally absent, a few patients have been reported with retarded bone age, mental retardation, stunted growth, and hearing defects (142). Similarly, persistent tachycardia, tremor, anxiety, and hyperactivity have been observed in some patients (143). These findings suggest that the degree of resistance to thyroid hormone may not be the same in all tissues.

The diagnosis of GRTH requires elevated serum levels of T_4 and free T_4. Serum T_3 and reverse T_3 levels are also elevated. The TBG level is normal and the resin T_3 uptake is elevated. As mentioned above, serum TSH is always detectable, and the TSH response to TRH is either normal or exaggerated (105). The RAI-U is increased. Laboratory tests of metabolic status such as basal metabolic rate, serum cholesterol and triglycerides, and carotene are usually normal. Most patients with GRTH require no treatment, but resistance to thyroid hormone may vary from tissue to tissue. Some patients may benefit from treatment with pharmacologic doses of T_4 or T_3; this is especially true in cases where the peripheral tissues are more resistant than the pituitary thyrotrophs. Affected children should be monitored closely for growth deceleration, delayed bone maturation, and impaired mental development; and thyroid hormone treatment in supraphysiologic doses should be instituted as necessary. It is important to monitor the effects of thyroid hormone treatment by measuring not only TSH and free thyroid hormone levels, but also several indices of peripheral thyroid hormone action such as sex hormone-binding globulin, soluble interleukin-2 (IL-2) receptor, angiotensin converting enzyme, and bone GLA protein (139). Any therapeutic maneuvers that may reduce the elevated circulating thyroid hormone levels are contraindicated in GRTH and should be avoided.

Peripheral conversion of T_4 to T_3 occurs through the activity of 5′-deiodinase. A variety of pathophysiologic conditions and pharmacologic agents have been associated with impaired T_4 to T_3 conversion. The clinical syndrome of type I iodothyronine-deiodinase deficiency has been reported but appears to be extremely rare (144). The reported patient was clinically euthyroid and had elevated serum levels of T_4 and reverse T_3, along with normal serum T_3 and TSH levels (145).

Alterations in serum thyroid hormone levels often accompany nonthyroidal illnesses (Vol. 2; Chap. 25). Although the T_4 level is typically low or normal, on occasion it may be elevated. The euthyroid sick or nonthyroidal illness syndrome is discussed in the chapter dealing with hypothyroidism (Vol. 2; Chap. 17). Various drugs have been found to cause an elevation of serum T_4 levels in adults, but the majority of these agents are not commonly used in children and adolescents. Examples include amiodarone, propranolol, heparin, oral cholecystographic agents, and amphetamines.

Elevated serum T_4 levels are sometimes seen in clinically euthyroid children who are receiving replacement or suppressive therapy with L-T_4. In these children, the serum T_3 level is normal. The mechanism responsible for normal T_3 levels despite increased T_4 concentrations has not been completely defined, but may be explained by the fact that 5′-deiodinase activity in peripheral tissues appears to be autoregulated by the levels of circulating T_4. Thus, as the serum T_4 concentration increases from low to elevated levels, the peripheral generation of T_3 from T_4 decreases, as reflected in the steady decline in the serum T_3/T_4 ratio (122). Therefore, in patients receiving L-T_4 therapy, the serum T_3 level is better than the serum T_4 level as an indicator of metabolic status.

Mild elevations in serum T_4 levels are observed in about 20% of patients hospitalized for acute psychiatric disorders (146). This situation is most commonly observed in patients with mania, schizophrenia, and other major affective disorders, but is also occasionally seen in patients with alcoholism or personality disorder. Both total and free T_4 levels are elevated, and, in an occasional patient, serum T_3 is also mildly elevated (146). The serum TSH is usually normal-to-mildly increased at baseline, and this finding helps to differentiate this condition from the most common forms of thyrotoxicosis. Often, the TSH response to TRH is blunted. These biochemical findings do not appear to represent thyrotoxicosis, and they usually resolve spontaneously within a few weeks without specific therapy. It has been proposed that a decrease in central nervous system dopaminergic inhibition results in activation of the hypothalamic–pituitary axis with enhanced TSH secretion and consequent elevations in serum T_4 levels (147).

T_3 AND T_4 TOXICOSIS

Increased serum concentrations of both T_4 and T_3 are observed in the majority of children presenting with hyperthyroidism. However, some thyrotoxic children may present with an increased serum T_3 concentration but a normal or occasionally low serum T_4 concentration (i.e., T_3 toxicosis), while others may

present with an elevated serum T_4 concentration and a normal or slightly decreased T_3 level (i.e., T_4 toxicosis). Just as in the usual presentation of thyrotoxicosis, the serum TSH level is suppressed in both these situations.

T_3 toxicosis can occur in the course of any disorder that causes hyperthyroidism. Most patients have elevations in both total and free T_3 concentrations, but some will present with elevated free T_3 levels while total T_3 levels are still within the normal range (148,149). During childhood, T_3 toxicosis is most often encountered early in the course of either initial or relapsing Graves' disease or in association with an autonomous nodule. In these situations, T_3 toxicosis reflects a predominant hypersecretion of T_3 by the thyroid gland, rather than an increase in the peripheral conversion of T_4 to T_3 (150). If left untreated, some patients with T_3 toxicosis due to true hyperthyroidism, over time, will develop elevated serum concentrations of both T_3 and T_4. T_3 toxicosis is also seen in thyrotoxicosis factitia related to ingestion of liothyronine (L-T_3).

T_4 toxicosis occurs in two circumstances, iodine-induced thyrotoxicosis and thyrotoxicosis accompanied by severe intercurrent illness. With iodine-induced thyrotoxicosis, about one-third of patients have elevated serum T_4 but normal serum T_3 levels, and the remainder have proportionate elevations of serum T_3 and T_4 levels (150). In severe illness, peripheral conversion of T_4 to T_3 is impaired because of marked reductions in 5′-deiodinase activity. This accounts for the normal or low serum T_3 levels in the presence of abnormally elevated serum T_4 levels. Further, serum reverse T_3 (rT_3) levels are also increased because of the impaired 5′-deiodinase activity. With resolution of the intercurrent illness, 5′-deiodinase activity normalizes with subsequent declines in serum rT_3 levels and increases of serum T_3 levels into the thyrotoxic range. On a clinical level, T_4 toxicosis of this type needs to be distinguished from the low serum T_3/elevated serum T_4 levels that are occasionally observed in the euthyroid sick syndrome. The serum TSH level will be suppressed in T_4 toxicosis, and it may also be very low or suppressed in the euthyroid sick syndrome. Therefore, serum TSH measurement may not be helpful initially in distinguishing between these two conditions.

GRAVES' OPHTHALMOPATHY

Ophthalmic abnormalities are clinically evident in over half of the children and adolescents with Graves' disease. In most of these patients, the signs and symptoms are relatively mild and include lid lag, lid retraction, stare, proptosis, conjunctival injection, chemosis, and periorbital and eyelid edema. Less commonly, patients may complain of eye discomfort, pain, or diplopia. Severe ophthalmopathy, associated with marked chemosis, severe proptosis, periorbital ecchymosis, corneal ulceration, eye muscle paralysis, and optic atrophy, is extremely rare during childhood and adolescence. The clinical onset of eye disease usually coincides with that of thyroid dysfunction, but it can precede or follow it by several months to years (151).

Lid lag, lid retraction, and stare most commonly result directly from thyrotoxicosis with enhanced sympathetic stimulation of Müller's muscle of the upper lid. These features can be found in patients with thyrotoxicosis of any etiology and generally improve with normalization of thyroid hormone levels. The other signs and symptoms, however, are characteristic of Graves' ophthalmopathy (GO) and can be explained by the mechanical effects of an increase in tissue volume within the bony orbit. Histologic examination reveals accumulation of glycosaminoglycans (GAGs) in the connective-tissue components of the orbital fat and muscles, as well as lymphocytic infiltration of the orbital tissues. The GAGs are hydrophilic macromolecules produced by orbital fibroblasts, and their accumulation results in enlargement of the extraocular muscles and surrounding fat (152). Enlargement of these tissues within the fixed space of the bony orbit leads to forward displacement of the globe (proptosis or exophthalmos). Chemosis and periorbital edema result from decreased venous drainage from the orbit and intraorbital inflammation. Extraocular muscle dysfunction results from accumulation of GAGs, edema, inflammation, and fibrosis of the endomysial connective tissues investing the muscle fibers (152).

Although information regarding its pathogenesis is limited, GO is generally considered to represent an organ-specific autoimmune disorder. Current evidence supports that orbital fibroblasts are the primary targets of the autoimmune attack (153). However, the nature of the autoimmune reaction is unclear, and a target orbital autoantigen has not been conclusively identified. The close association of GO with autoimmune thyroid disease strongly suggests that the orbital antigen(s) may share unique structural characteristics with antigens of the thyroid gland. Recent studies support that two such candidate antigens, the TSH receptor (154) and thyroglobulin (155), are present in orbital tissues from patients with GO. Therefore, it is possible that either of these two proteins could be the primary target antigen in GO, thus providing a common link between the thyroid and eye diseases. Because the TSH receptor is the primary target antigen in Graves' hyperthyroidism, most investigators currently consider it to be the leading candidate target antigen in GO. Several human and animal studies have provided compelling, although not yet definitive, evidence (153,156) to support this role ofthe TSH receptor in GO. However, more studies are needed to determine which, if either, of these two proteins is the target autoantigen in GO. Thus far, thyroid peroxidase has not been detected in orbital tissues (151).

Cell-mediated immunity appears to play a major role in the pathogenesis of GO. The extraocular

muscles and orbital connective tissues are infiltrated by lymphocytes and macrophages. The lymphocytes are predominately $CD4^+$ and $CD8^+$ T-cells with a few B-cells. Regardless of the target antigen that causes the lymphocytic infiltration, the proximal events in the pathogenesis of GO appear to be cytokine-mediated activation of orbital fibroblasts, secretion of GAGs by these cells, and ultimately, fibrosis. Immunohistochemical studies have demonstrated the presence of the cytokines, interferon-gamma, tumor necrosis factor-α, and IL-1α, in the cytoplasm of orbital-infiltrating mononuclear cells and in adjacent orbital connective tissue from patients with early active GO (157). These findings support that T-cells and antigen-presenting cells within these tissues are activated. Because transplacental passage of maternal thyroid-stimulating antibodies does not appear to cause infiltrative ophthalmopathy in neonates, and the presence of antibodies to orbital antigens is inconsistently related to eye disease, humoral autoimmunity appears to play at most a secondary role in the pathogenesis of GO (15).

Because ophthalmopathy is relatively mild and self-limited in the vast majority of affected children and adolescents, specific treatment is usually not necessary. In general, eye findings improve in association with control of the hyperthyroidism. Occasionally, local measures may be used to treat symptoms. For example, eye drop or ointment preparations containing methylcellulose may be necessary to prevent corneal drying. Sleeping with the head elevated may help reduce chemosis and periorbital edema. Other forms of treatment such as oral corticosteroids, orbital irradiation, and surgical decompression are rarely indicated in children and should be reserved for those with severe ophthalmopathy.

LABORATORY EVALUATION

The laboratory evaluation of thyrotoxicosis should be guided by the patient's clinical presentation as determined by the medical history and physical examination. In all causes of thyrotoxicosis, except for TSH-induced hyperthyroidism, the serum TSH level will be undetectable or very suppressed using modern second- or third-generation TSH assays. For the child/adolescent presenting with obvious signs and symptoms of Graves' disease, including a soft, diffusely enlarged, smooth goiter and proptosis, only a few laboratory tests are needed. In addition to the undetectable serum TSH, an elevated free T_4 (or free T_4 index) and the presence of TSHrAb (either TBII or TSI) substantiate the clinical diagnosis. When antithyroid drugs are selected as therapy, a baseline complete blood with differential white blood count should be obtained, for leukopenia occurs in untreated thyrotoxicosis and granulocytopenia is an occasional toxic reaction to antithyroid drugs.

In less severe presentations, however, further laboratory tests may be necessary. Serum T_3 levels will be elevated in nearly all patients with Graves' disease. Measurement of serum total and/or free T_3 levels can be useful in the occasional patient with early Graves' disease who presents with an undetectable TSH but a normal serum free T_4 level.

Table 5 Classification of Thyrotoxicosis by RAI-U

RAI-U usually elevated
Graves' disease
Toxic adenoma
Toxic multinodular goiter
Familial nonautoimmune hyperthyroidism
Thyrotropin-induced hyperthyroidism
Trophoblastic disease
RAI-U typically low
Subacute thyroiditis
Toxic thyroiditis of Hashimoto's disease
Thyrotoxicosis factitia
Iodine-induced hyperthyroidism
Metastatic thyroid carcinoma

Abbreviation: RAI-U, radioiodine uptake.

For the patient who presents with symptoms of thyrotoxicosis and a firm, mildly tender, asymmetric goiter, the RAI-U (Table 5) can differentiate Graves' disease from either the toxic thyroiditis of Hashimoto's disease or subacute thyroiditis (158,159). Further, the RAI-U also can help distinguish Graves' disease from thyrotoxicosis factitia.

Graves' disease is the most common cause of thyrotoxicosis during pregnancy (4). However, in the pregnant adolescent female with mild symptoms of thyrotoxicosis and a normal-to-slightly enlarged thyroid gland, the possibility of hCG-mediated hyperthyroidism should be considered. Biochemical and clinical hyperthyroidism can occur when serum hCG levels exceed 100,000 to 300,000 IU/L (4).

TSHrAb and autoantibodies to thyroglobulin and/or thyroid peroxidase are present in the majority of patients with Graves' disease and reflect the autoimmune nature of the disorder. However, none of these autoantibodies is specific to Graves' disease. While almost all hyperthyroid patients with serum TSHrAb will have Graves' disease, these antibodies also can be detected in patients with Hashimoto's thyroiditis (35,53,54) and in patients with subacute thyroiditis (160). Thyroglobulin and/or thyroid peroxidase antibodies are present in the majority of patients with Hashimoto's thyroiditis (161) and have been reported in several patients with subacute thyroiditis (160,162) and a few patients with FNH (102). Therefore, by themselves, none of these antibodies should be considered as absolute proof of Graves' disease in patients with thyrotoxicosis.

Several studies have suggested that the recently cloned and characterized Na^+/I^- symporter (NIS) may represent an important autoantigen in Graves' disease (163). Although earlier studies (based on relatively small sample sizes) suggested that NIS autoantibodies might be present in up to 60% to 80% of Graves' sera (163), a more recent study evaluating

177 Graves' sera found these antibodies in only 5% to 10% of the samples (164). Further, NIS antibodies also are present in 15% to 20% of sera from patients with Hashimoto's thyroiditis (163,164). At present, the functional roles, if any, that these antibodies play in either Graves' disease or Hashimoto's disease remains unclear. Although assays are not yet available for routine clinical use, based on current data, the measurement of NIS antibodies does not appear to offer any additional diagnostic benefit for patients with Graves' disease.

More detailed discussions of clinical features and laboratory studies that can be used to evaluate patients with other causes of thyrotoxicosis and to differentiate these disorders from Graves' disease are presented in the appropriate subsections discussed above.

PROGNOSIS AND TREATMENT

Introduction and Overview

The clinical course of Graves' disease is variable and unpredictable. However, the hyperthyroidism in untreated Graves' disease usually persists and progresses unless the thyroid gland has limited responsiveness as a result of coexisting chronic lymphocytic thyroiditis. Therefore, therapeutic intervention is recommended for all patients with active Graves' disease. Despite recent advances in our knowledge of the TSH receptor and TSHrAbs, none of the currently available treatments is specifically directed against the underlying immunological abnormality that causes Graves' disease. The three acceptable methods of therapy (antithyroid drugs, RAI ablation, and subtotal/total thyroidectomy) merely interrupt the disease process at the level of the thyroid gland, although treatment with thionamides has been reported to reduce levels of TSHrAb (165).

The treatment of Graves' disease in children and adolescents remains controversial. While all three therapeutic modalities represent effective treatments for Graves' hyperthyroidism, each has specific advantages and disadvantages that should be addressed when individual treatment plans are developed for affected individuals. The antithyroid drugs are generally well tolerated, their inhibitory effects on the thyroid are completely reversible, and some patients treated with them will achieve a long-term or permanent remission (166). Therefore, antithyroid drug treatment will allow some children to avoid surgery or exposure to RAI. However, antithyroid drugs usually take four to eight weeks to initially control hyperthyroidism, and a treatment period of several years is typically required to achieve a long-term remission. During this prolonged treatment period, noncompliance and drug toxicity (Table 6) can complicate patient management. Further, relapse of hyperthyroidism frequently occurs following discontinuation of therapy (166). As yet, there are no reliable clinical, biochemical, immunological, or genetic factors that have been identified that allow absolute prediction of those patients likely to do well, or poorly, in achieving a long-term remission with antithyroid drug therapy (167).

Table 6 Toxic Side Effects of Antithyroid Drug Therapy

Elevated liver enzymes	Nausea, abdominal discomfort
Granulocytopenia	Edema
Dermatitis, urticaria	Conjunctivitis
Arthralgia, arthritis	Thrombocytopenia
Lupus-like syndrome	Hypoprothrombinemia
Lymphadenopathy	Toxic psychosis
Peripheral neuritis	Sensorineural hearing loss
Fever	Loss of taste sensation
Hepatitis	Disseminated intravascular coagulation
	Pruritus

RAI represents the easiest form of treatment, and the majority of patients can be successfully treated with a single oral dose (166). However, RAI therapy is absolutely contraindicated during pregnancy and breast-feeding (167). Although hospitalization is not required, patients receiving RAI are usually advised to limit close contact with others and properly dispose of their urine for several days following treatment. RAI therapy is also slow to control hyperthyroidism; it usually takes 6 to 18 weeks for RAI to have its full effects on the thyroid. Some patients may require multiple doses of RAI to adequately treat their disease. Although RAI is generally well tolerated, radiation thyroiditis may occur. This is characterized by a transient increase in serum thyroid hormone levels with, occasionally, a worsening of hyperthyroid symptoms and thyroid gland tenderness. With ablative doses, the thyroid gland will shrink and hypothyroidism will develop in the majority of patients. Rarely, parathyroid dysfunction may develop after RAI therapy (168). Concerns regarding the potential long-term carcinogenic and genetic risks of RAI in children/adolescents continue to linger (166).

Surgical therapy represents the most rapidly effective form of treatment. Following at least 10 to 14 days of preoperative preparation with antithyroid drugs, stable iodine (e.g., SSKI or Lugol's solution), and β-adrenergic blockers; elective surgery consisting of either subtotal or total thyroidectomy can be performed. In clinical situations where more rapid preoperative control is necessary, a regimen of iopanoic acid, dexamethasone, and a β-adrenergic blocking agent, with or without a thionamide, can be used; this regimen has been shown to render patients with severe Graves' disease clinically euthyroid and ready for surgery in approximately one week (169). Both subtotal and total thyroidectomies are complicated procedures, and the long-term cure rates and the incidence of complications depend, in large part, on the skill and experience of the surgeon. Due to the continued reliance on antithyroid drugs and the increasing acceptance of RAI as primary therapies for juvenile Graves' disease, clinicians now infrequently

recommend surgery for children with Graves' disease. However, clinical indications for surgical therapy still exist. These include the patient with a very large goiter; the patient who fails medical treatment and refuses RAI; the very young patient (i.e., less than five years of age) who fails medical management; the pregnant patient with moderate to severe hyperthyroidism uncontrolled by medical treatment; and the patient with Graves' disease who develops a solid ''cold'' thyroid nodule that raises the suspicion of thyroid carcinoma (15,166–168,170). Hypothyroidism occurs in 60% to approximately 100% of children undergoing subtotal and total thyroidectomy, respectively (166). Hyperthyroidism recurs in 10% to 15% of patients following subtotal thyroidectomy, but in less than 3% of children who undergo total thyroidectomy (166). Potential surgical complications include pain, hemorrhage/hematoma, transient hypocalcemia, transient hoarseness, temporary tracheostomy, permanent hypoparathyroidism, vocal cord paralysis, keloid formation, and death (166). Because complication rates are comparable for total and subtotal thyroidectomy, relapse rates are higher from subtotal thyroidectomy, and subtotal thyroidectomy is associated with a high rate of hypothyroidism, many thyroid surgeons now recommend total/near-total thyroidectomy as the procedure of choice in patients requiring surgical therapy for Graves' disease (171,172).

Regardless of the modality that is ultimately used for the treatment of Graves' hyperthyroidism, patients will require long-term regular medical follow-up. Thyroid function studies will need to be monitored on a regular basis to aid in the maintenance of the euthyroid state. Patients will need to be treated and/or observed for other associated autoimmune disorders that may potentially accompany Graves' disease. Patients also will need to be monitored for the potential occurrence of thyroid tumors and any long-term consequences that may be associated with the preceding treatment(s) for hyperthyroidism. While it is reassuring that correction of hyperthyroidism appears to normalize musculoskeletal and cardiac manifestations of Graves' disease (64), recent studies in adults show that despite successful treatment of Graves' hyperthyroidism and a return to the euthyroid state, some patients will continue to exhibit symptoms of physical, emotional, and neuropsychological illness (173,174). Anxiety, depression, lack of energy, sleep disturbance, emotional lability, impaired memory, and forgetfulness are some of the difficulties experienced by patients who had remained euthyroid for more than a year (173). Other studies have demonstrated cognitive deficits in remitted hyperthyroid adults (175,176). It has been proposed that these impairments represent residual sequelae of elevated thyroid hormone levels on the brain (173). Although similar studies involving children/adolescents have not been published, it is highly likely that some previously hyperthyroid children and adolescents, despite a return to euthyroidism, also suffer from these, or similar, problems. Therefore, as part of the regular medical follow-up, previously hyperthyroid children and adolescents should be monitored for behavioral, emotional, and learning or other neuropsychological problems; and appropriate counseling and support services should be offered as necessary.

Discussions regarding specific treatments for other causes of thyrotoxicosis are presented in the appropriate subsections discussed previously.

β-Adrenergic Blocking Agents

From the preceding discussion, it is clear that each of the currently acceptable treatment options carries a lag time between the onset of therapy and the control of hyperthyroidism. This lag time ranges from as short as 10 to 14 days for elective surgery to as long as several weeks to months for both antithyroid drug and RAI treatments. β-adrenergic blockade with either propranolol or atenolol represents an important and very effective adjunctive therapy for the rapid control of adrenergic symptoms during the thyrotoxic course of the disease. These drugs should not be used alone for long-term management of hyperthyroidism in children because they do not significantly affect thyroid hormone secretion or correct the abnormal increases in metabolic rate and oxygen consumption (177). However, both propranolol (10–20 mg every six to eight hours) and atenolol (25–50 mg once or twice daily) are effective in controlling the distressing symptoms and signs of restlessness, tachycardia, heat intolerance, tremor, hyperhidrosis, diarrhea, and myopathy. In addition, propranolol (but not atenolol) reduces conversion of T_4 to T_3 by inhibiting the 5′-deiodination pathway, thus producing some decrease in serum T_3 levels (177). The doses of these medicines should be adjusted over time to return the pulse rate to normal. β-adrenergic blocking agents should be avoided or used very cautiously in patients with a history of asthma, hypoglycemia (including patients with insulin-dependent diabetes mellitus), heart block, or heart failure (178). Atenolol, in relatively small doses, represents the safer choice in hyperthyroid patients with a history of asthma and hypoglycemia because it is more cardioselective (i.e., β_1-adrenergic receptor specific) than propranolol (178). Neither drug should be discontinued abruptly because symptoms and signs of hyperthyroidism may acutely worsen. Instead, the dose should be decreased gradually over several days before stopping.

Other Adjunctive Therapeutic Agents

In addition to β-adrenergic blocking agents, a number of other agents are available as adjunctive therapies for patients with Graves' disease. None of these medications alone is intended for the long-term management of Graves' disease. Instead, these agents should be reserved for short-term management of specific situations that may arise during the course of Graves' disease. Among the agents to be considered

here are iodide preparations, iopanoic acid, lithium, glucocorticoids, cholestyramine, and l-carnitine.

The iodide preparations include Lugol's solution (8 mg iodide/drop) and saturated solution of potassium iodide (SSKI, 35–50 mg potassium iodide/drop) (179). When given acutely, excess iodide rapidly blocks the release of T_3 and T_4 from the thyroid gland and suppresses the activity of thyroid peroxidase, thus decreasing iodide oxidation and organification (Wolff-Chaikoff effect) and synthesis of new thyroid hormone (179). Iodide preparations also decrease the vascularity of the thyroid gland. However, the effects of iodides are short-lived, and the thyroid gland will escape inhibition within days to a few weeks. Therefore, prolonged use (>10–14 days) of iodides in patients with Graves' disease potentially can result in exacerbation of hyperthyroidism. Because of this, iodide preparations should be reserved for temporary treatment of patients with severe thyrotoxicosis and for patients being prepared for thyroidectomy. When used in patients with severe thyrotoxicosis, iodides should be given at least one hour after the first dose of PTU or methimazole (MMI) because iodides potentially can increase intrathyroidal hormone stores by providing more substrate for thyroid hormone synthesis. The dosage of either preparation during management of severe thyrotoxicosis/thyroid storm is four to eight drops orally every six to eight hours (180). When iodides are used along with PTU or MMI, serum T_4 levels fall rapidly and approach normal levels within four to five days (180). In patients undergoing elective thyroidectomy, iodides are generally added to the antithyroid drug regimen 10 to 14 days before the scheduled surgery; the usual doses of Lugol's solution and SSKI are three to five drops and one drop, respectively, orally three times daily. It should be recognized that iodide therapy impairs thyroidal RAI uptake and may preclude the use of RAI therapy for several weeks to months (180).

Iopanoic acid (Spectrum Chemical Mfg. Corp, Gardena, CA) is an oral cholescystographic agent that has multiple effects on thyroid hormone metabolism (121,181). It is a potent inhibitor of types I and II deiodinase and effectively blocks conversion of T_4 to T_3. In addition, the metabolism of iopanoic acid releases large amounts of inorganic iodine into the circulation (1 g of iopanoic acid contains 650 mg iodine); and this rapidly suppresses thyroid hormone synthesis and release (181). Iopanoic acid can also reduce the disposal rate of T_4 by inhibiting hepatic uptake of T_4 as well as displacing T_4 from hepatic-binding sites and plasma proteins. When used in patients who are thyrotoxic, iopanoic acid can achieve rapid reduction of thyroid hormone levels; serum T_3 levels can decrease 36% to 77% within 6 to 12 hours and normalize within two to five days (181). In patients with Graves' disease, serum T_4 levels fall more modestly, approximately 20% in the first 24 hours; and may or may not reach normal values within one to two weeks (181). Iopanoic acid has been used effectively in adult, childhood, and neonatal Graves' disease, as part of a preoperative regimen for thyroidectomy, in subacute thyroiditis, and in massive levothyroxine overdose (121,181). The reported treatment doses for iopanoic acid vary widely and depend on the clinical situation. Initial doses for severe thyrotoxicosis/thyroid storm are usually one gram orally every eight hours for the first 24 hours, and then 500 mg orally twice daily. In other clinical situations involving children and adults, daily doses of 500 to 1000 mg are usually effective. In neonatal Graves' disease, a dose of 250 to 500 mg orally every third or fourth day has been shown to be effective (182). Potential adverse effects include rash, thrombocytopenia, headache, nausea, vomiting, diarrhea, dysuria, uricosuria, and renal insufficiency (181). Iopanoic acid and iodide preparations should be avoided in patients with a history of iodine-induced anaphylaxis. Long-term therapy of Graves' disease with iopanoic acid alone is ineffective because a significant number of patients either do not respond to treatment or experience relapse; escape from the inhibitory effects of iopanoic acid occurs after 2 to 12 weeks (121,181). Other potential drawbacks to prolonged use of iopanoic acid in Graves' disease are related to its high iodine content and include worsening of hyperthyroidism and development of resistance to PTU and MMI (121,181). Therapy with iopanoic acid may also preclude subsequent RAI ablation therapy for several weeks to months, although return of ^{131}I-uptake can occur as early as one to two weeks after discontinuation of its use (121,181). A recent study in adults demonstrated that a seven-day course of iopanoic acid (500 mg/day) rapidly ameliorated hyperthyroidism and did not jeopardize subsequent RAI therapy; above 90% of these patients were able to undergo RAI therapy two weeks after stopping iopanoic acid, and the outcome of RAI therapy was in no way different than the outcome observed in patients who had been prepared with a six-week course of carbimazole (30–40 mg/day) therapy (183).

Although the exact mechanism of action remains unknown, lithium possesses antithyroid effects. Lithium appears to act similarly to iodide; it is taken up and concentrated by the thyroid gland and appears to inhibit the release of thyroid hormones (179). Like iodide, the antithyroid effects of lithium are transient because the thyroid tends to escape from its inhibitory actions. For this reason and because of the risk of significant adverse effects associated with the drug, long-term lithium therapy for Graves' disease is not recommended. However, lithium has been used in the treatment of thyroid storm, as part of a preoperative regimen for thyroidectomy, and as adjunctive therapy to prevent the rise of serum thyroid hormones following RAI therapy (180,184,185). Lithium can be used in patients with a history of iodine-induced anaphylaxis. The usual dose of lithium carbonate for treatment of these clinical situations is 300 mg orally every six to eight hours (180).

When used to prevent the rise of serum thyroid hormones following RAI therapy, lithium should be started three to five days before and continued for 10 to 14 days after administration of RAI (186). Significant nausea may develop in some patients late in the course of lithium therapy; this may be relieved by decreasing the dose of lithium. Although some investigators report that adjunctive lithium treatment increases the effectiveness of RAI therapy (184), others have been unable to demonstrate this (187).

In pharmacologic doses, glucocorticoids reduce T_4 to T_3 conversion in peripheral tissues due to an inhibitory effect on 5′-deiodination. In addition, they can also reduce serum concentrations of T_4 in patients with Graves' disease by reducing T_4 secretion either by a direct thyroidal effect or by lowering the production of TSI (188). Glucocorticoids have been used in the management of severe thyrotoxicosis/thyroid storm, in the short-term management of patients with poorly controlled Graves' disease, and in regimens designed to rapidly prepare thyrotoxic patients for thyroidectomy (169,180,189). Glucocorticoid therapy is also useful in the management of type 2 amiodarone-induced thyrotoxicosis, which is a form of destructive thyroiditis (11,12). Dexamethasone, betamethasone, prednisolone, and prednisone have all been employed in the management of thyrotoxicosis. In severe thyrotoxicosis, dexamethasone or betamethasone can be administered intravenously in doses ranging from 0.5 to 1 mg every 6 to 12 hours. In less acute situations, prednisone or prednisolone can be started orally in doses of 20 to 40 mg/day. Glucocorticoid therapy does not appear to influence the effect of RAI therapy in Graves' disease (190). The duration of pharmacologic glucocorticoid therapy should be limited to less than 10 to 14 days, if possible, to reduce the risk of significant adverse effects and adrenal suppression. Glucocorticoid therapy should be avoided in patients with systemic fungal infection or hypersensitivity to the drugs (191).

Cholestyramine, an ionic exchange resin, effectively binds thyroid hormone and prevents its intestinal absorption. Because of this, cholestyramine has been used in the management of acute overdoses of thyroid hormone (126). In addition, cholestyramine has been used as adjunctive therapy in patients with Graves' disease to hasten normalization of elevated thyroid hormone levels (192,193). In this situation, cholestyramine works by interfering with the enterohepatic circulation of thyroid hormone. Metabolism of thyroid hormones includes conjugation to glucuronides and sulfates in the liver, and these conjugates are excreted in the bile. Once the bile enters the intestine, free thyroid hormones are released and are reabsorbed into the blood stream. An enterohepatic circulation forms as a result. Thyrotoxic states are characterized by an increased enterohepatic circulation of thyroid hormones. Cholestyramine interferes with the intestinal reabsorption of thyroid hormone and thus reduces the enterohepatic circulation of thyroid hormone. When combined with either PTU or MMI therapy for two to four weeks, cholestyramine significantly hastens the fall in serum T_4 and T_3 levels in patients with Graves' disease (192,193). The usual dose of cholestyramine is 4 g orally two or three times daily. Treatment tends to be well tolerated; potential side effects are minor and include constipation, abdominal discomfort, heartburn, nausea, and transient elevations of serum aminotransferase levels.

L-carnitine (β-hydroxy-γ-trimethylammonium butyrate) is a quarternary amine that is ubiquitous in biological fluids and tissues of mammals, where it plays an important role in energy metabolism. In vitro studies have demonstrated that L-carnitine inhibits the entry of T_4 and T_3 into cell nuclei; therefore, it is a naturally occurring inhibitor of peripheral thyroid hormone action (194). Its cellular levels are decreased in several disease states, including hyperthyroidism (195). Therefore, in patients with thyrotoxicosis, the rationale for carnitine use appears to be twofold: to replenish tissue stores and to counteract thyroid hormones in the periphery. In a controlled trial of iatrogenic hyperthyroidism, L-carnitine therapy both prevented and reversed hyperthyroid symptoms (196). Reports of L-carnitine therapy in patients with naturally occurring hyperthyroidism are currently limited; but it has been used alone or in combination with conventional antithyroid drugs to relieve symptoms of thyrotoxicosis in patients with subacute and postpartum thyroiditis, Hashitoxicosis, autonomous thyroid nodule, and Graves' disease (197). Clinical improvement of symptoms (nervousness, tremors, tachycardia, palpitations, insomnia, etc.) generally occurs one to two weeks after starting L-carnitine therapy (196,197). Because it has no toxicity, teratogenicity, known contraindications, or interactions with drugs, it can be used for several weeks or months. In patients with Graves' disease, L-carnitine therapy may be useful for the control of hyperthyroid symptoms both during the initial weeks of thionamide treatment and following RAI therapy. The recommended oral dosage for children is 50 to 100 mg/kg/day in divided doses. For adolescents and young adults, the oral dosage is one to four grams daily in two divided doses. Potential side effects include transient nausea and vomiting, abdominal cramps, diarrhea, and body odor. Seizures have been reported to occur in patients with or without preexisting seizure activity receiving oral carnitine (198).

Antithyroid Drug Therapy

The antithyroid drugs for long-term therapy of children in the United States include PTU and MMI. In addition to these two agents, a MMI derivative, carbimazole, is available in Europe (199). These drugs block the incorporation of oxidized iodide into tyrosine residues of thyroglobulin by serving as substrates for thyroid peroxidase. The drugs are iodinated and degraded within the gland, thus diverting oxidized iodide away from thyroglobulin (199). Further, they

block the coupling of iodotyrosyl residues in thyroglobulin to form T_4 and T_3. They do not interfere with the thyroid gland's ability to concentrate iodide, nor do they block the release of stored thyroid hormone into the circulation (199). Because they do not block the release of preformed, stored thyroid hormones, most patients will require four to eight weeks of antithyroid drug therapy before a euthyroid state is achieved. PTU, but not MMI or carbimazole, also inhibits the peripheral conversion of T_4 to T_3 (199). Because of this, many clinicians tend to use PTU initially in patients with more severe hyperthyroidism.

In addition to their direct effects on thyroid hormone synthesis, the thionamides also may have immunosuppressive activity. Several investigators have reported significant reductions in circulating TSHrAbs during antithyroid drug treatment (165,200). Some studies suggest that thionamides have direct effects on thyroid autoantibody-producing lymphocytes, whereas others suggest that these drugs primarily act by reducing the antigenicity of thyrocytes (200).

MMI has a longer half-life (12–16 hours vs. 4–6 hours), and, on a weight basis, is about 10-fold more potent than PTU (166). To initially control hyperthyroidism, PTU is given every six to eight hours, whereas MMI can be given every 8 to 12 hours; after three to four weeks of therapy, dosing of MMI usually can be changed to once or twice a day (166,167). For patients with moderate-to-severe hyperthyroidism, the recommended starting doses for PTU and MMI are 5 to 10 mg/kg/day and 0.5 to 1.0 mg/kg/day, respectively. In patients with mild hyperthyroidism, smaller doses of antithyroid drugs may be effective. After starting antithyroid drug therapy, follow-up testing of thyroid function every four to six weeks is recommended, at least until thyroid function is stable or the patient becomes euthyroid. It should be recognized that serum TSH levels may remain suppressed for several weeks or months despite normalization of thyroid hormone levels. Therefore, serum TSH alone is a poor early measure of control. Further, some patients continue to have elevated serum T_3 levels despite normal or even low T_4 or free T_4 levels, indicating the need to increase, not to decrease, the dose of antithyroid drug (201).

Once clinical and biochemical euthyroidism have been achieved, maintenance therapy may proceed by either of two methods: (i) reduce the dosage by one-third to one half to maintain thyroid hormone levels in the normal range (titration regimen), or (ii) continue the initial therapeutic dosage to induce hypothyroidism, and initiate replacement L-thyroxine therapy (block-replace regimen). The block-replace method is preferred by many clinicians for children because euthyroidism seems easier to maintain, and it involves fewer clinic visits for monitoring of thyroid function. However, the titration method is associated with fewer adverse effects (202). Although a study from Japan demonstrated that the combined use of antithyroid drugs and L-thyroxine decreased the incidence of relapse in adult patients (203), several recent studies from around the world have been unable to reproduce this finding (204–206).

After one to three years of therapy, the medication is either slowly tapered or a T_3 suppression of serum T_4 using exogenous doses of oral T_3 (1.5 μg/kg/day in three divided doses) can be performed (53). The administration of exogenous T_3 for three weeks to normal children or patients in remission with Graves' disease will cause a decrease in the serum T_4 concentration to values below the normal range. The test is performed as follows:

1. While the patient is still receiving the antithyroid drug, T_3 is started at the dosage listed above. L-thyroxine therapy must be discontinued in those patients on combined therapy.
2. Three weeks later, serum T_4 and free T_4 levels are evaluated.
    a. If the results are normal or elevated, antithyroid drug therapy is continued.
    b. If the results are below normal, discontinue the antithyroid drug and exogenous T_3 therapy.
3. One or two weeks later, repeat the serum T_4 values.
    a. If normal or elevated, resume antithyroid drug therapy.
    b. If the serum T_4 values are low, discontinue T_3 therapy and monitor serum T_4 and T_3 levels at three-month intervals for the next year and annually thereafter. If relapse occurs, antithyroid therapy may be resumed, or the patient may be offered the choice of surgical or RAI therapy (13).

In adults, the presence of TSHrAb during antithyroid drug therapy may be associated with clinical relapse of disease on termination of therapy (46). Studies have shown that patients (adults and children) with negative TSHrAb values during antithyroid drug therapy remain in remission after cessation of therapy (53,207). One study in children with Graves' disease compared TSHrAb values and the clinical course with results of the T_3 suppression tests (53). TSHrAb values correctly predicted the subsequent clinical course in 72%, and the T_3 suppression tests accurately predicted the course in 64% of the patients. Furthermore, the TSHrAb values and the T_3 suppression tests were in agreement 75% of the time. This study suggests that TSHrAb values are as effective as T_3 suppression tests in determining when antithyroid drug therapy can be discontinued. However, both tests are limited in their ability to accurately predict the clinical course of the disease, and patients will continue to require periodic clinical evaluations and laboratory assessment after discontinuation of antithyroid drug therapy.

The major disadvantages of antithyroid drug therapy are prolonged duration of therapy required to achieve a long-term remission, high relapse rate

following cessation of therapy, and the risk of toxic side effects (13,166,199). In children, long-term remission rates are at best 50% to 60% after several years of drug therapy and are usually less than 20% to 40% (166,208). Furthermore, remission rates following antithyroid drug therapy are considerably less in prepubertal than in pubertal children (209). A recent study has demonstrated that the severity of hyperthyroidism at diagnosis, as determined by multiple clinical and laboratory variables, is important in predicting remission within two years of therapy (210). Specifically, this study found that within two years of treatment, patients with a minimal/small goiter and a body mass index score above –0.5 SD at time of initial diagnosis had a probability of 86% of achieving remission for at least six months, compared with only 13% for those with a moderate/large goiter and BMI below –0.5 SD (210). Other studies have shown that high levels of TSI at the time of diagnosis are associated with decreased long-term remission rates (211,212). A recent study showed that elevated serum IgE levels following 18 months of MMI therapy is associated with lower rates of remission (213). Although none of these factors can predict with absolute certainty which patients will achieve long-term remission with antithyroid drugs, they may be helpful in selecting specific treatment plans for individual patients.

Side effects of antithyroid drugs occur in up to 20% to 30% of treated children. The spectrum of side effects ranges from mild to potentially life-threatening or even lethal complications. The various side effects may be either idiosyncratic or dose-related (166). Side effects associated with MMI tend to be dose-related, whereas those of PTU are less clearly related to dose (199). The majority of side effects is mild and includes elevated liver enzyme levels, mild leukopenia, skin rashes, and mild gastrointestinal symptoms such as nausea. In the case of mild side effects, complications may be transient or may resolve after switching to an alternative antithyroid drug. Minor cutaneous reactions such as pruritus and hives may resolve when an antihistamine is added while drug therapy is continued. Fortunately, more serious side effects are either uncommon or rare. When a patient reports any serious toxic effect with antithyroid drugs, therapy must be stopped immediately, and the patient should be evaluated. The occurrence of serious side effects necessitates discontinuation of all antithyroid drugs, and an alternative mode of therapy (i.e., RAI or surgery) must be selected for subsequent treatment.

Although very rare, the most serious complication of antithyroid drug treatment is agranulocytosis (absolute granulocyte count $<500/mm^3$). Agranulocytosis is thought to be autoimmune-mediated and must be distinguished from the transient, mild granulocytopenia (absolute granulocyte count $<1500/mm^3$) that occasionally occurs in patients with Graves' disease. Most cases of agranulocytosis occur within the first 90 days of treatment, but it can occur a year or more after starting thionamide therapy (199). Further, agranulocytosis can develop after a prior uneventful course of antithyroid drug therapy. Patients should discontinue the antithyroid drug and have a complete blood count performed if sore throat, fever, or mouth ulcers develop. The drug should be discontinued if the granulocyte count is below $1000/mm^3$; patients whose granulocyte count is between 1000 and $1500/mm^3$ should be closely monitored. Treatment of agranulocytosis consists of immediate, complete discontinuation of the antithyroid drug and hospitalization for monitoring. Cross-reactivity between PTU and MMI for agranulocytosis is well documented; so the use of the alternative antithyroid drug is contraindicated. Treatment with IV broad-spectrum antibiotics (including coverage for possible pseudomonas infection) should be used in patients with fever or obvious infection (199). Treatment with granulocyte-colony stimulating factor (G-CSF) may shorten the time to recovery and length of hospitalization; a bone-marrow aspirate may be useful prognostically because severe depression of myeloid precursors suggests a prolonged recovery time and a failure to respond to G-CSF (199). Although a recent randomized trial in adults found no benefit of G-CSF treatment in antithyroid drug-induced agranulocytosis (214), most authorities still recommend G-CSF therapy for this indication (199).

Hepatotoxicity is another major side effect of antithyroid drugs. Cytotoxic hepatitis tends to occur with PTU, whereas cholestatic hepatitis is typically associated with MMI use. In either case, antithyroid drugs must be stopped immediately, and glucocorticoids may hasten recovery (166).

PTU-induced hepatotoxicity manifests as an allergic hepatitis accompanied by laboratory evidence of hepatocellular injury. The early recognition of PTU-induced hepatitis may be difficult because some patients with normal baseline SGOT and SGPT levels may experience transient acute increases (1.1–6 times the upper range of normal) in these levels with spontaneous resolution despite continued PTU therapy (199). Also, asymptomatic elevations in SGOT/SGPT levels occur frequently in untreated patients with hyperthyroidism and are not predictive of further increases after starting PTU therapy. Therefore, routine monitoring of liver function tests in patients being treated with PTU is generally not recommended. The average duration of PTU therapy before the onset of hepatotoxicity is about three months. It is clinically characterized by symptoms of hepatitis (e.g., nausea, vomiting, anorexia, malaise, and fatigue), jaundice, and marked serum elevations of SGOT/SGPT. Its onset may be severe and fulminant. Liver biopsy shows submassive or massive hepatic necrosis. Therapy consists of immediate cessation of PTU along with expectant management of the potential complications of liver failure, including hepatic encephalopathy. Referral to a specialized center is reasonable; some patients may require liver transplantation (199). PTU-induced hepatitis can be fatal.

Hepatic abnormalities associated with MMI are typical of a cholestatic process. Liver biopsy reveals preserved hepatocellular architecture, along with intracanalicular cholestasis and mild periportal inflammation (199). Complete, but slow, recovery is the rule after stopping MMI.

Systemic vasculitis is another rare, but major, complication of thionamide treatment. It is more commonly associated with PTU than MMI use. Serologic evidence consistent with lupus erythematosus develops in some patients, fulfilling the criteria for drug-induced lupus (199). Antineutropil cytoplasmic antibody (ANCA) -positive vasculitis has also been reported in children (215–217). ANCA-positive vasculitis may occur after several years of thionamide therapy and may be more common in Asians (199). Clinical features in children may include fever, arthritis, hematuria, proteinuria, acute renal failure, vasculitic rash, skin ulcerations, upper and lower respiratory symptoms (including sinusitis and pulmonary hemorrhage), and intracerebral hemorrhage (199,217). Although this syndrome generally resolves after drug cessation, high-dose glucocorticoid therapy or cyclophosphamide may be needed in severe cases. Some patients have required short-term hemodialysis (199). Childhood death has occurred due to complications of severe systemic vasculitis (217).

Radioiodine Therapy

Although still controversial, RAI therapy is becoming more acceptable for the treatment of juvenile Graves' disease as long-term experience with its use accumulates (166,170,204,218,219). RAI is administered as an oral solution or capsule containing Na-^{131}I. After absorption through the gastrointestinal tract, it is concentrated into the thyroid gland and organified. β-emissions from the ^{131}I result in extensive tissue damage; histologic findings after RAI therapy are consistent with acute inflammation and include epithelial swelling and necrosis, edema, and leukocyte infiltration (166). Within 4 to 10 days after RAI administration, serum levels of thyroid hormones may rise as a result of thyroid hormone release from degenerating follicular cells (220). The acute inflammatory phase is subsequently followed by extensive fibrosis and ablation of the thyroid gland within 6 to 18 weeks (221).

RAI may be used as either first- or second-line therapy in children/adolescents with Graves' disease. Because RAI is absolutely contraindicated during pregnancy, a pregnancy test must be obtained in the adolescent female before proceeding with RAI therapy. The authors consider RAI therapy to represent a definitive method for control of Graves' hyperthyroidism, and, therefore, recommend that it be used in ablative doses. When used as first-line therapy in patients with mild-to-moderate hyperthyroidism, a preceding course of antithyroid drugs is generally not necessary. β-adrenergic blocking agents can be used both before and after RAI administration to relieve thyrotoxic symptoms until the gland has been ablated. However, for the patient with severe hyperthyroidism, some clinicians recommend a four- to eight-week course of antithyroid drugs to reduce the severity of hyperthyroidism before RAI is administered (15). In these patients, and in those who have been receiving antithyroid drugs as first-line therapy prior to RAI ablation, the antithyroid drugs should be stopped at least four to seven days before RAI is administered (166,170). It should be pointed out that prior treatment with PTU, even when discontinued for several days before RAI dosing, reduces the effectiveness of RAI therapy (222); this should be considered when determining the therapeutic dose of RAI (222). Conversely, MMI appears to have very little or no effect on the success of RAI therapy when discontinued at least three to four days prior to RAI dosing (222,223). Again, β-adrenergic blocking agents are used both before and after RAI administration to control symptoms of hyperthyroidism.

Occasionally after RAI therapy, thyrotoxic symptoms cannot be adequately controlled with β-adrenergic blockers alone. In this situation, either antithyroid drugs or concentrated iodide solutions (SSKI or Lugol's solution) may be used until RAI takes effect (166). The immediate use of thionamide drugs following RAI therapy can attenuate the effects of ^{131}I (224). However, several studies have shown that thionamide drugs do not reduce the effectiveness of RAI as long as they are started more than 7 to 14 days after ^{131}I administration (225,226). The use of a concentrated iodide solution (e.g., SSKI, five to six drops by mouth daily) beginning seven days after RAI administration has been shown to effectively treat thyrotoxicosis more rapidly than ^{131}I alone without adversely affecting the outcome of RAI therapy (227).

A single ablative dose of RAI will successfully treat hyperthyroidism in the majority of patients within 6 to 18 weeks (166,221). A dose of above 300 μCi ^{131}I/gram of thyroid tissue has been recommended to insure ablation of the thyroid gland in children with Graves' disease (228). Some patients will require more than one dose of RAI to take care of their hyperthyroidism. Repeat doses of RAI can be given at intervals of two to six months if hyperthyroidism persists beyond the first RAI treatment (166).

Acute complications following RAI therapy are uncommon and tend to be mild. Radiation thyroiditis with mild pain over the gland may develop within the first three to five days, and can generally be relieved with nonsteroidal anti-inflammatory agents such as ibuprofen. Symptoms of hyperthyroidism may worsen temporarily during the first two weeks due to the release of preformed thyroid hormones from degenerating follicular cells. Typically, these symptoms can be controlled with the β-adrenergic blocking drugs. Rarely, thyroid storm can occur after RAI treatment (73,74,166).

Although there is some concern in adult patients regarding progression of ophthalmopathy following

RAI, there is currently no evidence to support that RAI promotes progression of eye disease in children (166,170). Current studies suggest that childhood ophthalmopathy characteristically improves after RAI therapy; but eye disease may progress in 3% to 5% of children with GO after RAI (64). However, this rate of progression is very similar to the rates observed in children undergoing either antithyroid drug or surgical therapy (166). Rarely, parathyroid dysfunction may occur after RAI therapy (166,168). Up to 90% or more of patients who receive ablative doses of RAI will eventually develop primary hypothyroidism (166,170). Thyroid function tests should be monitored every two to three months after RAI so that thyroid hormone replacement can be started before the onset of clinical symptoms.

Traditionally, the reluctance of clinicians to recommend RAI as first-line therapy has stemmed from the theoretical possibility of increased risk for subsequent thyroid tumors (both malignant and benign), other nonthyroid malignancies, infertility, and genetic defects among progeny (218). Patients with Graves' disease have a higher incidence of thyroid tumors than the general population (166). However, Graves' patients receiving RAI appear to have lower rates of thyroid cancer than those receiving long-term antithyroid drug therapy; this most likely is explained by the presence of more thyroid tissue in patients treated with drugs than in those treated with RAI (166). Currently available studies involving the long-term follow-up (from less than 5 years to about 36 years) of approximately 1000 children and adolescents who received RAI therapy for hyperthyroidism have not revealed an increased risk of thyroid malignancy (166,229). Although an increased incidence of benign thyroid adenoma has been observed in children treated with low dose RAI (50 μCi ^{131}I/g thyroid tissue) (230), the incidence of thyroid adenoma is not increased when higher doses (100–200 μCi/g) are used (230). There is currently no evidence for an increased risk of leukemia. With the possible exception of a small increase in the incidence of stomach cancer, there is currently no evidence for an increased risk of other nonthyroid malignancies in adults who have received RAI (166). However, a comprehensive follow-up study of nonthyroid cancer risks has not yet been performed for children treated with RAI. There is no evidence for decreased fertility in patients who have received RAI, and the incidence of congenital anomalies among the offspring of patients treated with RAI is not different from that observed in the general population (166,229).

Based on current experience and knowledge, RAI represents a convenient, effective, and apparently relatively safe therapeutic option for childhood Graves' disease. After considering that antithyroid drug therapy is associated with disappointingly low long-term remission rates despite prolonged therapy and relatively high rates of adverse side effects, it is easy to understand why RAI is becoming more acceptable for the treatment of childhood Graves' disease. However, it should be remembered that RAI is absolutely contraindicated during pregnancy and breast-feeding. ^{131}I readily crosses the placenta, and if administered to a pregnant female after 10 to 12 weeks gestation, it will be concentrated by the fetal thyroid gland. This can result in ablation of the fetal thyroid and subsequent fetal hypothyroidism (170). RAI should probably be avoided in children less than five years of age. This is because experience with RAI is currently quite limited in this age group, and the risks of thyroid cancer after external irradiation are highest in children less than five years of age (166).

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Thyromegaly

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INTRODUCTION

Thyromegaly is defined as enlargement of the thyroid gland. The term goiter refers to any thyromegaly, diffuse or nodular, and the term simple goiter (or nontoxic goiter) describes goiter that is not associated with thyroid dysfunction or autoimmune thyroid disease (1,2). Thyromegaly can involve the entire gland (diffuse thyromegaly) and/or result from thyroid nodules (nodular thyromegaly). Types of thyromegaly can be classified according to cause (Table 1).

Estimates of the incidence of thyromegaly reported by surveys of apparently healthy school-age children range from 1.9% to 6.8% (3–5). Its prevalence in childhood peaks during adolescence with a female to male predominance, and, outside of iodine deficient regions, autoimmune thyroid disease is the most common cause (6). Although some goiters are idiopathic, the finding of an enlarged or firm thyroid obligates an investigation for underlying pathology, and a focused evaluation will identify a cause in the majority of children.

PATHOPHYSIOLOGY

Thyromegaly can develop as an adaptation to any factor that impairs thyroid hormone synthesis and thus leads to compensatory thyroid-stimulating hormone (TSH) secretion. The induction of goiter in rats by thiouracil administration is preceded by vascular proliferation, and the in vitro stimulation of cultured thyroid follicular cells with TSH or Graves' IgG increases VEGF expression, indicating that the induction of thyroid growth by TSH likely requires the paracrine stimulation of nonfollicular cells by angiogenic and other growth factors (7,8). Stimulation of the thyrotropin receptor by either TSH or TSH-receptor antibodies can explain the thyromegaly that is observed in hypothyroid conditions, iodine deficiency, Graves' disease, germline-activating mutations of the TSH-receptor gene, and syndromes of inappropriate TSH secretion (9). The latter includes both TSH-secreting tumors and pituitary resistance to thyroid hormone in patients with germline mutations of the thyroid hormone receptor (10–12).

Separate from TSH-receptor stimulation, thyroid enlargement can also occur as the result of glandular inflammation (thyroiditis) or infiltrative disease (nodules). Even in the context of exposure to a known goitrogen, goiter formation is a complex trait, and the importance of host susceptibility is illustrated by the variation in thyroid size observed in endemic goiter (2,13).

CAUSES OF GOITER

Thyroiditis

The term thyroiditis is defined as evidence of ''intrathyroidal lymphocytic infiltration'' with or without follicular damage (14). Chronic autoimmune thyroiditis (also known as chronic lymphocytic thyroiditis) is the most common cause of thyroiditis, affecting approximately 2% of the female population and 0.2% of the male population (15). Its overall prevalence peaks in adulthood, but it is the most common etiology of both acquired thyroid dysfunction and thyromegaly in children (6). The childhood prevalence of chronic autoimmune thyroiditis peaks in early to mid puberty with a female preponderance of 2:1 (16). Presentation is rare under the age of three years, but cases have been described even in infancy (17). Measurements of circulating autoantibodies have replaced the need for biopsy in the diagnosis of autoimmune thyroid disease, and the nomenclature itself has been redefined in recent years (Table 2) (2,14).

A goiter or firm thyroid is the first physical sign of chronic autoimmune thyroiditis. Thyromegaly is typically diffuse with a ''pebbly'' or ''seedy'' surface that evolves into a firm and nodular consistency. Two types of autoimmune thyroid disease, Type 2A autoimmune thyroiditis (classic Hashimoto's disease) and Graves' disease, present with thyromegaly and are associated with persistent thyroid dysfunction. Accordingly, the finding of diffuse thyromegaly warrants the measurement of both thyroid antibodies and thyroid function. Chronic autoimmune thyroiditis may be the initial presentation of an autoimmune polyglandular syndrome, and the possibility of

Table 1 Causes of Thyromegaly

- Diffuse thyromegaly
  - Thyroiditis
    - Autoimmune thyroid disease (Table 2)
      - Type 1A autoimmune thyroiditis
      - Type 2A autoimmune thyroiditis
      - Type 2C autoimmune thyroiditis
      - Graves' disease
    - Painless thyroiditis
      - Painless sporadic thyroiditis
      - Painless postpartum thyroiditis
    - Subacute painful thyroiditis
    - Riedel's thyroiditis
  - Environmental goitrogens
    - Iodine deficiency
    - Food and drinking water
      - Brassica vegetables
      - Cassava plants
      - Resorcinol?
      - Perchlorate anion?
    - Medications
      - Thionamides
      - Lithium
  - Familial goiter
    - Dyshormonogenesis
    - Pendred syndrome
  - Inappropriate TSH secretion
    - TSH-secreting pituitary adenoma
    - Resistance to thyroid hormone
  - Idiopathic
- Nodular thyromegaly
  - Hypofunctioning thyroid nodules
    - Benign adenoma
    - Differentiated thyroid cancer of follicular cell origin
    - Medullary thyroid cancer
    - Thyroid metastases from extrathyroidal primary cancer
    - Cyst
  - Hyperfunctioning thyroid nodules
  - Suppurative thyroiditis with or without abscess

Abbreviation: TSH, thyroid-stimulating hormone.

coexisting autoimmune diseases such as Type 1 diabetes, Addison's disease, and pernicious anemia must be addressed by the past medical history and the review of systems (Vol. 2; Chap. 26).

Table 2 Classification of Autoimmune Thyroid Disease

- Type 1 Autoimmune Thyroiditis (Hashimoto's disease Type 1)
  - 1A: goitrous
  - 1B: nongoitrous
  - Status: euthyroid with normal TSH
- Type 2 Autoimmune Thyroiditis (Hashimoto's disease Type 2)
  - 2A: goitrous (classic Hashimoto's disease)
  - 2B: nongoitrous (primary myxedema, atrophic thyroiditis)
  - Status: persistent hypothyroidism with high serum TSH
  - 2C: transient subacute thyroiditis (example postpartum thyroiditis)
  - Status: may start as transient, low radioiodine uptake thyrotoxicosis followed by recovery with transient hypothyroidism
- Graves' disease
  - Status: hyperthyroid or euthyroid with low serum TSH. Stimulatory autoantibodies to the TSH receptor are present (autoantibodies to thyroglobulin and thyroid peroxidase are also usually present)

Abbreviation: TSH, thyroid-stimulating hormone.
Source: Adapted from Refs. 14,18.

Chronic autoimmune thyroiditis is characterized by high serum concentrations of thyroid autoantibodies and varying degrees of thyroid dysfunction. In Types 1 and 2 autoimmune thyroiditis, the activation of CD4 (helper) T-lymphocytes specific for thyroid antigens is believed to be the first step in pathogenesis. Once activated, self-reactive CD4 T-cells recruit cytotoxic CD8 T-cells as well as autoreactive B-cells into the thyroid. The three main targets of thyroid antibodies are thyroglobulin, thyroid peroxidase, and the thyrotropin receptor. Anti-TPO antibodies have been shown to inhibit the activity of thyroid peroxidase in vitro, but direct killing by CD8 T-cells is believed to be the main mechanism of hypothyroidism in vivo (19). Anti–TSH-receptor antibodies may contribute to hypothyroidism in a minority of adult patients with the atrophic form of chronic autoimmune thyroiditis (Type 2B), but this has not been proven in children (20,21). The presentation of Type 2 autoimmune thyroiditis includes hypothyroidism or goiter, or both. As the disease progresses, subclinical and then overt hypothyroidism appears. In Graves' disease, autoantibodies directed against thyroglobulin, thyroid peroxidase, and the sodium–iodine symporter may be present, but hyperthyroidism is caused by thyroid-stimulating antibodies that bind and activate the thyrotropin receptor, leading to follicular cell hyperplasia and the hypersecretion of thyroid hormone. Lymphocytic infiltration of the thyroid is present, hence its classification as a form of thyroiditis. Occasionally, germinal centers form, which can develop as major sources of intrathyroid autoantibodies. Lymphocytic infiltration and the accumulation of glycosaminoglycans in the orbital connective tissue and skin cause the extrathyroidal manifestations of Graves' ophthalmopathy and dermopathy, respectively.

In addition to chronic autoimmune thyroiditis, painless sporadic thyroiditis, painless postpartum thyroiditis, Riedel's thyroiditis, and subacute painful thyroiditis can also present with thyromegaly (22,23). Painless sporadic thyroiditis and painless postpartum thyroiditis are both associated with circulating thyroid antibodies, and they are clinically indistinguishable from one another except by the relationship of the latter to pregnancy (onset within one year postpartum) (22). Either form of painless thyroiditis can cause transient thyrotoxicosis due to the release of preformed hormone from the damaged gland. In some cases, this thyrotoxic phase is followed by a period of hypothyroidism, and rarely hypothyroidism without thyrotoxicosis is observed (24). A small, nontender, firm goiter is present in most cases of painless postpartum thyroiditis and in about 50% of patients with painless sporadic thyroiditis (23). Riedel's thyroiditis is a rare progressive fibrotic disorder of the thyroid gland that presents with a hard, fixed, painless goiter. Most patients with Riedel's thyroiditis are euthyroid upon presentation, but hypothyroidism can develop due to replacement

of the normal thyroid parenchyma. These forms of thyroiditis should be distinguished from painful subacute thyroiditis and suppurative thyroiditis, which typically present with pain or a discrete tender thyroid mass rather than with diffuse thyromegaly. Painful subacute thyroiditis is often preceded by symptoms of upper respiratory infection, but its etiology is unknown. Like painless sporadic thyroiditis, painful subacute thyroiditis can cause transient thyrotoxicosis followed by hypothyroidism. Suppurative thyroiditis is usually due to bacterial infection of the thyroid, although fungal, mycobacterial, and parasitic infections have been described. Thyroid function is usually normal in patients with suppurative thyroiditis. The patients present with complaints of fever and local thyroid pain or tenderness. In childhood, persistence of the pyriform sinus fistula is the most common route of infection, and it is left sided in approximately 90% of cases (22,25).

The existence of multiple classification schemes has led to variable terminology, and this is a potential source of confusion in the literature. Postpartum thyroiditis and subacute lymphocytic thyroiditis are used as synonyms for painless postpartum thyroiditis. Silent sporadic thyroiditis and subacute lymphocytic thyroiditis are used as synonyms for painless sporadic thyroiditis. The terms deQuervain's disease, deQuervain's thyroiditis, subacute thyroiditis, subacute nonsuppurative thyroiditis, giant cell thyroiditis, subacute granulomatous thyroiditis, pseudogranulomatous thyroiditis, and struma granulomatosa are all variably used as synonyms for painful subacute thyroiditis (22,26).

Environmental Goitrogens

The association of goiter and cretinism with specific geographic regions long preceded the discovery of iodine in the thyroid by Baumann in 1896 and the subsequent acceptance of iodine deficiency as the cause of endemic goiter (27). Iodine is the nutritional precursor of thyroxine, which contains 65% iodine by weight, and iodine deficiency causes a spectrum of disorders that includes goiter, hypothyroidism, and mental retardation (18). Approximately 38% of the world population was iodine deficient in 1999, and iodine deficiency remains the most prevalent preventable cause of goiter and delayed intellectual development in the world today (28). When dietary iodine intake is low, thyroxine secretion decreases and the compensatory increase in TSH causes thyromegaly. Iodine administration has little effect in adults with long-standing endemic goiter, but it greatly reduces or prevents the incidence of endemic goiter and the other iodine-deficiency disorders in children.

Several investigators have described the persistence of goiter in certain geographic regions after successful iodine supplementation. Study of these populations has led to the identification of naturally occurring goitrogens including vegetables of the genus Brassica (Cruciferae family), which contain thiocyanates and isothiocyanates that inhibit thyroidal iodine uptake. This group includes cabbage, brussel sprouts, and turnips (27). It has been suggested that the process of cooking decreases the goitrogenic properties of these plants, and so it is notable that certain new nutritional supplements contain large quantities of freeze-dried Brassica vegetables (29–31). Foods that contain cyanogenic glucosides such as cassava may also contribute to goiter rates in iodine deficient regions (27,32).

In addition to the above foods, resorcinol and perchlorate anion are goitrogens that are present in the drinking water and foodstuffs of certain regions. However, convincing evidence that the amounts ingested by the inhabitants of these regions can cause goiter or hypothyroidism is currently lacking (27,33,34). Tobacco smoking is associated with an increased prevalence of goiter, particularly in areas of iodine deficiency (35). Certain medications are goitrogens. The most common of these are the thionamide antithyroid medications, propylthiouracil, and methimazole, which inhibit the oxidation and organic binding of thyroid iodide. Lithium impairs the glandular release of thyroid hormone, a property that was exploited in the past to treat hyperthyroidism and that is used today to prolong the retention time of therapeutic radioiodine (36,37). This same property causes hypothyroidism or goiter in up to half of adults on long-term lithium therapy (38–40). Chronic lithium therapy is also associated with an increased risk of thyrotoxicosis, which, when due to hyperthyroidism, is associated with thyromegaly (41).

Familial Goiter

As reviewed in Vol. 2; Chap. 16 (Thyroid Disorders of Infancy), the mutation of genes involved in thyroid hormone synthesis (thyroglobulin, thyroid peroxidase, sodium iodine symporter, pendrin, and thyrotropin receptor) can cause dyshormonogenesis and congenital hypothyroidism. Studies of familial goiter indicate that abnormalities of these same genes can also cause euthyroid goiter, presumably due to milder disruption of gene function (42). Mutations of thyroid peroxidase and the sodium iodide symporter have been reported in children with euthyroid goiter (43–46). For the thyroglobulin gene, a point mutation in exon 10 has been associated with nontoxic goiter in three families, and the finding of one patient with goiter and monoallelic deletion of the 5′ region of the thyroglobulin gene is evidence that goiter can also arise from haploinsufficiency (47,48). Pendred syndrome is an autosomal recessive disorder defined by congenital sensorineural hearing loss and goiter. It is the most common form of syndromic deafness and is caused by mutations of the pendrin gene, which encodes the transmembrane protein pendrin, normally expressed in the kidney, inner ear, and the apical border of the thyroid cell where it is believed

to transport iodide into the follicular lumen. The loss of pendrin function leads to variable impairment of thyroid hormone synthesis with goiter, but not often hypothyroidism, and a positive perchlorate discharge test (49,50). The above conditions demonstrate that goiter can arise from the dysfunction of several different genes. For the majority of familial goiters, no specific genetic mutation has been identified, presumably because of multigenetic inheritance and the contribution of unidentified environmental factors. One recent study of sonographically measured thyroid size in 104 monozygotic twin pairs and 153 dizygotic twin pairs concluded that genetic factors accounted for 71% of individual differences in thyroid volume (51). Genetic influences are, therefore, important in the regulation of normal thyroid growth as well as goiter formation.

Nodular Goiter

The term nodular goiter refers to enlargement of the thyroid with deformation of the normal parenchymal structure by the presence of one (uninodular goiter) or more (multinodular goiter) thyroid nodules. The frequency of nodular thyroid disease increases with age (Vol. 2; Chap. 20). Thyroid nodules are, therefore, rare in children (estimated frequency of 0.05–1.8%) and common in adults (present in up to 50% of adults after the sixth decade of life) (4,52,53). In comparison to the 5% to 10% cancer prevalence cited for adults, early series reported a 40% to 60% prevalence of thyroid cancer in children with thyroid nodules (2). More recent studies estimate the prevalence of cancer among children with thyroid nodules to be 5% to 33% (54–63). The reason for this discrepancy is not clear, although differences in cohort size, geographic variation, thyroid screening, and the discontinuation of the practice of neck irradiation for benign conditions are speculated to be contributors. Thyroid nodules may be detected by the child or parent on physical examination, or as an incidental finding on radiographic studies. Regardless of how a thyroid nodule is discovered, all nodules of significant size should be evaluated for the possibility of malignancy (a threshold of $\geq$1 cm is used at our center). As thyroid cancer prognosis depends in part upon tumor size, the early identification of differentiated thyroid cancer is the primary goal in the evaluation of patients with thyroid nodules (64).

Upon referral, the medical history should include inquiry into prior neck irradiation, determination of whether there is a family history of thyroid cancer or endocrine neoplasia (MEN2B), and if there are extrathyroidal manifestations suspicious of other syndromes associated with thyroid cancer (Cowden's syndrome, Bannayan–Riley–Ruvalcaba syndrome, familial adenomatous polyposis, etc.) (Vol. 2; Chap. 27) (65–67). A complete review of systems should include symptoms of thyroid dysfunction and neck compression (dysphagia, hoarseness, pain, etc.). Physical examination should include palpation of both the thyroid gland and the cervical lymph nodes. Nodules that are hard, large, adherent to adjacent structures, or associated with lymphadenopathy should heighten the suspicion of cancer. The initial laboratory evaluation should include thyroid function testing (measurement of serum TSH) to screen for an autonomous/hyperfunctioning nodule. Some investigators have advocated the routine measurement of serum calcitonin to increase the sensitivity of medullary thyroid cancer (MTC) detection; however, individual institutions should consider the frequency of MTC/MEN2 in their referral population, and clinicians should recognize that benign conditions such as autoimmune thyroiditis and high serum TSH concentrations may cause elevations (68). Diffuse goiter or high serum TSH concentrations should prompt the measurement of antithyroid antibodies. However, autoimmune thyroid disease does not exclude the possibility of coexistent thyroid cancer, illustrated by the report of autoimmune thyroiditis in 18% (7 of 39) of children with papillary thyroid cancer cited by a recent study (69).

Ultrasonography is the recommended imaging study to confirm the presence of a thyroid nodule. Referral to a center with specific expertise in thyroid ultrasonography facilitates both the accurate identification of thyroid nodules and subsequent follow-up (either the noninvasive monitoring of nodules with benign cytology or the surveillance for local recurrence in patients with thyroid cancer). In a retrospective review of 173 adults referred for suspected nodular thyroid disease, ultrasound altered management in 63% due to the detection of nonpalpable nodules or the determination that no nodules met criteria for biopsy. In this series, 4 of the 12 patients with thyroid cancer were diagnosed by the biopsy of nodules that were not palpated by the referring physician and, therefore, would not have been detected without ultrasonography, illustrating the limitations of physical examination (70). Thyroid ultrasonography should be performed in all children with suspected thyroid nodules before any attempt at biopsy. Nodules that are cystic or homogeneously hyperechoic carry a lower risk of malignancy. Conversely, a solid hypoechoic echotexture, calcifications, irregular shape, or the absence of a halo are features associated with cancer (2,4,53). However, no sonographic findings reliably predict the likelihood of cancer, and so biopsy is indicated for all thyroid nodules of significant size.

Ultrasound-guided fine-needle aspiration is the procedure of choice for the cytologic evaluation of thyroid nodules. In a recent survey of the American Thyroid Association, 100% of clinicians surveyed advised fine-needle aspiration for the evaluation of thyroid nodules, and none recommended large-needle biopsy (71). Ultrasound guidance improves the diagnostic accuracy of fine-needle aspiration guided by palpation alone and reduces the likelihood of accidental penetration into the trachea or the great

vessels (72–75). Furthermore, ultrasound guidance is necessary to biopsy nodules that are primarily cystic or nonpalpable due to their location in the posterior aspect of the gland. Up to 20% of fine-needle aspirations are insufficient, with cystic content as the primary predictor of nondiagnostic cytology. Repeat aspiration is usually successful (63% on the first repeat ultrasound-guided biopsy) and is indicated for all patients with insufficient or nondiagnostic cytology on initial biopsy (70). Papillary thyroid cancer is the most common malignant tumor of the thyroid (85–90% of thyroid cancers in children) and is characterized by nuclear abnormalities that are readily identifiable by cytology (76). However, even under optimal conditions, cytology alone cannot accurately differentiate follicular adenomas from follicular carcinoma because the latter diagnosis requires the documentation of capsular and/or vascular invasion. Despite this limitation, cytology should be obtained in all patients prior to considering surgery. Benign cytology usually obviates surgical resection. Conversely, in patients with abnormal cytology, the degree and type of cytologic abnormality allows a more specific assignment of cancer risk and facilitates the discussion of surgical options with the family.

CLINICAL PRESENTATION

Children with thyromegaly that occurs in conjunction with thyroid dysfunction will have symptoms referable to hypothyroidism (see Vol. 2; Chap. 17, Hypothyroidism) or hyperthyroidism (Vol. 2; Chap. 18, Hyperthyroidism). However, other children present with symptoms referable to thyromegaly itself. A thyroid mass may be noted by the physician, the patient, or the parent, or as an incidental finding by radiologic studies obtained for other reasons. Large goiters can cause compressive symptoms related to compression/displacement of the trachea, esophagus, neck vessels, or recurrent laryngeal nerve. Accordingly, a history should be obtained with specific attention to stridor, dysphagia, or changes in vocal quality (hoarseness). Thyroid pain may result from glandular inflammation or acute hemorrhage with resultant stretching of the thyroid capsule.

Examination of the Thyroid Gland

The normal thyroid gland is bilobed and connected by an isthmus that overlies the second through fourth tracheal cartilages. The two main lobes of the thyroid are usually equal in size, but the right lobe tends to enlarge to a greater degree than the left in patients with diffuse goiter. Physical examination of the thyroid is best performed with the patient seated and the neck in moderate extension. The examiner should first inspect the neck visually and then palpate the thyroid gland and the cervical and supraclavicular lymph nodes. Because the thyroid is ensheathed in the pretracheal fascia, it moves on swallowing, and this feature differentiates thyroid tissue from other neck structures. Accordingly, the patient should be provided a cup of water and instructed to swallow sips at appropriate intervals during palpation. Some clinicians prefer to stand behind the seated patient and palpate the thyroid with their fingertips. Alternatively, the clinician can face the seated patient and use gentle pressure with the thumb to locate the thyroid isthmus and then move laterally to compress the lobe of the thyroid against the trachea as the patient sips water (114). The latter technique facilitates identification of the gland's lateral borders and the detection of small nodules that are not easily palpated with the posterior approach. With practice, one should be able to palpate the normal thyroid in nearly all school-age children. For infants, the child can be placed in the supine position with the examiner's hand under the patient's shoulders. The examiner can then gently raise the infant's shoulders above the examination table until the neck is in moderate hyperextension and palpate the thyroid using the opposite hand.

Assessment of goiter should take into account the normal thyroid size for age. The equation of $T = 1.48 + 0.054A$, where T is the weight of the thyroid in grams and A is the age in months, describes the average thyroid weight from birth to 20 years (77). In North America, the normal adult thyroid weighs approximately 14 to 20 g (average weight 14.4 g for women 20–69 years of age, 16.4 g for men 20–29 years of age, and 18.5 g for men 30–69 years of age) (78,79). A helpful rule created by the World Health Organization in 1960 to facilitate palpation surveys for endemic goiter was the definition of thyromegaly as ''lobes larger than the terminal phalanxes of the (patient's) thumbs'' (80). In patients with diffuse goiter by palpation, adjunctive imaging is not necessary. However, if the goiter is asymmetric or the question of a mass is raised, thyroid ultrasonography is the best test to address the possibility of a nodule. Sonographic measurements of the gland can also be used to estimate thyroid size more precisely by using the formula for a rotational ellipsoid [length (cm) × width (cm) × depth (cm) × pi/6] for each lobe (81,82).

Suggested Approach to the Evaluation of Diffuse Thyromegaly

Serum TSH and free T4 (or free T4 index) should be measured in all patients with goiter (Table 3).

Serum antithyroid peroxidase antibodies are the most sensitive test for chronic autoimmune thyroiditis and should be measured in all hypothyroid or euthyroid patients with goiter (83). Little further benefit is gained by the additional measurement of serum antithyroglobulin antibodies (84). The typical patient with hypothyroidism secondary to chronic autoimmune thyroiditis has a high serum TSH (over 10 μU/mL), a low FT4I, and a high antithyroid peroxidase antibody value. In early stages of the disease, serum TSH may be normal and antithyroid

Table 3 Thyroid Function Tests in Diffuse Thyromegaly

	Serum TSH	Serum FT4	Serum FT3	Radioiodine uptake
Chronic autoimmune thyroiditis	High or normal	Normal or low	Normal or low	Not applicable[a]
Graves' hyperthyroidism	Low	Normal or high	Normal or high	High
Painless thyroiditis (thyrotoxic phase)	Low	Normal or high	Normal or high	Low
Painless thyroiditis (recovery phase)	High	Normal or low	Normal or low	Not applicable[a]
Inappropriate TSH secretion	Normal or high	High	High	High[a]

[a]In the United States, dietary iodine intake renders the radioiodine uptake values in hypothyroidism indistinguishable from the lower end of the normal range, and so radioiodine uptake measurements are usually not helpful in the evaluation of hypothyroidism (114). Radioiodine uptake is high in syndromes of inappropriate TSH secretion, but the measurement is not clinically useful in distinguishing TSH-secreting pituitary adenomas from resistance to thyroid hormone.
Abbreviation: TSH, thyroid-stimulating hormone.

peroxidase antibodies may be high with goiter (Type 1A). Later, serum TSH is slightly high (between 5 and 10 μU/mL) with a normal FT4 (subclinical hypothyroidism). Up to 90% of patients with hypothyroidism secondary to autoimmune thyroiditis have a high serum antithyroid peroxidase antibody value. It should be noted that 10% to 15% of the general adult population have a high serum antithyroid peroxidase antibody value, and that low titers (less than 1/100 by agglutination methods or less than 100 IU/L by immunoassays) are less specific for autoimmune thyroid disease (15). If the serum anti-TPO antibody value is normal, less common causes of primary hypothyroidism such as transient hypothyroidism (postsubacute thyroiditis) and consumptive hypothyroidism should be considered (85–87).

Thyrotoxicosis is recognized by high serum free T4 (or FT4I) and low serum TSH (typically less than 0.1 μU/mL) values. Serum free triiodothyronine should be measured if serum TSH is low and the serum free T4 is normal. In patients with mild hyperthyroidism or iodine deficiency, serum free T4 concentrations may be normal or low despite high serum triiodothyronine concentrations. These are the only situations in which a serum triiodothyronine needs to be measured. If serum TSH is low and free T4 normal to high in a patient with diffuse goiter, distinction between painless thyroiditis and the more common Graves' disease is critical because antithyroid drugs have no role in the treatment of the former. Once biochemical derangement has been documented, it is helpful to address the duration of thyrotoxicosis to facilitate the differentiation of Graves' disease from painless thyroiditis. Onset may be documented by prior laboratory studies or inferred from the history. The differential diagnosis of thyrotoxicosis includes transient thyroiditis, hyperfunctioning nodule(s), and exogenous thyroid hormone (Vol. 2; Chap. 18). Thyromegaly will be absent in the latter. In the majority of cases, the presence of a symmetrically enlarged thyroid coupled with the chronicity of symptoms will be adequate to allow a diagnosis, but radionuclide studies can provide confirmatory data (Table 3). If symptoms have been present for less than eight weeks, painless thyroiditis should be considered. These forms of thyroiditis are self-limited and refractory to therapy with antithyroid drugs. The radioiodine uptake will be low, distinguishing them from the more common Graves' disease (88). For thyrotoxicosis that has been present for more than eight weeks, Graves' is by far the most likely etiology. The constellation of thyrotoxicosis, goiter, and ophthalmopathy is pathognomonic of this condition. If thyromegaly is subtle and eye changes are absent, an ^{123}I uptake, with or without a scan, should be performed. Autonomous nodules must be large to cause hyperthyroidism (typically 2–3 cm or more in diameter), so radioiodine scanning should be reserved for patients in whom a discrete nodule(s) is palpable. In patients with a toxic nodule, ^{123}I uptake will localize to the nodule and the signal in the surrounding tissue will be low, secondary to TSH suppression.

If serum free T4 and T3 are high with a normal or high serum TSH concentration, causes of inappropriate TSH secretion should be explored. Inappropriate TSH secretion can be caused by a pituitary tumor or by resistance to thyroid hormone. Distinction between these two entities is critical because therapy is different. Given the rarity of these disorders, the possibility of laboratory artifact should be addressed before the patient is subjected to an extensive evaluation (Vol. 2; Chap. 17) (89). The best test to distinguish between these is a pituitary MRI (10,11,90). Resistance to thyroid hormone is nearly always due to inheritance of a dominant-negative mutation of the β-thyroid hormone receptor, so obtaining thyroid function tests in first-degree relatives is an inexpensive and useful screen.

Suggested Approach to the Evaluation of Nodular Thyromegaly

A serum TSH should be measured in any patient with nodular thyroid disease (Fig. 1).

If the patient's serum TSH concentration is low, a thyroid scan should be obtained to address the possibility of a hyperfunctioning nodule (see Vol. 2; Chap. 18, Hyperthyroidism). ^{123}I is the ideal radionuclide for thyroid scintigraphy. Due to its short half-life and the absence of beta radiation, it delivers only 1% of radiation to the thyroid per milliCurie administered, compared with ^{131}I, and the energy of its main γ-ray is ideal for detection by standard gamma cameras. ^{99m}Tc-pertechnetate (TcO4) is a

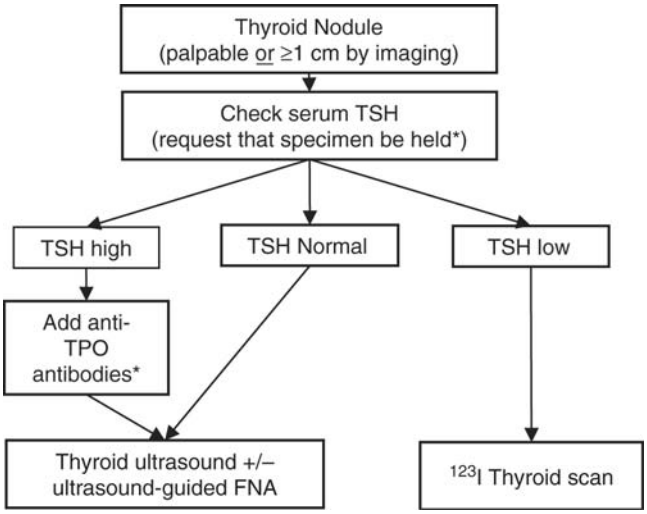

Figure 1 Suggested approach to the evaluation of nodular thyromegaly.

monovalent anion that, like iodine, is actively transported by the sodium–iodine symporter and can be used to measure thyroid uptake. Unlike iodine, it is not oxidized and organically bound, and therefore it rapidly diffuses out of the thyroid as its plasma concentration falls. Approximately 5% to 10% of thyroid tumors that are hypofunctioning on radioiodine scintigraphy appear to be functioning with ^{99m}TcO4, presumably because these nodules can trap but not organify iodine (91). This can lead to the false conclusion that a thyroid nodule is functioning and therefore benign. Accordingly,^{99m} TcO4 scintigraphy should not be used in the evaluation of nodular thyroid disease.

Children with thyroid nodules who have normal to elevated serum TSH concentrations should be referred directly to a center with experience in the management of thyroid nodules and cancer, and any thyroid nodule $\geq$1 cm in diameter should be biopsied by ultrasound-guided fine-needle aspiration. Categorization of biopsy interpretations into cytologic risk categories facilitates the discussion of surgical approach, based upon the likelihood of cancer associated with the patient's specific category and an individual assessment of the child's operative risks. Total or near-total thyroidectomy is the optimal surgery for children with a biopsy diagnosis of papillary thyroid cancer, because this facilitates radioiodine ablation and subsequent monitoring for recurrence and disease progression (Vol. 2; Chap. 20) (92,93). However, for patients whose cytology predicts a <50% likelihood of cancer, thyroid lobectomy is the preferred initial surgery, followed by completion thyroidectomy only if lobectomy confirms the diagnosis of cancer. This approach reduces the risk of complications for the patients with benign nodules. Referral to a surgeon with a low personal complication rate and extensive experience with thyroidectomy is required even for lobectomy because surgeon experience is the primary determinant of operative morbidity (94). Although unilateral vocal cord paralysis or parathyroid injury may not compromise activities of daily life, these complications increase the risk of permanent morbidity with future surgeries (completion thyroidectomy or neck dissection for local recurrence) in those patients who have cancer. Nodules greater than 4 cm should be resected even with benign cytology because even optimal biopsies may not represent the composition of the entire nodule. Children with thyroid nodules $<$1 cm or with benign cytology should be followed chronically by serial ultrasonography every 6 to 12 months, and ultrasound-guided fine-needle aspiration should be repeated if the nodule enlarges or other concerning sonographic features develop.

THERAPY OF NONTOXIC DIFFUSE GOITER

For most patients with goiter, interventions that are indicated to address coexisting thyroid dysfunction will decrease thyroid size. In Type 2A autoimmune thyroiditis, the normalization of serum TSH with levothyroxine is associated with a mean 32% decrease in thyroid size, with almost half attaining normal thyroid size after two years (95). For Graves' hyperthyroidism or hyperfunctioning "hot" nodules, most patients fail to achieve lasting remission with antithyroid drug therapy (see Vol. 2; Chap. 18), and destructive therapy will be needed. For patients with hypofunctioning nodules, surgery (lobectomy or subtotal thyroidectomy) is indicated if there are suspicious cytologic abnormalities, or the nodule has caused compressive symptoms.

For children with idiopathic nontoxic diffuse goiter, observation and reassurance is appropriate for the majority because the goiter regresses spontaneously in up to 60% (3). Another 5% to 10% of these children are eventually diagnosed with autoimmune thyroiditis (96). For children with large nontoxic diffuse goiters, debate exists as to the optimal therapy (3). Little data are available in children. The following section summarizes reports of goiter therapy mostly in adult patients. The theoretical concerns of radioiodine therapy for nontoxic goiter in childhood and the practical risks of thyroidectomy in children favor deferral of definitive therapy until adulthood when possible.

Thyroid Hormone

Thyroid hormone is sometimes given to reduce the size of nontoxic goiters. Efficacy correlates with TSH suppression, and this explains the finding of one study in children that goiter was significantly decreased only in children who were hypothyroid before treatment and the general observation of studies in adults that nontoxic goiters tend to relapse once thyroid hormone therapy is withdrawn (97,98). With

this limitation in mind, children with goiters can be offered levothyroxine if observation alone is not acceptable. The serum TSH concentration should be reduced to a low normal value (0.2–0.3 μU/mL). Based upon experience in patients with thyroid cancer, this degree of TSH suppression is not associated with hyperthyroxinemia or symptoms of hyperthyroidism. Chronically low serum TSH concentrations less than 0.1 μU/mL are associated with an increased risk of atrial fibrillation in patients over 60 years of age, and some studies have raised the concern of osteopenia in postmenopausal women, but no similar risks have been identified in children (99). Withdrawal of thyroid hormone therapy can be considered in early adulthood when the risks of surgery or radioiodine are lower.

Surgery

Thyroid resection is used to relieve the cosmetic and compressive symptoms of large goiters, even in the absence of hyperthyroidism or cytologic abnormalities. Surgery has the potential advantages of rapid goiter reduction and, for patients with nodular goiter, definitive pathologic diagnosis. However, the potential complications of thyroidectomy are substantial, illustrated by a recent metanalysis of thyroidectomy performed in children with Graves' hyperthyroidism, which cited a 2% incidence of permanent hypoparathyroidism, 2% incidence of vocal cord paralysis, and 0.08% mortality (100). Large goiter size and substernal extension further increase the likelihood of respiratory complications (101,102). Surgery should be deferred until adulthood except for the rare child with progressive symptoms despite levothyroxine therapy. Surgeon experience is the primary determinant of operative morbidity, so referral to a surgeon with a low personal complication rate and extensive experience with thyroidectomy is required if this is the desired procedure (94,103). ^{131}I is recommended for patients in whom goiter recurs after surgery, due to the high complication rate of secondary thyroidectomy.

Radioiodine

The observation that radioiodine therapy for Graves' hyperthyroidism decreases thyroid volume has led several groups to treat patients with nontoxic goiters with ^{131}I. One recent study reported goiter resolution in 26 of 34 adults with nontoxic diffuse goiter (average size 67.9 mL) after radioiodine treatment (average dose 600 MBq), although the description of baseline serum TSH concentrations as low as 0.02 μU/mL indicates that some of these patients had subclinical hyperthyroidism (104). The efficacy of radioiodine therapy is dependent upon both thyroid mass and uptake, so the treatment of large nontoxic goiters presents a unique challenge because glandular mass is greater and uptake is lower than in most patients with hyperthyroidism. Two studies have described the successful stimulation of radioiodine uptake with recombinant TSH in patients with nontoxic multinodular goiter, and it is likely that the effect on nontoxic diffuse goiter would be similar (105,106). Concerns over the potential long-term complications of radiation exposure in children have traditionally made endocrinologists cautious in giving radioiodine to children, but ^{131}I is gaining wider acceptance due to reports that the incidence of thyroid carcinoma and leukemia is not increased after ^{131}I for children with Graves' hyperthyroidism (107–110). Nonetheless, experience with X-rays and the Chernobyl nuclear power plant accident indicate that the carcinogenic effects of radiation to the thyroid are highest in young children (Vol. 2; Chap. 31) (111–113). Furthermore, unlike in children with Graves' hyperthyroidism where ^{131}I doses are calculated to eliminate future cancer risk by destroying all thyroid follicular cells,^{131}I treatment of nontoxic goiters generally leaves a substantial remnant, arguing for the deferral of radioiodine therapy for nontoxic goiter until adulthood when possible.

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Thyroid Tumors in Children

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EPIDEMIOLOGY

Thyroid Nodules

Thyroid disorders affect 3.7% of children in the United States between the ages of 11 and 18 years; the incidence of thyroid nodules is 0.46% to 1.5% (1,2). Postmortem studies and ultrasound evaluation of the thyroid gland demonstrate an incidence of thyroid nodules of up to 50% in adults (2). Ezzat et al. (3) demonstrated that 21% of asymptomatic adults had a palpable thyroid nodule on physical examination and that 67% had abnormal thyroid ultrasound findings. In another study of adults between the ages of 18 and 39 years, postmortem thyroid nodules were present in 13% (4). These studies suggest that the true incidence of thyroid nodules in children might be higher than the incidence found in clinical studies.

Solitary nodules in children have an 18% to 21% prevalence of malignancy (5,6). This malignancy risk in children is fourfold greater than the risk among adults. In addition, the finding of a solitary nodule on physical examination is associated with multinodule on ultrasonographic findings in 50% of the cases (7). Often a multinodular gland is considered less likely than a gland with a solitary nodule to be malignant. However, a study of 5637 patients showed that the frequency of thyroid cancer was 4.1% in nodules judged to be solitary and 4.7% in nodules judged to be multiple by palpation (7).

Risk factors for the development of thyroid nodules include female sex, pubertal age, family history of thyroid disease, previous or coexisting thyroid disease, history of a medical condition that may be steroid-related or endocrine-related, and previous radiation exposure (8).

Thyroid Cancers

Thyroid cancers, particularly, papillary cancers are found at autopsy in a large minority of individuals, who died of other causes. The prevalence varies from 2% in Guatemala to 35.6% in Finland (9,10). The autopsy prevalence of thyroid cancers is relatively low in children and adolescents. The prevalence in Finnish youth less than 20 years of age is 13.6% and no cases were detected in Finnish children less than 10 years of age (11).

According to the 2005 U.S. Cancer Statistics, estimated new thyroid cancer cases are 25,690 per year (6500 in males and 19,190 in females). Thyroid cancer causes 1490 (630 in males and 860 in females) deaths each year. The incidence of thyroid cancer in children and adolescents has been estimated to be between 0.2 and 3 cases per million per year (12,13).

Thyroid carcinoma is the third most common solid malignancy of childhood. Differentiated thyroid carcinoma is the most common pediatric endocrine tumor and constitutes 0.5% to 3% of all childhood malignancies (14). Thyroid is one of the most frequent sites of secondary neoplasms in children who receive radiation therapy for other malignancies. Differentiated thyroid carcinoma has been studied extensively in adults; however, the pediatric literature on differentiated thyroid carcinoma is much less complete. Thyroid cancer in adults is two to four times more frequent in females than in males (15). This female predominance is less evident in children and is not observed in children less than 11 years of age (13).

PREDISPOSING FACTORS

Environment

The best-established environmental risk factor promoting thyroid cancer is radiation exposure (Vol. 2; Chap. 31). During the 1st half of the 20th century, when radiation treatment was used in benign conditions such as thymic enlargement of infancy, tonsillar and adenoid infections and tinea capitis, approximately 36% of the pediatric pateints diagnosed with thyroid cancer were exposed to radiation treatment earlier in life (17). The incidence of thyroid

cancer in children decreased after discontinuation of radiation for these indications; however, the incidence of thyroid nodules was unchanged.

Hancock et al. (16) reviewed the records of 1677 patients with Hodgkin's disease who received radiation treatment and found that 573 had evidence of thyroid disease. In addition to increased risk for hypothyroidism and Grave's disease in these patients, 44 patients were found to have thyroid nodules, six of whom had papillary or follicular thyroid cancers. The risk of thyroid cancer was 15.6 times than in the normal population. A decade later, Sklar et al. (17) confirmed similar findings of increased thyroid disease in this population. In their study, Hodgkin's disease survivors had thyroid nodules 27 times more frequently than did their siblings. Among 1791 patients studied, the relative risk of developing thyroid cancer was 18.3-fold greater than the risk in the general population. Ninety-five percent of the Hodgkin's disease patients who developed thyroid cancer in this study received irradiation doses $\geq$1000 cGy. Other studies have reported the development of thyroid cancer even when lower doses of radiation are used. A recent study in cancer survivors after radiation therapy suggested that the absolute risk for thyroid neoplasms was increased in females; the female to male ratio was 11:2. The interval from irradiation to the detection of tumor was between 6 and 30 years (18).

A number of reports suggest that patients undergoing bone marrow transplantation preceded by radiation therapy are at an increased risk of developing thyroid cancer (19,20). There are also reports of thyroid adenoma/carcinoma developing after treatment for childhood leukemia and other malignancies (21,22). Therefore, these children should be followed closely for the development of thyroid cancer.

During the Chernobyl accident in 1986, large populations were exposed to radioactive iodine. An increase in thyroid cancer among exposed children was detected as early as four years after the accident (23). Short-latency-period tumors resulted from oncogenes such as RET-PTC-3, which cause a high growth rate, structural de-differentiation, and tumor aggressiveness. In comparison, long-latency tumors resulted from oncogenes such as RET-PTC-1, which caused a slower tumor growth rate, greater differentiation, and less aggressiveness (24). Pacini et al. (25) compared the post-Chernobyl thyroid carcinoma in Belarus children and adolescents with the naturally occurring thyroid carcinoma in children and adolescents in France and Italy. Thyroid cancers affecting the Belarus children were less influenced by gender, occurred in younger children, had greater aggressiveness at presentation, and were more frequently papillary and more frequently associated with thyroid autoimmunity.

In iodine-replete areas, thyroid cancers are usually papillary, whereas tumors in iodine-deficient areas tend to be follicular or anaplastic. The ratio of papillary to follicular thyroid cancer ranges from 3.4 to 6.5:1 in areas with high iodine intake, 1.6 to 3.7:1 in areas with moderate iodine intake, and significantly decreased to 0.19 to 1.7:1 in iodine-deficient areas (26).

Another environmental factor predisposing to thyroid cancers is the residence in a volcanic area. Hawaii and Iceland have high incidence of thyroid cancers (27).

Thyroid carcinoma may occur in the context of autoimmune disease. Up to 38% of patients with thyroid cancer can have coexistent Hashimoto's thyroiditis (28). A recent study showed that BRAF mutations, which are significantly correlated with age, might be a predictor for the progression of Hashimoto's thyroiditis to papillary cancer (29). Cancer rate is estimated to be between 1% and 9% in patients with Grave's disease (30). Therefore, prominent nodularity in the thyroid gland of a patient with autoimmune thyroiditis warrants evaluation.

Recently, two patients with Turner's syndrome were reported to have papillary thyroid carcinoma after growth hormone treatment. The investigators detected Growth hormone receptor expression in the papillary carcinoma cells (31).

Genetics

Nonmedullary thyroid carcinoma is less frequently inherited than is medullary thyroid carcinoma. Up to 5% of nonmedullary cancers are inherited (32). Heritable nonmedullary thyroid cancers include oxyphilic papillary cancers. Classic papillary thyroid carcinoma may be heritable without other abnormality or in association with nontoxic multinodular goiter (33,34). A recent analysis from the Swedish National Database showed a high predisposition for thyroid papillary carcinoma, especially among the sisters of probands with papillary carcinomas. Thyroid adenocarcinoma was noted to be associated with melanoma and with connective tissue tumors. Associations were also found between thyroid carcinoma and cancers of the colon, breast, ovary, and kidney. The number of Hurthle cell tumors was too small to conclude an association between them and Hodgkin's and non-Hodgkin's lymphoma despite suggestive findings (35).

These inherited nonmedullary thyroid cancers may occur in a number of heritable syndromes. Familial adenomatous polyposis (FAP) is an autosomal dominant precancerous condition. It is characterized by multiple adenomatous polyps of the colon and rectum, osteoma of the bones (in a subtype referred to as ''Gardner syndrome''), epidermoid cysts of the skin, desmoid tumor of the abdominal wall, congenital hypertrophy of the retinal pigmented epithelium, and thyroid carcinoma. FAP is caused by germline mutations of the *APC* gene. The *APC* gene is a tumor suppressor gene located on chromosome 5 (5q21–22). The incidence of FAP is one case per 10,000 people.

One to 2% of patients with FAP have thyroid carcinoma; this occurs in female patients (85%) and may be found in patients younger than 30 years of age. More than 95% of FAP-associated thyroid cancers are papillary, and some show a cribriform pattern. The thyroid carcinoma occurs in mutations occurring within codons 1 to 1220 of the *APC* gene. The mutation in the *APC* gene causes activation of β-catenin, a signal transducing molecule, which is thought to contribute to the development of the cribriform–morular variant of papillary thyroid cancer seen in this syndrome (36). Tomoda et al. (37) suggested that the presence of cribriform–morular variant should prompt a suspicion of FAP, and that it can thus lead to the early detection of colon cancer. In the same study, three out of seven patients with the cribriform–morular variant had FAP, two of these three patients with colon polyposis had bilateral multiple thyroid tumors whereas others without polyposis had a solitary tumor. Two of the three patients with familial polyposis were asymptomatic at the time of diagnosis.

Another hereditary setting for nonmedullary thyroid cancers is Cowden disease, an autosomal dominant condition presenting with hamartomas and other benign tumors of the skin. The most characteristic features are facial papules (trichilemmomas and verrucae), oral papillamatosis, and palmoplantar keratoses (Fig. 1). Malignancies associated with Cowden disease include thyroid cancer in 7% of patients as well as breast carcinoma, non-Hodgkin's lymphoma, melanoma, colon and uterine adenocarcinoma, basal cell skin carcinoma, and acute myelogenous leukemia. The genetic cause is a germline mutation of the tyrosine phosphatase tumor suppressor gene PTEN on chromosome 10q23.3. PTEN is a lipid phosphatase; its enzymatic activity primarily serves to remove phosphate groups from key intracellular phosphoinositide signaling molecules. This activity normally serves to restrict growth and survival signals by limiting the activity of the phosphoinositide-3 kinase pathway. The loss of PTEN activity results in constitutive activation of the pathway leading to unrestricted cell growth. The thyroid abnormalities present as early as in childhood. Eighty-five percent of Cowden disease patients with thyroid disease present with multinodular goiter (38). Significant thyroid lesions are multicentric follicular adenomas and adenomatous nodules showing a wide range of nonspecific cytoarchitectural patterns. Lesions may comprise oxyphil or clear cells, hyalinizing trabecular adenoma, or adenolipoma. Most of the thyroid cancers are of papillary or Hurthle cell types. Occasionally, follicular carcinomas, in addition to multiple benign follicular cell proliferations, occur in this setting. Harach et al. (39) suggested that multiple adenomatous goiters or multiple follicular adenomas occuring in children and young adults should alert the pathologist and physician to the possibility of an inherited trait such as Cowden disease. The tumors are usually benign and well demarcated, but because of multi-centricity and increased risk of recurrence or progression to carcinoma, total thyroidectomy is advocated.

Bannayan–Ruvalcaba–Riley syndrome (BRRS), which is also secondary to germline point mutation in the PTEN gene, is characterized by neonatal-onset macrocephaly, mental retardation, Hashimoto's thyroiditis, lipomatosis, hemangiomas, hamartomatous polyps and pigmented macules of the glans penis, and the risk of developing benign and malignant tumors of the breast and thyroid gland. Mostly, follicular adenomas of the thyroid and papillary thyroid carcinomas are seen. Zambrano et al. (40) suggested that PTEN-associated tumor syndrome should be considered in the differential diagnosis of thyroid C-cell hyperplasia after describing two cases of overlapping Cowden disease (CD)/BRRS phenotypes with C-cell hyperplasia.

Carney complex is an autosomal dominant syndrome, which includes spotty skin pigmentation, myxomas, endocrine overactivity, and schwannomas. Pigmented skin lesions include pinpoint brown to black macules (also present on mucosal membranes), café-au-lait spots, and blue nevi (Fig. 2). Myxomas occur in heart, skin, breast, and in other tissues. Multiple endocrine organs may be affected. Micronodular adrenocortical disease produces Cushing syndrome. Other endocrinopathies include growth hormone secreting pituitary tumors, male precocious puberty caused by hormonally active testicular tumors, and thyroid abnormalities. Thyroid abnormalities include hyperthyroidism, thyroid nodules in 67% of the patients and thyroid carcinoma in 3.8% (41). Thyroid

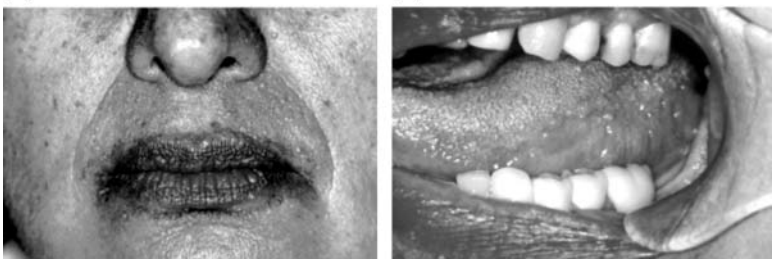

Figure 1 Cowden disease has a number of skin and mucous membrane hamartomas. Facial trichilemmomas are seen on the left. Cobblestone-like papules are seen on the buccal mucosa at the base of the tongue.

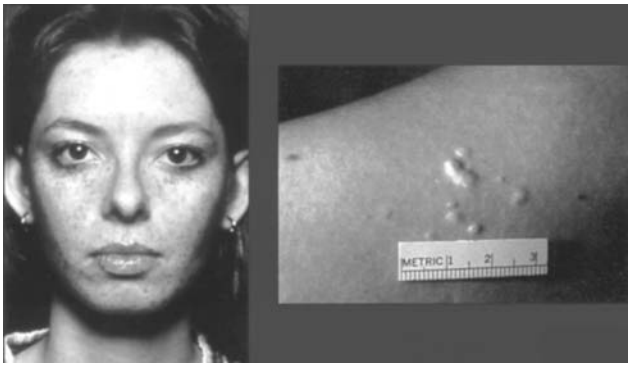

Figure 2 Principal skin findings in Carney complex (myxoma, spotty pigmentation, and endocrine overactivity) include ephelides or melanin spots in and around the lips.

carcinoma may be papillary or follicular. This condition is linked to at least two chromosomal loci. One locus is the gene for the Type 1A regulatory subunit of protein kinase A (PPKAR1A).

Patients with ataxia-telangiectasia are at higher risk to develop primary carcinomas including those of the thyroid (mostly papillary or follicular) and parathyroid. Patients with tuberous sclerosis also tend to have a higher incidence of thyroid cancers.

The vast majority of medullary thyroid cancers detected in childhood are components of genetic syndromes: multiple endocrine neoplasia Type 2 or familial medullary thyroid carcinoma (FMTC) (Vol. 2; Chap. 27). Medullary thyroid carcinoma comprises 5% of childhood thyroid carcinomas. Multiple endocrine neoplasia (MEN) Type 2A includes multicentric and usually bilateral medullary thyroid carcinoma, unilateral or bilateral pheochromocytoma, and hyperparathyroidism due to parathyroid hyperplasia or adenoma (Vol. 2; Chap. 27). Some patients have cutaneous lichen amyloidosis. MEN Type 2B includes medullary thyroid cancer, pheochromocytoma, mucosal neuromas of the alimentary tract and subconjunctival areas, and skeletal abnormalities including marfanoid habitus, pectus excavatum, and slipped capital femoral epiphysis (Fig. 3). MTC in MEN Type 2B presents at younger ages than does that in MEN 2A and in FMTC; not infrequently, it is detectable in infancy. MEN 2B-associated medullary thyroid cancer is also more aggressive than the medullary thyroid cancer associated with MEN 2A or with familial thyroid carcinoma. MTC occurring in all age groups is inherited in approximately 25% of cases (43).

PATHOLOGY

Benign Thyroid Nodules

Follicular adenomas, adenomatous nodules, Hurthle cell adenoma, lymphocytic thyroiditis, and thyroglossal duct cyst are among the benign thyroid nodules. Some benign thyroid nodules even can cause over activation of the gland or compression signs of the nearby organs in addition to causing cosmetic problems. Some nodules associated with Hashimoto thyroiditis are also associated with hypothyroidism.

Adenomas are identified using the following morphologic criteria: complete fibrous encapsulation, clear distinction in architecture between the inside and outside of the capsule, compression of the thyroid parenchyma around the adenoma, uniformity within the internal architecture, and the absence of

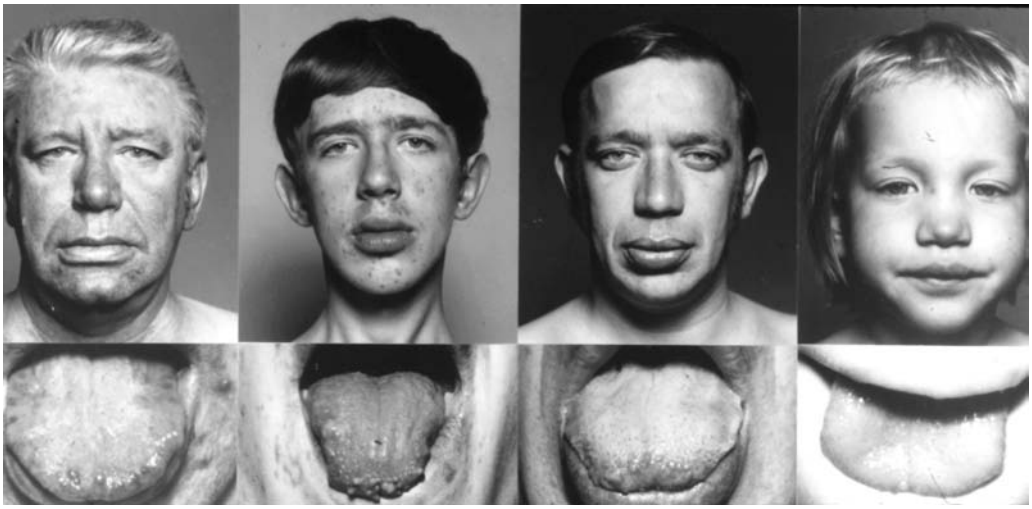

Figure 3 MEN 2B is characterized by a Marfanoid body habitus and by characteristic facies. The facies include thickened lips with nodules due to ganglioneuromas. The tongue has nodular ganglioneuromas, as do the eyelids.

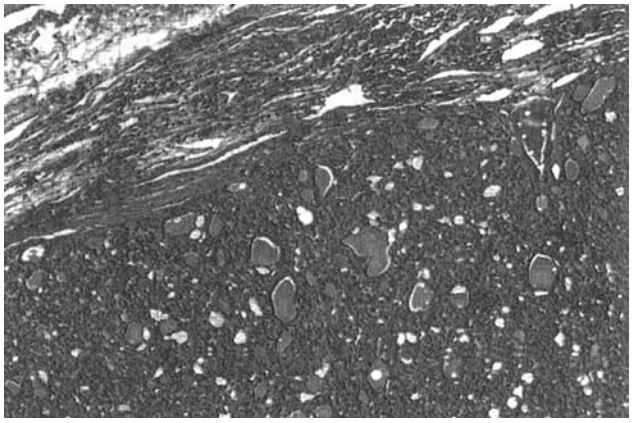

Figure 4 Follicular adenomas of the thyroid are encapsulated and have a uniform structure.

multi-nodularity in the remaining thyroid gland (44). On macroscopic examination, adenomas may measure from 1 mm to several cm in diameter. All adenomas can be classified under follicular adenomas (Fig. 4).

Some adenomas are atypical and might be hypercellular with some mitotic figures. In such cases, careful attention is required to differentiate from carcinoma. Follicular adenomas with papillary hyperplasia some of which can be functional should be classified as papillary hyperplastic nodules. An adenomatous nodule, also known as colloid or hyperplastic nodule, is composed of large and small follicles usually with some amount of colloid (Fig. 5). They may contain giant follicles or colloid cysts of irregular shapes. Cyst formation tends to accompany the formation of papillae. The cells may be columnar to cuboidal to flat. They are uniform, rounded, and small. The stroma is loose and edematous with signs of chronic inflammation such as macrophage groups, hemosiderin, fibrosis, and even calcifications. Hyalinizing trabecular adenoma is a small and well-circumscribed tumor. Trabecular and nesting patterns with elongated cells are seen surrounding hyaline connective tissue. Cytoplasmic inclusions might be seen within the nuclei. Small psammoma bodies might be visualized. Gradation between typical follicular adenomas, trabecular adenomas, and hyalinazing trabecular adenomas can be seen (45). Hurthle cell nodules are diagnosed when more than 75% of the cells composing the nodule are of this cell type (also known as Askenazy or oncocytic cells). Accumulation of altered mitochondria leads to cellular enlargement with increased eosinophilic granular cytoplasm. Oncocytic changes may also occur in inflammatory disorders such as thyroiditis. With the hematoxylin and eosin stain, Hurthle cells appear as large polygonal to square cells with distinct cell borders. The cytoplasm appears to be large, granular, and eosinophilic, whereas the nucleus appears to be hyperchromatic with cherry-pink macronucleoli. Hurthle cell adenomas are encapsulated lesions without evidence of capsular or vascular invasion or of papillary carcinoma (46). Toxic adenomas, also known as hyperfunctioning adenoma or Plummer adenomas, are follicular adenomas with a clinical evidence of usually mild degree of hyperfunction. In this type, the cells more likely to be tall-cuboidal and the follicles are of small or normal size. Approximately 1% of thyroid adenomas are toxic adenomas. The diagnosis of toxic adenoma is based on clinical findings rather than on microscopic findings.

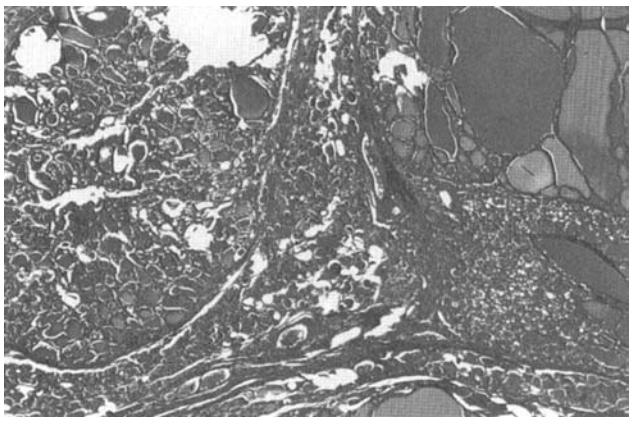

Figure 5 Benign adenomatous nodules have more varied internal structures than follicular adenomas.

Thyroid Cancers

Some thyroid cancers arise from the thyroid follicular epithelium-papillary, follicular, and insular carcinomas. This epithelium produces thyroglobulin, thyroxine, and triiodothyronine. Insular thyroid carcinoma is less well differentiated than are follicular and papillary carcinomas. Medullary cancers arise from C-cells that derive from the ultimobranchial bodies. These cells are located between the basal lamina and the follicular epithelial cells. Unlike follicular epithelial cells, C-cells do not extend into the follicular lumen. They produce calcitonin in addition to a number of other peptides such as somatostatin and calcitonin gene-related peptide but do not make thyroid hormone or thyroglobulin.

Papillary Thyroid Carcinomas

These make up approximately 72% of the thyroid cancers in children (47). The tumors often infiltrate surrounding tissue and are only infrequently encapsulated. Most contain papillae with a fibrovascular core and a single layer of typical epithelial cells (Fig. 6).

Often this pattern forms only a small proportion of the tumor. The epithelial cells contain large

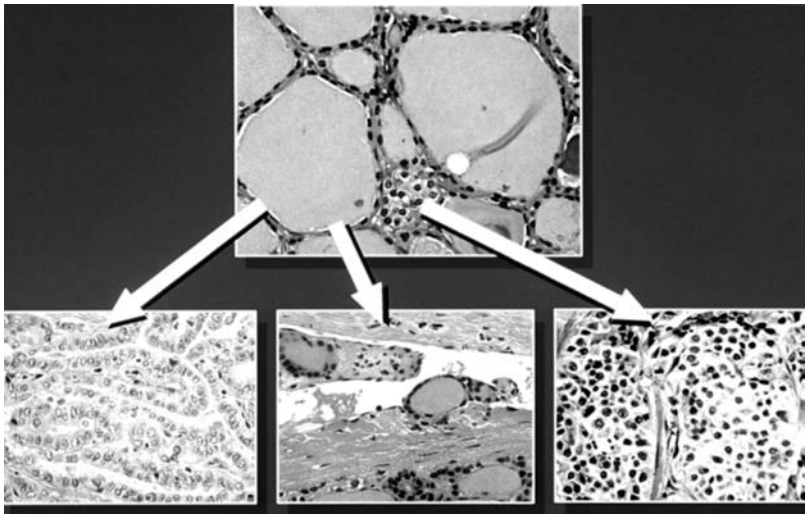

Figure 6 Although both papillary (*bottom left*) and follicular carcinomas (*bottom center*) are derived from the thyroid-hormone-producing cells of the thyroid follicle, medullary thyroid carcinoma (*bottom right*) is derived from the calcitonin-producing cells of the interstitial areas between thyroid follicles. *Source*: Courtesy of the Mayo Clinic Foundation.

irregular nuclei that are folded and indented with cytoplasmic inclusions. Because the nuclear heterochromatin is concentrated near the nuclear membrane, the central portion has a pale ground-glass appearance. This appearance, which is also called orphan-Annie-eyed nuclei, is well seen in fixed tissue but not in frozen section. The nuclei are often aligned similarly on the basal or apical part of the cell and, therefore, overlap one another. The nuclei of the epithelial cells are irregular, large, and are folded and indented with apparent intranuclear inclusions that comprise invaginations of cytoplasm. Psammoma bodies, which are found in 40% to 50% of these tumors, are focal calcifications (48). Their presence in lymph nodes is a strong sign of metastasis, and the lymphatic route is the principle route of metastasis of PTC. PTCs are usually un-encapsulated.

PTC has several subtypes. Children less than 10 years of age appear to have a distinctive ''childhood type of papillary thyroid cancer,'' in which the nuclei are more rounded rather than elongated, smooth rather than grooved, and not crowded or overlapping. The predominant pattern of this type of papillary cancer is solid rather than pure papillary or a mixture of papillary and follicular. Another variant of papillary thyroid carcinoma occurring most commonly in younger patients is the diffuse sclerosing type (Fig. 7). The tumor involves all lymphatics of one or both thyroid lobes and is associated with severe lymphocytic thyroiditis and with interstitial fibrosis. The clinical course is more aggressive with more frequent lymph node and pulmonary metastasis. Other patterns found in papillary cancer include follicular, trabecular, and cribriform. In the ''follicular variant of PTC'', the most common variant, the neoplastic cells have the same nuclear features as in the typical PTC. However, follicular structures are interspersed among papillary structures. The clinical behavior is similar to pure PTC. Tall cell variant and the columnar cell variant are rare and more aggressive types of PTC. ''In the tall cell variant'', papillae are well formed with the height of cells being at least twice their width. ''In the columnar type'', there is prominence of nuclear stratification. ''Papillary thyroid microcarcinoma'' is the term given to PTC 1 cm or less in diameter. Even though the autopsy incidence is as high as 36%, the clinical diagnosis is 1 per 100,000 (49).

Papillary thyroid cancers are frequently multifocal and bilateral in up to 80% of cases (50). Papillary thyroid carcinoma metastasizes to cervical lymph nodes in 90% of affected children and to lungs in approximately 7% (51). Only rarely does the tumor invade blood vessels or metastasize to distant sites other than to lung.

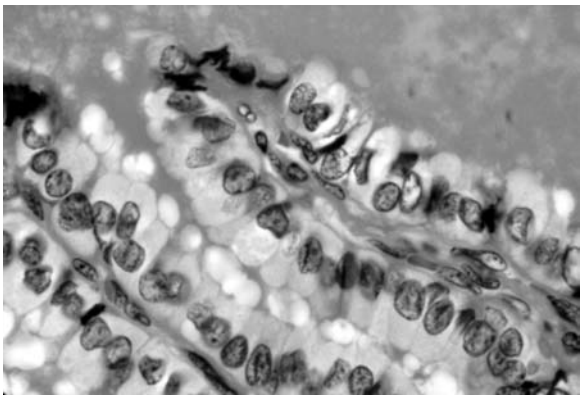

Figure 7 The finger-like projections, or papillae, of papillary thyroid carcinoma have a fibrovascular core and single layer of epithelial cells located basally. The nuclei appear to overlap.

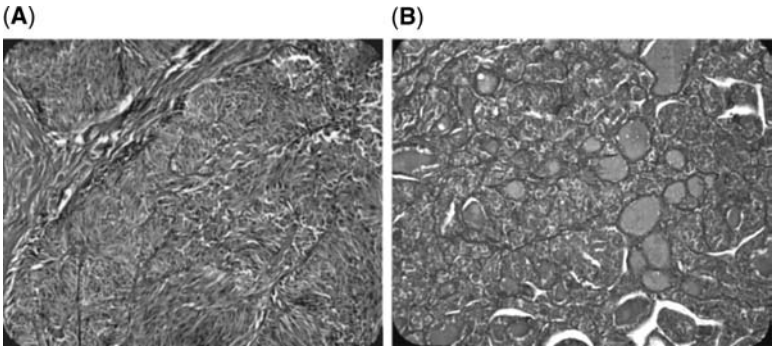

Figure 8 Follicular thyroid carcinoma frequently invades blood vessel walls.

Follicular Carcinoma of the Thyroid

These make up 18% of the thyroid cancers in childhood (47). FTC is found in higher prevalence in areas with iodine deficiency. This could be one of the reasons FTC has decreased in the United States in recent years. It is more common among African-Americans than in Asians or Caucasians. It is usually unifocal and unilateral. Grossly, the tumor may contain foci of hemorrhage, fibrosis, or calcification. These tumors may be well circumscribed and may be hard to differentiate from adenomas.

Diagnosis is made by observing invasion of the capsule or of structures beyond the capsule such as blood vessel walls (Fig. 8).

Histologically, FTC has a solid growth pattern and is composed of uniform cells forming small follicles containing colloid. It may look quite similar to normal thyroid. In other cases, follicular differentiation may be less apparent. Unlike PTC, FTC metastasizes by the vascular route to the lungs, liver, bone, and brain.

One important variant of follicular thyroid cancer is the oncocytic cell type (also called oxyphilic or Hurthle cell type). The cytoplasm is granular and eosinophilic because of the presence of a large number of mitochondria (Fig. 9). These tumors exhibit intrathyroidal extension, and lymph node and distant metastases more frequently than do other follicular carcinomas.

Insular Thyroid Carcinoma

These are poorly differentiated carcinomas that are very rare in childhood. Most patients with these tumors are older than 50 years old. The youngest patient described with insular carcinoma is a 10 year-old female (52). We follow a patient with insular carcinoma who was nine years-old at the time of diagnosis; and the thyroid nodule of the patient was presented clinically at the age of eight years. Clinically, insular thyroid carcinoma presents as a rapidly growing mass, which may produce symptoms of pressure such as dyspnea, hoarseness, and dysphagia. Approximately two-thirds of insular thyroid carcinomas may present with papillary or follicular features. Under the microscope, it presents as small follicles within solid clusters of tumor cells and foci of necrosis. The prognosis is not as well delineated as are those of more common thyroid tumors because of the relatively small case series, but it is poorer than the prognosis of differentiated carcinomas. The recurrence rate may be as high as 80% (52). Most insular cancers present with involvement beyond the thyroid gland. Regional lymph node, lung, and bone metastases are common, and these often lead to the patients' death. Therefore, aggressive treatment is warranted. Thyroglobulin can be used as a marker for tumor recurrence. The tumor

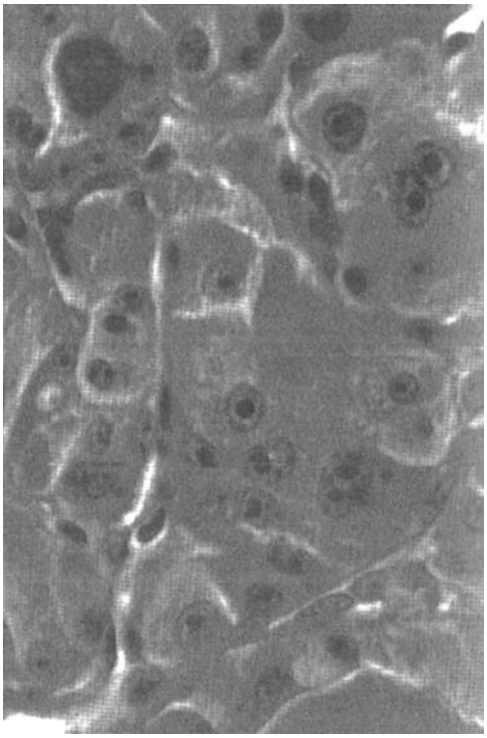

Figure 9 Oncocytic cell thyroid carcinoma has granular cytoplasm that is intensively eosinophylic.

appears to be intermediate in aggressiveness between differentiated cancers and anaplastic cancers (53).

Anaplastic Carcinoma

This is an extremely aggressive tumor and has been described as comprising 2.6% of childhood thyroid cancers (47). Insular carcinomas have islands of small, dense cells that aggregate in solid, follicular, or papillary patterns.

Medullary Thyroid Carcinoma

Medullary thyroid carcinoma make up approximately 2.6% of childhood thyroid cancers (47). These tumors arise most frequently at the junction of the upper one-third and the lower two-thirds of the thyroid gland: the thyroid region most densely populated with C-cells. The cells in this tumor may be round, spindle shaped, or polygonal and form sheets separated by fibrous stroma (Fig. 10).

It may have papillary or follicular patterns. Between the cells, amyloid, calcifications, or psammoma bodies may be seen. Sixty to 80% of the tumors contain amyloid. FNA cytology shows lack of cellular cohesiveness. The C-cells forming medullary carcinoma produce calcitonin and carcinoembryonic antigen in addition to a number of other substances.

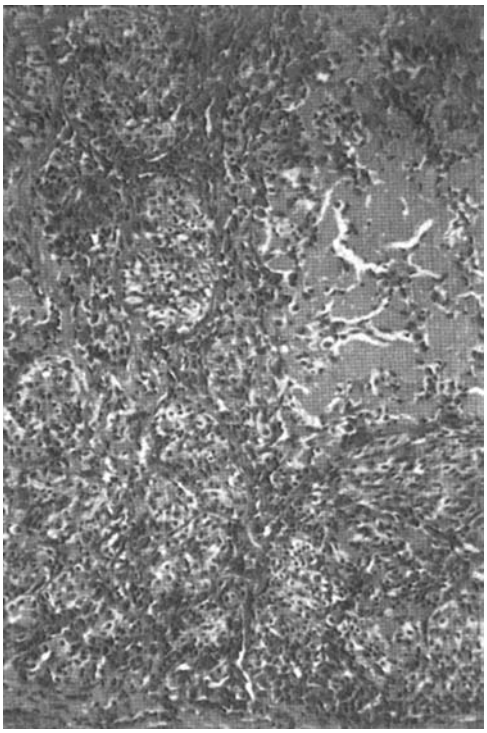

Figure 10 The C-cells that make up medullary thyroid carcinoma may be round, spindle shaped, or polygonal. They form sheets separated by fibrous stroma and may produce amyloid.

Medullary tumors can be immunostained for calcitonin if adequate material is available. If less than 10% of a tumor stains for calcitonin and if necrosis is prominent, the 10-year survival is reduced significantly (54). FMTCs are multicentric, bilateral, and are associated with C-cell hyperplasia. In the sporadic form, tumors tend to be solitary.

Others

Other cancers of the thyroid gland include the thyroid lymphomas that arise from intrathyroidal lymphocytes. Thyroid teratomas are found most commonly in the newborn. Large teratomas may be associated with polyhydramniosis because they may interfere with fetal swallowing of amniotic fluid.

PATHOGENESIS

Thyroid Nodules

Multiple genes have been implicated in the pathogenesis of thyroid nodules. Approximately 12% of thyroid nodules are autonomously functioning; they produce thyroid hormone in the absence of thyroid-stimulating hormone (TSH). A large majority of these nodules are caused by activating mutations of the TSH receptor. These changes in the TSH receptor molecule result in adenylate cyclase activation in the absence of TSH. Less commonly, autonomous nodules can be due to Gsa mutations (55). Clinical and experimental data suggest low thyroid hormone synthesis in cold thyroid nodules (CTNs). The first step in thyroid hormone synthesis, iodine trapping by the Na^+/I^--symporter (NIS), was studied as to examine its role in the pathogenesis of cold nodules. Compared to the surrounding tissue, a significantly reduced NIS expression was detected in 86% of CTNs (56). Some cold nodules have mutations in the RET-proto-oncogene.

A common sequence of events leading to nodular formation is as follows: (i) Diffuse thyroid hyperplasia occurs as a result of TSH hypersecretion triggered by iodine deficiency or other goitrogens. (ii) In the proliferation stage, growth factor expression (such as Insulin growth factor-1 and Epidermal growth factor) is increased. (iii) During this stage, because of increased proliferation, mutagenesis is increased, leading to more cells with mutations. This results in cell division and formation of new clones. (iv) After the discontinuation of the increased growth factor expression, the small clones with mutations may transform into small foci developing into thyroid nodules if adequate self-stimulation from the expression of growth factors has been received (57).

Thyroid Cancers

The tumorigenesis of cancers can be explained mainly by two mechanisms: activation of proto-oncogenes and inactivation of tumor suppressor genes. In thyroid

cancers, the most frequent proto-oncogenes include mutations of the *RET* gene in papillary and medullary thyroid cancer, and of the *BRAF* gene in papillary thyroid cancer. Tumor suppressor genes involved in follicular thyroid carcinoma are *p53* and PTEN. Angiogenesis stimulators or inhibitors also appear to facilitate tumor growth.

Mitogen-activated protein kinases (MAPK) are widely expressed serine-threonine kinases. The members of various MAPK groups contribute to the generation of different cellular responses such as gene transcription, induction of cellular death, maintenance of cell survival, malignant transformation, and regulation of cell-cycle progression. Mutations causing constitutive activation of effectors along the MAPK pathway play a critical role in the formation of papillary thyroid cancers. Once activated by signals arising from the interaction with peptide ligands, transmembrane tyrosine kinase receptors activate the monomeric G-protein RAS via autophosphorylation. This, in turn, binds to BRAF, a serine-threonine kinase which induces the sequential engagement of a series of phosphoproteins that, in turn, induce MEK and ERK. Induction of MEK and ERK results in transcriptional upregulation of target genes and promotion of cell-cycle progression and cell division. In papillary thyroid carcinomas, mutations of six different genes that code for effectors signaling in this pathway have been described. Intrachromosomal inversions or translocations of the *RET* and *NTRK* genes may lead to constitutive activation of these kinases (Fig. 11).

RET is located on chromosome 10q11 and is not normally expressed in thyroid follicular cells. It is found in tissues derived from the neural crest. RET is a transmembrane tyrosine kinase; its extra cellular portion is stimulated by proteins such as, glial cell line-derived neurotrophic factor, and similar ligands such as neurturin, artemin, and persephin, members of the transforming growth factor β family (Fig. 12).

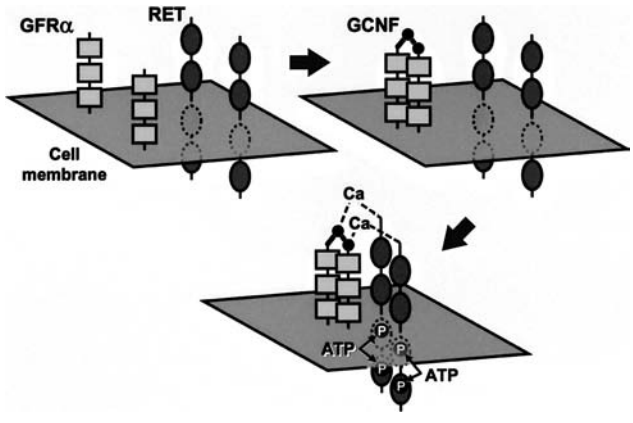

Figure 11 The RET proto-oncogene is a transmembrane molecule. It collaborates with specific receptors for each of the members of the glial cell line-derived neurotrophic factor family (e.g., GCNF receptor depicted by a line with square domains) in transducing signals to the interior of the cell. *Abbreviation*: ATP, adenosine triphosphate.

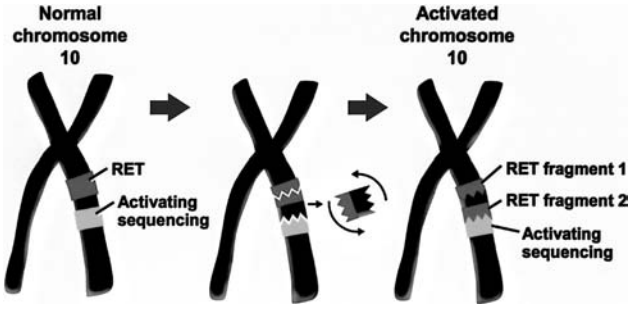

Figure 12 Activation of the RET in papillary thyroid carcinoma results from chromosomal rearrangements involving the 10th chromosome (which contains RET gene). This diagram shows an inversion of a segment of chromosome 10 resulting in RET activation.

These ligands interact with specific receptors (GFR) α-1, -2 and -3. The combined ligands and receptors, in turn, interact with and activate RET. The expression of RET in follicular cells is dependent on chromosomal rearrangements, which appose regulatory subunits of other genes to the tyrosine kinase domain of the *RET* gene. In addition, these oncogenes code for protein coiled-coil domains that predispose to constitutive dimerization of the proteins with one another and, in this manner, activate the tyrosine kinase. RET/PTC oncogenes are thought to play an important role in the pathogenesis of two subsets of the papillary thyroid cancer: those arising in the pediatric population and those arising secondary to radiation exposure. Somatic rearrangements involving the *RET* gene portion of chromosome 10 lead to activation of the *RET* gene in thyroid follicular cells, in which this is normally inactive. Some *RET* rearrangements also involve translocation of a fragment of chromosome 10 to a different chromosome such as chromosome 17. RET/PTC are formed by attachment of the tyrosine kinase domain of RET to the five-prime sequence of other genes. Ten *RET/PTC* genes have been described so far. *PTC/RET* genes 1 and 3 are the most common. Attachment to five-prime sequence of H4 is seen in RET/PTC1; attachment to ELE1 is seen in PTC3. These combinations result in ligand-independent dimerization and constitutive activation of these RET proteins. RET/PTC is believed to be one of the key steps in thyroid cancer pathogenesis for the following reasons: (i) There is a high prevalence of RET/PTC mutations in occult and microscopic PTC, which suggests its activation in the early stages of the tumor. (ii) In transgenic mice, the thyroid-specific over-expression of RET/PTC1 or PTC3 leads to the development of tumors with histologic features of PTCs. (iii) RET/PTC rearrangements are induced

within hours when cell lines and fetal thyroid extracts are exposed to ionizing radiation. (iv) The breakpoints in RET/PTC3 expressed in radiation-induced pediatric thyroid cancers from Chernobyl are consistent with direct double-strand DNA breakage resulting in illegitimate reciprocal recombination. (v) Even though the *H4* and the *RET* genes are quite far from each other on chromosome 10, during interphase they are spatially juxtaposed. This probably presents a target for simultaneous double-strand breaks in each gene after exposure to ionizing radiation (58).

Bounacer et al. (59) have demonstrated that RET rearrangements are also present in patients receiving radiation treatment for therapeutic reasons. In childhood PTCs due to radiation exposure, the incidence of RET mutations is reported to be between 50% and 70%, whereas in sporadic adulthood thyroid carcinomas, the incidence is between 5% and 30% (60). Nikiforov et al. (61) demonstrated that, in children with radiation-induced PTCs, the solid variant of papillary carcinoma was found in 37%, follicular in 29%, typical papillary in 18%, and mixed and diffuse sclerosing variants in 8%. In the sporadic group, the solid variant was found in 4%, diffuse sclerosing in 9%, follicular in 17%, and a typical papillary pattern in 70%. The prevalence of RET rearrangements was high in both groups, with significant differences in the frequency of specific types of rearrangements. RET/PTC3 was found in 58%, RET/PTC1 in 16%, and RET/PTC2 in 3% among radiation-induced tumors. Among sporadic tumors, RET/PTC3 was found in 18% and RET/PTC1 was found in 47%. They also observed differences in the morphological variants of papillary carcinoma in specific types of RET rearrangement. Seventy-nine percent of solid variant tumors had RET/PTC3, whereas only 7% had RET/PTC1. Among typical papillary tumors, RET/PTC3 was found in 19%, RET/PTC1 in 38%, and RET/PTC2 in 5%. However, another study failed to demonstrate evidence for a predominant occurrence of the solid subtype in PTCs with RET rearrangements (62). The same group also suggested that RET/PTC3 may be typical for radiation-associated childhood PTC with a short-latency period, whereas RET/PTC1 may be a marker for later-occurring PTC of radiation-exposed adults and children (62).

NTRK1 gene encodes the receptor for nerve growth factor and plays an important role in the development of the nervous system. The gene for this proto-oncogene is on chromosome 1q22. Oncogenic activation of the NTRK1 follows the fusion of the NTRK1 TK domain and the 5′ sequences of other genes leading to constitutive activation. NTRK1 mutations occur through intrachromosomal inversions or translocations. NTRK1 rearrangements are less frequent in papillary thyroid carcinoma (12%) than are RET mutations (46%). Both are more common in younger patients than in older ones (63,64).

RAS proteins are known to regulate cell growth, differentiation, and apoptosis, and to induce processes such as cell migration and neuronal activity. RAS proteins are guanosine triphosphate (GTP) -binding proteins that regulate a number of signaling molecules by translocating them to the plasma membrane for activation. Constitutively active forms of RAS protein result from mutations in the guanosine (G) codons G12 and G13 in the GTP-binding domain and G59 and G61 in the GTPase domain via converting inactive GDP-bound RAS to the active GTP-bound state. Point mutations in the RAS genes (H-ras, K-ras, and N-ras) are less prevalent, and the data regarding its role in thyroid carcinogenesis of PTCs are variable. A recent study failed to demonstrate RAS mutations in papillary thyroid carcinomas (65). Fenton et al. (66) demonstrated RAS mutations in 6.5% of sporadic papillary thyroid cancers in children. The follicular variant of papillary thyroid carcinoma, which is characterized by higher prevalence of tumor encapsulation, angiovascular invasion, and poorly differentiated areas and lower rate of lymph node invasion, has a higher incidence of RAS mutations (43%) (67). Differences in methodologies or environmental factors such as exposure to radiation and iodine deficiency could account for these different findings (66). Other studies suggest that mutation of N-ras gene at codon 61 is an independent prognostic factor for aggressiveness of papillary thyroid carcinomas (68).

In adults, *BRAF* mutations are the most common mutations in papillary thyroid carcinomas and account for 36% to 69% of the cases (69). The majority of these mutations consist of a T to A transversion at nucleotide 1796 in exon 15 on the *BRAF* gene, resulting in a valine-to-glutamine substitution at residue 599. This mutation leads to constitutive activation of the BRAF leading to increased activity through the MAPK pathway, in turn, resulting in cellular proliferation. Several studies have failed to demonstrate BRAF mutations in other types of thyroid carcinoma.

It is also suggested that increased frequency of the BRAF mutations in adults but not in children could account for the age-relevant prognostic difference in adults and children with papillary thyroid carcinoma (70).

A recent study failed to show any BRAF mutations in PTC patients younger than 21 years of age. However, only the most common mutation site was examined. Therefore, mutations in other sites of the *BRAF* gene could not be excluded (71).

Ciampi et al. (69) demonstrated a chromosomal rearrangement in papillary thyroid cancers leading to BRAF fusion to *AKAP9* gene. This fusion protein lacks the regulatory domains of BRAF and functions as an oncogene, resulting in RAS-independent constitutive kinase activation. This mutation was harbored particularly in children with histories of radiation exposure.

No overlap among the RET/PTC, BRAF, and RAS mutations have been shown; all together they account for approximately 70% of papillary thyroid carcinomas.

The *PAX8* gene, which encodes a thyroid transcription factor, plays an important role in thyroid

differentiation, growth, and function. Translocation t(2;3) (q13;p25) leads to PAX8–PPARγ (peroxisome proliferator-activated receptor) fusion and its overexpression in thyroid follicular cells. This fusion oncoprotein acts as a dominant negative inhibitor of the wild-type PPARγ. Over 60% of follicular thyroid carcinomas demonstrate mutations causing PAX8–PPARγ fusion (72). PAX8–PPARγ fusion is also more common in the radiation-exposed group. Later, it was also demonstrated in follicular adenomas (73). Recently, 37.5% of papillary thyroid carcinomas with the follicular variant were detected to have the PAX8–PPARχ fusion proto-oncogene. The same study also suggested that multifocality and vascular invasiveness were more common in this group (74).

Lemoine et al. (75) initially described activation of all three RAS oncogenes (H-ras, K-ras, and N-ras) in follicular thyroid cancers. Recently, it was suggested that ras mutations are a marker for aggressive cancer behavior and that ras genotyping might have a role in identifying thyroid carcinoma subsets associated with poor prognosis (76).

RET mutations have been found in medullary carcinoma of the thyroid (77–79). *RET* gene mutations in MEN 2A and in FMTC most frequently produce a change from cysteine (which, in the unmutated molecule, participates with another cysteine in an intramolecular disulfide bond) to another aminoacid. This transversion frees the other usually intramolecularly coupled cysteine to couple with the corresponding uncoupled cysteine in a neighboring mutated RET molecule (Fig. 13). This intermolecular cysteine coupling dimerizes RET and results in constitutive activation (80).

RET mutations in MEN 2B occur in the intracellular tyrosine kinase domain rather than the cysteine-rich extracellular domain of the RET molecule (81). These tyrosine kinase mutations activate RET without the need of dimerization (Fig. 14). This nondimeric structure of activated RET in MEN 2B

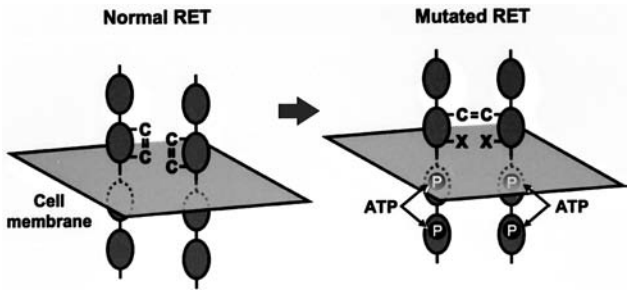

Figure 13 In MEN 2A, most of the RET mutations involve a nucleic acid transversion resulting in a change in the cysteine-rich portion of the extracellular portion of RET. Since one of the intramolecular disulfide bonds does not form, a "spare" cysteine is available for intermolecular disulfide bonding. This binding results in dimerization of RET molecules. This dimerization, in turn, constitutively activates RET.

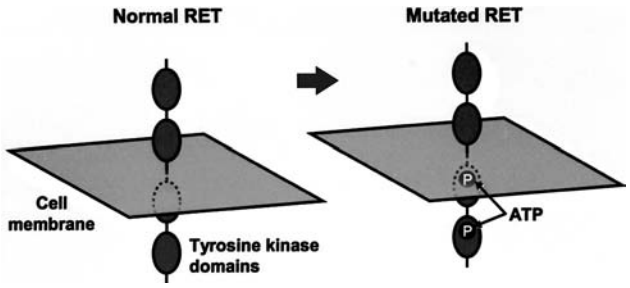

Figure 14 In MEN 2B, RET mutations occur in the portion of the gene coding for the portion of the RET protein containing thyrosine kinase activity. These mutations are constitutively active despite absence of dimerization of the RET proteins.

alters the substrate specificity of tyrosine kinase. In addition, unlike MEN 2A RET mutations, MEN 2B mutations allow persistence of RET ligand responsiveness (82). Perhaps, these differences contribute to phenotype differences between MEN 2A and 2B.

Intestinal ganglioneuromatosis, characteristic of MEN 2B, reflects the embryologic role of RET in the development of the enteric nervous system (83). Ganglioneuromatosis results from constitutive RET activation. Inactivating RET mutations are found in 23% to 33% of patients with Hirshsprung's disease, a condition characterized by absence of ganglia within a portion of the distal colonic wall (84). Some kindred's have RET mutations that predispose to MEN 2A (or FMTC) and Hirschsprung's disease. Such mutations may produce constitutively active RET molecules that are numerically limited, causing constitutively active C-cells but numerically insufficient RET signals to effect gangliogenesis (85).

CLINICAL PRESENTATION

In children, thyroid nodules usually present as an incidentally noted asymptomatic mass. Children can complain of difficulty in swallowing, hoarseness, or systemic symptoms of hypothyroidism or hyperthyroidism. Tenderness may suggest an inflammatory process, or hemorrhage into a cyst or tumor. Hardness of the thyroid nodule and its fixation to the surrounding tissue suggests malignancy.

Up to 90% of children with thyroid carcinoma may present with cervical lymphadenopathy (51). Grigsby et al. (86) reported a 29% incidence of cervical adenopathy in children with thyroid carcinoma clinically. In their series, 73% of the patients had cervical lymph node disease. In some children, the only presentation of thyroid cancer is the presence of lymph nodes in the lower neck. When an unexplained cervical or neck lymph node is palpated, the thyroid gland needs to be examined carefully. In rare cases, the primary tumor in the thyroid may be too small to

palpate and the diagnosis may be based on the lymph node biopsy findings.

Thyroid cancers do not affect the surrounding thyroid tissue; therefore, most patients with thyroid cancer have normal thyroid function. Although thyroid antibodies have been detected in 25% of patients with thyroid cancer, the autoimmune process does not usually attenuate thyroid function (87).

DIAGNOSIS

The differential diagnosis of a thyroid nodule includes congenital thyroid anomalies such as hemiagenesis of the thyroid gland, dyshormonogenesis, abscess, autoimmune thyroiditis, nodular goiter, cyst, adenoma, and malignancy.

In patients presented with a thyroid nodule, a detailed history including radiation exposure, and an extensive family history should be obtained. Family history of the nonthyroidal components of multiple endocrine neoplasia type 2 should also be probed. Physical examination should include looking for signs of the syndromes described above. Laboratory evaluation should include TSH, free thyroxine, triiodothyronine, calcitonin, thyroglobulin, and thyroid antibody levels.

Thyroid ultrasonography may show nonpalpable nodules (in addition to those palpated) and indicates whether the nodule is cystic or solid. The risk of malignancy in solitary nodules in children is approximately 30% to 50%. Purely cystic lesions are rarely malignant. Ultrasound guided fine needle aspiration is usually the initial approach to diagnosis in children with thyroid nodules. Sampling of the wall of partially cystic lesions is most likely to give a meaningful cytologic result.

The risk of malignancy of the thyroid nodules is higher in children than in adults. Eighteen percent to 25% of solitary thyroid nodules are cancerous in children, and 5% are cancerous in adults (5,88,89). Fine needle aspiration of such nodules is generally favored to avoid unnecessary surgery in the adult population (90). Some investigators caution that aspiration may be misleading. False negative results of aspiration may be obtained in 2.3% to 3.6% of thyroid nodules (89,91). Therefore, follow-up of the physical examination as well as the ultrasound findings are helpful in ascertaining the benign nature of a nodule thought to be benign in FNA. Any change would warrant repeat fine needle aspiration or surgery. A recent study of 218 aspirates from children and adolescents showed a thyroid needle aspiration sensitivity of 100% and a specificity of 65% (92).

Thyroid radionuclear scanning findings provides information on iodine trapping function. Thyroid nodules can be classified as hyperfunctional/hot, normofunctional/warm or hypofunctional/cold nodules. In cold nodules the uptake and absorption of the radioactive material is reduced compared with the remaining of the thyroid. Hung (88) reported that 19.9% of the CTNs were malignant in patients under the age of 18. Hot nodules are rarely malignant. At times FNA results are inconclusive. This is frequently true when cytology indicates a follicular neoplasm. Scan may help to determine the probability of malignancy in such neoplasms.

Aspirates from medullary thyroid cancers show hypercellularity, spindle-shaped, and poorly cohesive cells. Other typical aspirate findings include anisonuclesis, binucleation, eccentric nuclei, and coarse chromatin. It is common practice to immunostain for calcitonin, and this is the gold standard for diagnosis of MTC. In patients who have family history or have features of MEN, mutations of the RET oncogene should be evaluated. The presence of elevated serum levels of calcitonin and carcinoembryonic antigen confirms C-cell disease. However, the normal of serum calcitonin levels do not rule out the disease. Especially in C-cell hyperplasia and early tumor stages, basal computerized tomography (CT) concentrations may be mildly increased or within the reference values. Measurement of calcitonin levels after the administration of calcium or pentagastin (currently unavailable) can be used to determine the presence of C-cell hyperplasia or carcinoma. These tests are also used to document the extent of tumor preoperatively and postoperatively.

TREATMENT

Thyroid Nodules

Most pediatric practice guidelines are derived from studies on adults. In our practice, evaluation of a solitary thyroid nodule almost always warrants a fine needle aspiration under ultrasound guidance (Fig. 15). Thyroid nodules which are found on aspiration cytology to be benign can be followed by repeated physical

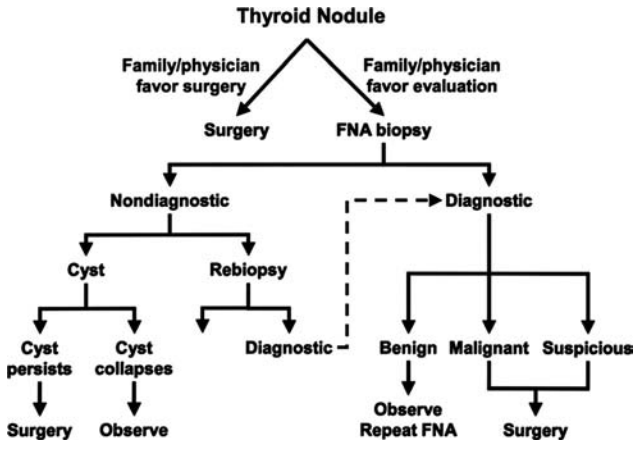

Figure 15 Thyroid nodules in children may be removed surgically if this approach is favored by the family or the physician. Otherwise, fine-needle-aspiration is the most cost-effective test to guide further evaluation and treatment.

examination and neck ultrasounds. Any enlargement in the size of the nodule would warrant a repeat fine needle aspiration or surgical removal. For many years thyroxine therapy trying to suppress the size of the nodule has been used. This approach was thought to differentiate benign versus malignant nodules. A recent study by Gharib and Mazzeferi (93) showed that only 10% to 20% of thyroid nodules responded to thyroxine suppressive therapy, by decreasing the size by at least 50%. In fact, a decrease in size is observed in 13% to 15% of the cancers; this is likely due to TSH responsiveness in malignant cells (94,95). Fine needle aspiration, which is a safe and simple procedure, is better than suppressive therapy in differentiating benign versus malignant nodules. In 1993, Gharib and Goellner (96) reported an overall sensitivity of 83% and a specificity of 92% with a diagnostic accuracy of greater than 95%. In a 1995 study among 57 patients below the age of 17 who had fine needle aspiration of the thyroid for the evaluation of nodular thyroid, there were no false positive results and one false negative result (91). The accuracy of the fine needle aspiration depends on careful aspiration, smear preparation and microscopic evaluation, and on the experience of the cytologist.

In the untreated individuals with thyroid nodules, approximately 50% of thyroid nodules decrease in size or disappear, 30% remain the same, and 20% increase in size. Those that decrease in size are mostly cystic lesions and those that grow are particularly likely to be malignant (93). In our practice, we use thyroxine treatment to suppress TSH to low-normal levels in patients with benign FNA cytology and elevated TSH levels. This treatment option brings with it the possible disadvantages especially on the skeletal system. A recent study suggested that chronic exogenous subclinical hyperthyroidism has no adverse effects on BMD in adolescence females (97). However, the studies in younger children are limited.

Thyroid Cancer

The surgical procedure most frequently advocated for follicular-based thyroid carcinoma is near-total (total on the side of the lesion and near-total contralaterally) or total thyroidectomy in PTC. Unilateral procedures are associated with a higher incidence of local tumor recurrence because PTCs have a high tendency to be multifocal (98). Other studies show that unilateral surgeries are associated with higher mortality only in high-risk patients (advanced age, large tumor size, local invasion, and/or distant metastases and high-tumor grade) (99,100). Bilateral thyroid surgery significantly decrease the rate of tumor recurrence in a study of 1685 patients of all ages evaluated for a mean of 18 years (100). Study of a subset of this patient group comprising the 90 children less than 17 years of age also demonstrated reduced recurrence in patients undergoing bilateral surgical procedures (51). A more recent study of a different patient population has also confirmed these results (101). Another study of 329 patients younger than 21 years of age evaluated for a mean of 11.3 years did not report a difference between unilateral and bilateral surgeries. This study demonstrated a higher frequency of permanent hypoparathyroidism in bilateral operations than in unilateral ones (102). In addition to the probable advantage in preventing tumor recurrence offered by bilateral surgery, this surgical approach facilitates the usefulness of thyroglobulin measurement and of radioiodine scanning in evaluating thyroid cancer patients postoperatively for recurrence of tumor in both papillary and follicular cancers (103). The role of the surgical procedure in bolstering the sensitivity of the postoperative management techniques is particularly important in evaluating patients with follicular thyroid carcinoma, which not infrequently metastasize by the hematogenous route. The recurrence rate of follicular thyroid carcinoma may not be significantly affected, however, by bilateral rather than by unilateral surgical procedures.

Total thyroidectomy can be carried out safely by experienced hands. Complications include transient or permanent hypoparathyroidism, major bleeding, damage to the laryngeal nerve, facial paralysis, airway compromise, or Horner's syndrome. In children younger than four years the risk of complications is higher.

Lobectomy may be adequate for micropapillary cancers (less than 1 cm in diameter) with a normal contrlateral lobe. Some surgeons favor lobectomy for FTCs less than 2 cm in diameter with minimal capsular invasion. This approach remains controversial. Subtotal thyroidectomy may be contraindicated in the presence of a history of radiation exposure.

During the surgical procedure, the lymph nodes of the tracheoesophageal groove are palpated. Those on the side of the tumor are biopsied. If these nodes are positive, then a modified neck dissection is performed. In addition, lymph nodes in the supaclavicular area are palpated and if large, these are also biopsied. If a neck node area has metastases, then that area is carefully dissected and neighboring areas are sampled. Even though lymph node metastasis is rare with FTCs, nodes should be palpated and if clinically involved should be resected (104) (Table 1).

Another controversial aspect of the treatment of papillary thyroid carcinoma is the use of radioiodine ablation of the postoperative normal thyroid remnant. There appears to be a substantial agreement that ^{131}I treatment of tumors smaller than 1.5 cm does not reduce the rate of tumor recurrence (105). One large series strongly suggests decreased tumor recurrence and decreased mortality in ^{131}I-treated patients; another did not demonstrate such benefits (106).

The results of a number of studies seem to support the view that total or near-total thyroidectomy reduces the apparent benefit of ^{131}I ablation (107). Remnant ablation renders thyroglobulin levels more specific in the follow-up for persistent and recurrent

Table 1 TNM Classification of Malignant Tumors of the Thyroid Gland

T stage: Primary tumor		
TX	Tumor cannot be assessed	
T0	No clinical evidence of tumor	
T1	Tumor ≤ 1 cm	
T2	Tumor > 1 and < 4 cm	
T3	Tumor ≥ 4 cm	
T4	Tumor extending beyond thyroid capsule	
N stage: Regional lymph nodes		
N0	No palpable nodes	
N1	Regional nodal metastases	
	N1a	Ipsilateral nodes
	N1b	Contralateral, bilateral, or mediastinal nodes
M stage: Distant metastases		
MX	Metastases cannot be assessed	
M0	No evidence of distant metastases	
M1	Distant metastases present	

disease. Although children with PTC have survival rates essentially the same as the normal population during the first 30 years following initial surgery, mortality increases remarkably between 30 and 40 years of follow-up. Patients develop unusual cancers and succumb to them. More recent data indicate that the patients who develop those late cancers are specifically the ones treated with radiation treatment followed by radioablation (I.D. Hay, personal communication). This observation raises the possibility that ablation at the thyroid remnant should be undertaken less readily in children than in adults. If ablation is chosen, in most cases, an outpatient dose of 29.9 mCi is adequate for a successful treatment. In some cases with locally advanced disease at diagnosis, some endocrinologists prefer a higher inpatient dose of 150 mCi of ^{131}I (104). In five to seven days posttherapy scans are obtained as baseline studies along with thyroglobulin levels.

Measurement of serum thyroglobulin levels is a cornerstone of postoperative monitoring. Approximately 25% of patients with thyroid cancer have antibodies directed against thyroglobulin (87). These antibodies may interfere with thyroglobulin measurement, often resulting in the underestimation of thyroglobulin levels. Patients with thyroglobulin antibodies have been studied using a polymerase chain reaction (PCR) -based assay for thyroglobulin mRNA. However, the relationship between circulating thyroglobulin mRNA and tumor load has been challenged. At present, thyroglobulin is usually measured with either immunoradiometric assays or radioimmunoassay. Immunoradiometric assays have shorter incubation times and automation. A recent study showed that immunoradiometric assays were prone to underestimation of serum thyroglobulin levels and that the radioimmunoassay appeared to be relatively more resistant to interference with thyroglobulin antibodies (107).

Withdrawal of thyroid hormone replacement allows the detection of thyroglobulin in almost all patients with tumor in neck lymph nodes and with distant metastasis (108). Measurement of thyroglobulin postoperatively or following withdrawal of thyroid hormone replacement is frequently preceded by L-T3 replacement (in doses of approximately 25 μg/1.73 m^2 body surface area three times daily or 1 mg/kg/day divided three times daily) for four weeks. Thereafter, L-T3 is withdrawn for a period of two weeks. This practice allows L-T4 levels to decline gradually (in light of the long T4 half-life of one week) while prolonging TSH suppression by T3. T3 has a much shorter half-life (approximately 24 hours), allowing robust TSH release over a presumable safer and shorter period of time. A recent study showed that, in children with follicular cell-derived thyroid carcinoma, the time to reach hyperthyrotropinemia of > 25 mIU/ml after discontinuation of L-T4 was shorter than anticipated, 12.3 ± 0.7 days. A more rapid clearance of T4 in children or a higher TSH/FT4 ratio in children could explain this sharp increase (109).

Instead of withdrawing thyroid hormone, patients may be given recombinant TSH by intramuscular injection before the measurement of thyroglobulin levels. This method obviates the need to produce hypothyroidism (110,111). Most children and adolescents with thyroid carcinoma do not require age-appropriate dose adjustments of recombinant human TSH injections (112). Side effects include nausea and headache. A retrospective study showed that thyroglobulin levels after the administration of recombinant-TSH stimulation in combination with neck ultrasonography has the highest diagnostic accuracy in detecting persistent disease in the follow-up of differentiated thyroid carcinoma (113).

In addition to thyroglobulin measurement, thyroid cancer is monitored by total body scanning employing 0.5 to 2 mCi ^{131}I or 300 to 400 μCi of ^{123}I. Using higher doses of ^{131}I may result in thyroid stunning (114,115). Maximum doses ≤ 2 mCi are recommended to avoid thyroid stunning (116). For adequate scanning sensitivity, TSH levels should be greater than 30 mIU/l. This can be accomplished either by withdrawing patients from thyroid hormone or by injecting them with recombinant TSH.

Tumor detected by ^{131}I scanning and inaccessible to neck surgery may be treatable with radioiodine (117). Treatment of tumor detected by thyroglobulin but not by diagnostic scan remains controversial (105,118). [^{18}F]Fluorodeoxyglucose positron emission tomographic scanning with simultaneous CT may be used for the detection of these metastases (119).

High-resolution real-time ultrasonography is useful in monitoring patients for tumor recurrence in the neck. Suspicious masses may be aspirated under ultrasound guidance for cytological study. Confirmed tumor may then be surgically removed.

Thyroid hormone replacement should be adjusted to suppress TSH (120). Low-risk patients

may not require marked suppression (121). It has therefore been recommended that high-risk patients have suppression of TSH below 0.1 mIU/l whereas low-risk patients should have TSH levels between 0.1 and 0.4 mIU/l (122).

Six months after radioiodine ablation, patients are reevaluated by a whole-body scan, thyroglobulin levels and thyroglobulin antibody levels, neck ultrasonography, chest radiography, and CT to reassess for persistent or metastatic disease. Residual enlarged lymph nodes are best treated with surgery two to three months after the initial procedure. This interval should provide adequate healing time. Microscopic neck involvement or disease in the chest is best treated with therapeutic radioiodine. Recommended doses are as follows: 150 mCi/1.73 m^2 for isolated soft tissue metastases, 175 to 200 mCi/1.73 m^2 for multiple or diffuse pulmonary metastases and 200 mCi/1.73 m^2 for bone metastases (104). Doses are adjusted for body surface area of the children.

Optimally thyroglobulin levels will become unmeasurable (measured while TSH levels are elevated), neck ultrasonography, chest radiography, and whole-body scan will become negative. Once these goals are achieved, patient can be followed-up on yearly basis. If the neck US and scan findings are negative, modest thyroglobulin elevations to 2 to 10 ng/mL within the first year after the radioablation can be observed to ascertain that thyroglobulin levels are stable or progressively decrease (123).

The outcome of treatment of papillary and follicular thyroid carcinoma in children is generally favorable. Mortality tends to be in the range of 1% at 10 years (51). The patients most likely to die from thyroid cancer seem to be those younger than 10 years of age at the time of diagnosis (124,125).

In patients with thyroid carcinoma, the risk of breast and kidney cancers are increased. This increase is not dependent on radioiodine administration. Others such as leukemia, colorectal cancer, and salivary gland cancers are increased in parallel to the dose of radioiodine. The accumulation of radioactive iodine can be reduced by encouraging increased liquid intake and by use of laxatives (126). Other side effects from ^{131}I treatment includes painful swelling of the tissues that uptake the radioiodine, nausea, vomiting, sialadenitis, transient loss of smell or taste, and transient bone marrow suppression that mandates checking complete blood count in six to eight weeks after the procedure.

Management of medullary thyroid cancer in children focuses on the detection of inherited diseases before appearance of symptoms or signs. In the past, affected family members were detected by provocative tests stimulating calcitonin secretions. Formed medullary thyroid carcinoma may be detected in children with MEN 2 as early as two to three years of age (127). The need to perform surgery early in these patients is highlighted by the finding of lymph node metastases of medullary thyroid cancer in a child as young as five years of age (128,129). These reports suggest that thyroid surgery should be performed in RET-positive children with MEN 2A by five years of age and within the first few months in MEN 2B.

Approximately, 50% of the patients with MEN 2A or 2B develop pheochromocytoma. Owing to this possibility in MEN 2 patients, all should undergo appropriate measurement of serum and urine catecholamines and catecholamine metabolite studies (Vol. 2; Chap. 27) (130). Children with MEN 2B associated pheochromocytomas have a higher risk of malignancy compared with MEN 2A or spordiac diseases (130). In addition, patients who may have MEN 2A should have studies of calcium because hyperparathyroidism can be treated at the same time when thyroidectomy is being performed. Preoperative calcitonin levels should be measured.

There is a general agreement that patients with medullary thyroid carcinoma should undergo total thyroidectomy. Thereafter, the patients will undergo thyroid replacement therapy, with the goal of achieving normal rather than suppressed circulating TSH, C-cells are TSH unresponsive. Patients with familial disease have bilateral multifocal tumors. Similar multifocal disease may be found in 5% to 30% of patients with apparently sporadic medullary thyroid cancer (128,131).

If serum calcitonin levels remain elevated postoperatively, physical examination and ultrasonography may reveal persistent cervical lymph node metastases that should be surgically excised. Aggressive surgical therapy of patients without obvious disease in the neck, mediastinum, liver, or lungs may render approximately 30% of postoperation hypercalcitonemic patients normocalcitonemic with a follow-up of approximately six years (132). A more conservative approach to such patients has been advocated by others in view of their 10-year survival rate of 86% (133).

Survival rates of all patients with MTC are 78.2% and 61.4% at 5 and 10 years, respectively. MEN 2A patients have a better survival rate than do sporadic MTC patients. Patients with MEN 2B presenting with MTC have 85% and 75% survival rate at 5 and 10 years, respectively (134).

Deeper understanding of the pathogenesis of the thyroid cancers will help to develop selective oncogenic inhibitors. Pharmocologic compounds that inhibit RET kinase activity and MAPK pathway in MTC and papillary thyroid cancer, respectively, are possible candidate pharmacologic agents in the treatment of these cancers.

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Calcium Disorders in Children and Adolescents

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INTRODUCTION

Adequate concentrations of extracellular calcium help to sustain a number of normal physiological processes as diverse as bone formation and turnover, neuronal cell excitability, muscle contractility, and blood clotting. Significant shifts in extracellular fluid calcium concentration can have adverse effects on these physiological functions. The extracellular concentration of calcium is normally affected by intermittent changes in calcium absorption in the gut, continuous mineral turnover in bone, and calcium losses in the urine. Extracellular calcium levels are set within a very narrow range by the concerted action of regulatory ''calciotropic'' hormones on calcium handling by the gastrointestinal tract, bone, and kidney. In children and adolescents, maintenance of adequate calcium balance is particularly important because bone accretion and growth are closely linked to calcium availability. Children require an adequate supply of calcium to sustain bone mineralization and achieve an adequate peak bone mass without disrupting the tight regulation of extracellular calcium. Abnormalities in the organs that express calciotropic hormones, the abnormal function of these hormones or the failure of the gastrointestinal tract, bone or kidney to handle calcium properly can cause either hypo or hypercalcemia. This chapter will describe the physiology of calcium homeostasis, the clinical disorders linked to alterations of calcium concentration in the extracellular compartment, their evaluation and treatment.

HORMONAL REGULATION OF SERUM CA^{2+}

Calcium is among the most abundant mineral ions in the body but greater than 98% of total calcium is present as mineral salts in bone. The remaining fraction of calcium is distributed between the intracellular and extracellular compartments. The small fraction that is present in soluble form is derived from (1) the continuous exchange of calcium between bone and the extracellular compartment during bone remodeling; (2) the intestinal absorption of calcium ingested as part of the oral diet; and (3) the continuous reabsorption of calcium from the glomerulal filtrate in the kidney. Calcium in serum exists in three forms: (i) a protein-bound fraction (30–50% of total serum calcium), primarily bound to albumin; (ii) complexed with serum anions such as phosphate, citrate, and bicarbonate (5–15%); and (iii) ionized Ca (Ca^{2+}) (40–60%). Ca^{2+} is the metabolically active form and is the soluble fraction that is tightly regulated. The concentration of serum Ca^{2+} remains relatively constant with age and dietary intake. In adults, under normal circumstances, the daily absorption of calcium is countered by a comparable loss through the urine and less significantly through the gut to avoid a positive balance of calcium intake. Bone growth requires a positive calcium balance directed to bone mineralization in children and adolescents. The concentration of serum Ca^{2+} is maintained within a narrow range by a homeostatic feedback mechanism, primarily active in the parathyroid glands, that detects alterations of serum Ca^{2+} concentration and regulates the secretion of parathyroid hormone (PTH). A decrease in serum Ca^{2+} is sensed by the parathyroid glands, which secrete PTH in response to hypocalcemia. In the kidney, PTH promotes the retention of calcium, blocks the reabsorption of phosphate, and increases the synthesis of calcitriol, the bioactive form of vitamin D, which promotes the absorption of both calcium and phosphate in the small intestine. PTH also activates osteoclasts to release calcium and phosphate from bone. The combined effect of PTH secretion is to restore serum calcium levels to the normal range (Fig. 1).

The main organ responsible for regulating serum Ca^{2+} is the parathyroid gland. There are four paired parathyroid glands, usually positioned in the superior and inferior poles of the thyroid, derived from the fourth and third pharyngeal pouches, respectively. Chief cells, the major cell type of the glands, are arranged in cords and sheets within the parathyroid gland. Chief cells express and store PTH in secretory granules. These cells regulate serum

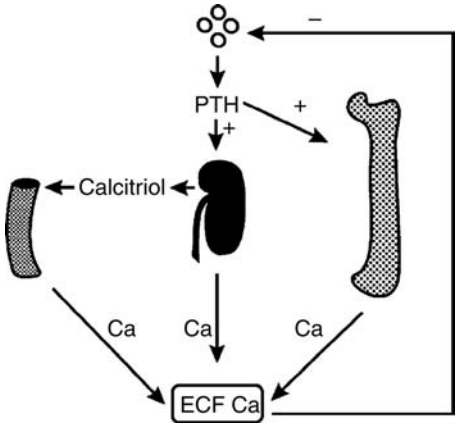

Figure 1 Schematic diagram of calcium homeostasis. The parathyroid glands control extracellular fluid Ca^{2+} concentration by regulating the fliud of Ca^{2+} from the kidneys and skeleton and, through adjusting the production of calcitriol by the kidneys, from the intestine. This control is mediated by PTH; its secretion is regulated trough feedback sensing of serum Ca^{2+} concentration by the parathyroid glands.

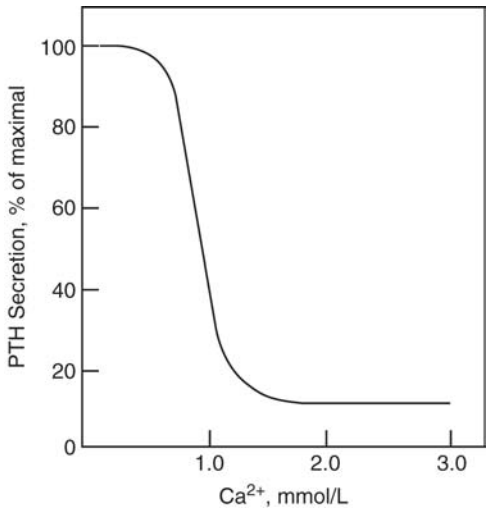

Figure 2 Serum Ca^{2+} concentration determines the secretory rate of PTH following a sigmoidal dose–response curve. The normal level of serum Ca^{2+} concentration corresponds approximately to the midpoint of the slope, "set point." The minimum secretory rate is not zero.

Ca^{2+} concentration on a minute-to-minute basis by rapidly adjusting their secretion of stored PTH in response to changes in Ca^{2+} concentration (1,2). Ca^{2+} sensing in the parathyroid gland is mediated by a G protein-coupled, calcium-sensing receptor (CaR) (3,4). This receptor is also expressed in the kidneys, in the intestine, parts of the brain, the thyroid parafollicular cells, breast, and bone. The human 1078 amino acid protein, encoded by CaR gene in chromosome 3q13, consists of three structural regions: a large amino terminal extracellular domain that contains clusters of acidic amino acids thought to be involved in binding of Ca^{2+}, seven transmembrane helices characteristic of the G protein-coupled receptors, and a cytoplasmic carboxy terminal domain. Once activated by Ca^{2+}, the CaR activates phospholipase C, which in turn mediates the intracellular accumulation of inositol triphosphate and, secondarily, a rise in intracellular Ca^{2+} (4). Inhibition of adenyl cyclase-mediated production of cAMP has also been reported. CaR activation appears to mediate the acute inhibition of secretion of PTH as well as the overall rate of PTH expression, but the specific signal transduction pathways responsible for these effects are still poorly understood.

There is an inverse sigmoidal relationship between serum Ca^{2+} concentration and the release of intact PTH, with the normal set point (the 50% response) at approximately 1.25 mmol/l. The normal level of serum Ca^{2+} concentration corresponds approximately to the midpoint of the slope and the minimum secretory rate is not zero (Fig. 2). The overall effect of changes in calciotropic hormones on calcium handling by the kidney, gastrointestinal tract, and bone is to maintain the extracellular Ca^{2+} concentration around the normal range or set point. Because this is primarily achieved by the regulation of PTH secretion in parathyroid cells, the CaR's binding affinity for Ca^{2+} is the major determinant of serum Ca^{2+} set point. The most compelling evidence in support of this important role of the CaR is that activating and inactivating mutation of the CaR are associated with a raise and a decrease in serum Ca^{2+} set point, respectively (5). The CaR is highly expressed in the kidney tubule, where Ca^{2+} exerts a direct role in regulating its own absorption in the distal tubule (6); high serum Ca^{2+} inhibits the absorption of luminal calcium by blocking the activity of the Na/K/Cl transporter and decreasing the luminal positive charge. The CaR is also expressed in the collecting duct and its activation appears to block the expression of water channels on the luminal surface, providing at least one mechanism for the polyuria noted in hypercalcemia (7). CaRs are also expressed in intestinal epithelia and various bone-derived cells, but its role in calcium homeostasis in these sites has not been clearly defined yet.

The CaR is also stimulated by other divalent cations, most importantly Mg^{2+}, although concentrations above the normal physiologic range are needed to activate the receptor. Certain amino acids and various polyamines are able to bind and activate the receptor but the physiologic significance of these findings has not been fully elucidated (8). The identification of compounds that can alter the affinity curve of the receptor has led to the development of drugs that can shift the sensitivity curve to a lower Ca^{2+} set point (calcimimetics) or to a higher set point (calcilytics) (9).

CALCIOTROPIC HORMONES

Parathyroid Hormone

PTH is the main secretory product of parathyroid chief cells. The gene for PTH, located in chromosome 11p15, consists of three exons and two introns encoding pre-pro-PTH of 115 amino acids including a 25-residue signal presequence, and a 6-residue prosequence. These sequences direct the protein into the secretory pathway. The prehormone is cleaved sequentially during posttranslational processing to the 84 amino acid mature PTH and stored in secretory granules. A significant portion of stored PTH is degraded by proteases within the secretory granules and secreted as carboxy-terminal fragments (1). Hypercalcemia increases the intracellular degration of PTH. Although Ca^{2+} is the major regulator of PTH secretion, other calciotropic factors also affect its secretion. The active form of vitamin D, calcitriol, inhibits PTH synthesis while high serum phosphate has been recently shown to stimulate PTH secretion (10). Profound hypomagnesemia inhibits both PTH secretion and action by affecting intracellular signaling function (11); hypermagnesemia also inhibits PTH secretion, a process likely mediated by the CaR.

PTH has a serum half-life of less than 8 min. It is exquisitely sensitive to degradation both intracellularly in the parathyroid cell and in serum, especially as it traverses the liver and kidney. Inactive proteolytic fragments are always present in blood and accumulate to high concentrations in conditions that decrease their clearance (i.e., renal failure). Thus, an accurate measurement of active PTH requires an immunoassay that measures intact PTH, presently achieved by a sandwich immunoradiometric assay or an immunofluorometric assay. The bioactive site in PTH resides within the first 27 amino acids of the peptide. PTH binds to a G protein-coupled receptor (PTH1R) that activates the production of cAMP and, in some cells, the release of intracellular calcium stores via activation of phospholipase C. This receptor is present in osteoblasts and kidney tubular epithelium, cells that play a direct role in calcium homeostasis. Two additional receptors (PTH2R and PTH3R) with some homology to the first characterized receptor have been recently described, but their role in calcium homeostasis may not be significant (12).

The net effect of PTH on calcium homeostasis is to activate mechanisms that increase serum Ca^{2+} levels. PTH promotes calcium mobilization from bone by osteoblast-mediated activation of bone-resorbing osteoclasts. In the kidney's proximal tubule, PTH activates 1-α-hydroxylase to synthesize calcitriol ($1,25(OH)_2D$), increases the absorption of sodium, calcium, and bicarbonate while inhibiting phosphate transport and promoting phosphaturia. In the distal tubule, PTH has its most significant effect in the distal convoluted tubule where it activates calcium absorption. In the gut, PTH indirectly promotes, through the action of calcitriol, the absorption of both calcium and phosphate.

Another peptide has been shown to have PTH-like effects by nature of its binding affinity to the PTH receptor. PTH-related protein (PTHrP) was initially characterized for causing hypercalcemia when secreted by some malignant tumors (13). The amino terminus of this peptide has high homology with the bioactive amino terminus of PTH and binds the PTH receptor. Besides its role as a calciotropic hormone when present in high serum concentration, PTHrP appears to serve important functions in cartilage formation and the differentiation of several organs during fetal and postnatal development (14). It is present in very high concentration in maternal breast milk and its increased maternal serum levels during the breastfeeding period may contribute to the mobilization of maternal calcium from bone. The mid region of PTHrP appears to regulate active transplacental transport of Ca^{2+} via an unidentified receptor. Since PTHrP is expressed and secreted in the parathyroid gland during the fetal period, it is likely that transplacental calcium transport is under parathyroid control (15).

Vitamin D

Calcitriol is the hydroxylated metabolite of vitamin D with significant calciotropic properties. Vitamin D_3 (cholecalciferol) is the product of photolysis of cholesterol to 7-dehydrocholesterol under ultraviolet B irradiation (280–305 nm wavelength) followed by isomerization in the skin (16). A number of hydroxylation steps are required to produce a bioactive form of vitamin D. It is hydroxylated to 25-hydroxyvitamin D (25OHD) in the liver, a step that is largely substrate-dependent. 25OHD has a long half-life in serum and its measurement constitutes a useful assessment of vitamin D stores. The additional hydroxylation step by a 1-α-hydroxylase in the renal proximal tubule produces $1,25(OH)_2D$, calcitriol, the bioactive form of vitamin D. PTH, hypophosphatemia, and to a lesser extent hypocalcemia are the major inducers of 1-α-hydroxylase activity in the proximal tubule. An increase in calcitriol production becomes apparent hours after exposure to a stimulus and the half-life of $1,25(OH)_2D$ is only several hours. The proximal tubule also has 24-hydroxylase activity; hypercalcemia, hyperphosphatemia, and $1,25(OH)_2D$ induce this enzyme, promoting the production of the inactive metabolite $24,25(OH)_2D$ from 25OHD. 1-α-hydroxylase is also expressed in placenta, a significant source of calcitriol for the fetus, keratinocytes, and activated mononuclear cells. 1-α-hydroxylase activity in mononuclear cells is thought to be responsible for the hypercalcemia and elevation of $1,25(OH)_2D$ levels seen in granulomatous disorders such as sarcoidosis (17).

Vitamin D and its metabolites are transported in serum bound to Vitamin D binding protein, showing greatest affinity for 25OHD. This protein provides a

reservoir of vitamin D metabolites and prevents their rapid clearance in the urine. Megalin, a lipoprotein-like receptor that binds DBP, has been shown to mediate the uptake of vitamin D metabolites in the proximal tubule, suggesting a role for this protein in ensuring 25OHD availability for 1-α-hydroxylation in the kidney (18).

$1,25(OH)_2D$ binds to vitamin D receptors (VDR), a member of the retinoid family of nuclear receptors, expressed in intestine, distal renal tubular cells, osteoblasts, parathyroid cells, and other tissues not directly involved in calcium homeostasis. In bone, binding to VDR promotes the activation of osteocalcin and alkaline phosphatase production by osteoblasts and the differentiation of osteoclasts precursors, having a net effect in mobilizing calcium and phosphate from bone. In the kidney, calcitriol facilitates the action of PTH on distal tubule calcium absorption. In the small intestine, calcitriol promotes the absorption of calcium and phosphate in the duodenum and jejunum. Thus, the net result of calcitriol action is to promote the rise of both calcium and phosphate levels in serum (16).

Calcitonin

Calcitonin is a 32 amino acid peptide produced by thyroid parafollicular C cells and in lesser amounts by other neuroendocrine cells (19). Parafollicular cells have the ability to sense changes in serum Ca^{2+} because they also express CaR. Activation of the CaR by elevations of serum Ca^{2+} elicits a rise in calcitonin secretion. In almost all instances, calcitonin antagonizes the effect of PTH on bone and kidney while no measurable effects have been reported on intestinal handling of mineral ions. Calcitonin exerts its peripheral effects via its binding to a G protein-coupled receptor of the same family as the PTH receptor. Calcitonin levels rise abruptly at birth but decrease rapidly after that (20) and, in children older than three years of age, normal serum levels are often below detection unless elicited by hypercalcemia or in the setting of medullary thyroid carcinoma. The role of calcitonin in normal calcium homeostasis is uncertain, since in the absence of parafollicular cells (i.e., thyroidectomy), no significant alterations in calcium homeostasis have been observed; however, calcitonin has been used as a pharmacological agent in the acute treatment of hypercalcemia and osteoporosis for its role in calcium deposition in bone.

HYPOCALCEMIA

Etiology

Hypocalcemia develops as a consequence of either decreased inflow of calcium from bone, kidney, or the gastrointestinal track into the vascular and extracellular space, or excessive losses of calcium from these spaces into urine, bone, or stool. Causes of hypocalcemia can be grouped under several broad categories that include various forms of parathyroid dysfunction or hypoparathyroidism, tissue insensitivity to PTH, abnormalities of vitamin D metabolism and alterations of organs involved in calcium homeostasis (Table 1).

Parathyroid Dysfunction/Hypoparathyroidism

Parathyroid-related causes of hypocalcemia can be the consequence of changes in serum Ca^{2+} sensitivity by the parathyroid gland; abnormal parathyroid gland development; destruction of the gland; defects in PTH synthesis or secretion; or decreased tissue responsiveness to PTH. Lack of adequate parathyroid gland development or PTH production is a frequent cause of hypocalcemia in neonates and early childhood (Vol. 2; Chap. 22).

Patients with autosomal dominant hypocalcemia harboring activating mutations of the CaR show a shift in the curve of inhibition of PTH secretion to change the set point of serum Ca^{2+} to a concentration that can be sufficiently low to elicit adverse effects. Heterozygous gain of function mutations have been described in the extracellular domain (Ala116Thr, Glu127Ala, Glu191Lys) and transmembrane segments (Phe806Ser, Gln681His) of the CaR (Fig. 3) (22). Correction of hypocalcemia with the addition of vitamin D metabolites causes significant hypercalciuria as the ability of CaR to decrease tubular absorption of calcium also increases, augmenting the risk to develop urinary stones when compared to other forms of hypoparathyroidism. PTH levels in these patients can be low or inappropriately normal and their presentation can be indistinguishable from other forms of hypoparathyroidism showing high serum phosphate levels. Since this disorder is transmitted in autosomal dominant fashion, a history of familial hypocalcemia in either parent and/or particularly high urinary calcium readings with treatment should raise suspicion about this diagnosis.

In some instances, mutations of the PTH gene lead to inappropriate expression of PTH and dyshormonogenesis. In a family with autosomal dominant hypoparathyroidism, a point mutation in the signal peptide sequence (Cys18Arg) results in inefficient translocation of PTH into the endoplasmic reticulum and posttranslational processing, thus resulting in the release of an inactive PTH molecule (23). The dominant pattern of genetic transmission suggests that the mutant form of PTH has a dominant negative effect on the synthesis of normal PTH or blocks the normal processing of the wild type peptide.

A form of autosomal recessive hypoparathyroidism has been associated with a homozygous G–C transversion in nucleotide 1 of intron 2 of the PTH gene, resulting in skipping of exon 2 and reduced PTH expression. Because exon 2 encodes the initiation codon and the signal peptide, loss of this exon presumably prevents translation of the PTH mRNA and translocation of the peptide. In one kindred, each of the three affected members experienced hypocalcemic

Table 1 Causes of Hypocalcemia

- *Parathyroid dysfunction/hypoparathyroidism*

Alterations in Ca^{2+} sensing
  Autosomal dominant hypocalcemia
Hypoparathyroidism
  Parathyroid agenesis/dysfunction
    Familial forms of isolated PTH deficiency
      Autosomal recessive
        Familial isolated
        With dysmorphism, growth and mental retardation (Sanjad–Sakati syndrome)
      Autosomal dominant
        Familial isolated
        With renal dysplasia and sensorineural deafness (Barakat syndrome)
      X-linked recessive
    DiGeorge's Syndrome
    Kenney–Caffey syndrome
    Dyshormonogenesis
  Acquired hypoparathyroidism
    Autoimmune
      Sporadic
      Polyglandular autoimmune disease type I
      Mitcochondrial myopathies (Kearns–Sayre syndrome)
      Disorders of infiltrative/metal ion deposition (Hemochromatosis, thalasemia, Wilson's disease)
    Radiation exposure/postsurgical
    Idiopathic
  Abormal PTH secretion
    Hypomagnesemia
    Critical illness
  Peripheral resistance to PTH
    Blomstrand Chondrodysplasia (loss-of-function mutation PTHR1)
    Pseudohypoparathyroidism types IA, IB, II
    Pseudopseudohypoparathyroidism

- *Vitamin D*

  Vitamin D deficiency
    Nutritional deficiency
    Liver disease
    Iatrogenic (e.g., phenobarbital use)
  Vitamin D resístanse
    Hydroxylase deficiencies
    Vitamin D receptor dysfunction

- *Alterations of organs involved in calcium homeostasis*

  Kidney
    Acute renal failure
    Renal tubular acidosis
    Hypercalciuria
  Intestine
    Malabsorption
  Skeleton
    Hungry bone syndrome

Other causes
  High phosphate load
    Tumor lysis syndome
    High phosphate formula
    Rhabdomyolysis
  Acute illness
    Acute pancreatitis
    Organic acidemias
    Toxic shock syndrome
  Drugs
    Furosemide
    Calcitonin
    Bisphosphonates
    Antineoplastic agents (plicamycin, asparaginase, cisplatin, cytosine arabinose, doxorubicin)
    Exchange blood transfusion

seizures in the neonatal period and had undetectable circulating PTH concentrations with normal renal response to PTH1-38 (24). In another large pedigree, hypoparathyroidism manifested with seizures in the neonatal period or infancy. It was associated with a homozygous point mutation in exon 2 of the PTH gene, located at the first nucleotide of position 23 in the 25-amino acid signal peptide. Since this is the −3 position in the signal peptide, the resulting pre-pro-PTH mutant is presumably not cleaved by signal peptidase at the normal position, and it might be degraded in the rough endoplasmic reticulum (25).

There are familial, autosomal dominant, autosomal recessive, and X-linked recessive forms of hypoparathyroidism. An autosomal dominant form of hypoparathyroidism associated with renal malformations, congenital heart disease, deafness, immune defects as well as growth, and developmental delays (Barakat syndrome) has been linked to deletions of the terminal segment of the short arm of chromosome 10 (26). This disorder has been linked to heterozygous deletion or loss-of-function mutations of GATA3, a zinc finger transcription factor important in embryonic development of the inner ear, kidneys, and parathyroid glands (27). Isolated GATA3 defects are not associated with the congenital heart malformations, dysmorphic features, immune defects, and growth retardation noted with larger terminal segment deletions of 10p.

Hypoparathyroidism is also common in patients with Kenny–Caffey syndrome, characterized by medullary stenosis of the long bones, short stature, hyperopia, and basal ganglia calcifications. Both autosomal dominant and recessive forms of this syndrome have been described and are linked to chromosome 1q42-q43 (28). A point mutation (Gly63Ser) in the DNA binding domain of the parathyroid-specific transcription factor GCMB has been shown to cause a form of autosomal recessive hypoparathyroidism (29). Sanjad–Sakati syndrome is an autosomal recessive form of hypoparathyroidism associated with intrauterine and postnatal growth failure, developmental delay, seizures, and dysmorphic facial features. This syndrome has been linked to chromosome 1q42-q43 (30).

Kindreds with X-linked recessive isolated HP have also been reported (31). Only male subjects were affected; they had hypocalcemic seizures starting in the neonatal or early infantile period. Circulating immunoreactive concentrations of PTH were undetectable, and renal response to bovine PTH was normal. In a careful search at autopsy of one of the patients who died accidentally as a teenager, no PTH tissue could be identified (32). The mutant gene that causes an embryonic defect in parathyroid development links to chromosome Xq26-q27 (33).

There are sporadic and familial forms of hypoparathyroidism caused by parathyroid agenesis or dysfunction. The DiGeorge malformation complex (DMC) and its variants under the more generalized description of velocardiofacial syndrome (DMC

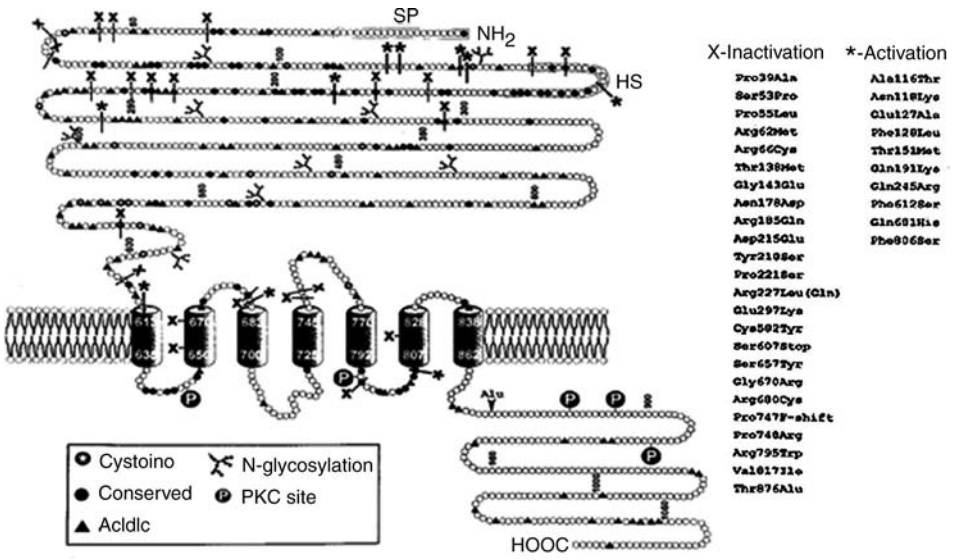

Figure 3 Schematic view of the CaR.* Gain- and (X) loss-of-function missense and nonsense mutations associated with autosomal dominant hypoparathyroidism, familial hypocalciuric hypercalcemia, and neonatal severe hyperparathyroidism (HPT) are depicted with format normal amino acid-codon-mutation. *Source:* From Ref. 21.

MIM 188400; DiGeorge syndrome, Dysbranchiogenesis, Shprintzen, Velocardiofacial syndrome, Conotruncal anomaly face syndrome) are a more generalized embryological abnormality that occurs either sporadically or with variable autosomal dominant penetrance, involving the development of the third and fourth branchial pouches. A spectrum of phenotypes, often collectively called CATCH22 (for cardiac defect, anomaly of face, thymic hypoplasia, cleft palate, hypoparathyroidism), is associated with heterozygous deletions of chromosome 22q.11.2 (monosomy 22q.11.2, the DiGeorge critical region I [DGCR-I]). This spectrum includes the overlapping entities DMC, the Shprintzen velocardiofacial syndrome (34) so-called conotruncal anomaly face, and isolated outflow tract defects of the heart (conotruncal heart defects: tetralogy of Fallot, truncus arteriosus, and interrupted aortic arch) (35,36). Most of the cases are sporadic, but many families show autosomal dominant transmission. The same deletion may cause phenotypes that vary within families from normal to syndromic (37,38) and differ even between identical twins (39). In fact, over 90% of patients have the same deletion breakpoints (40).

DMC main components are hypoplasia of the parathyroid glands, which may present as neonatal tetany or seizures; a deficit of T cells with susceptibility to infection, due to hypoplasia or aplasia of the thymus; congenital defect of the heart or the great arteries, particularly affecting the outflow tract; and a characteristic facial dysmorphism. At least three of these four components are required for the clinical diagnosis of the syndrome (41). However, the prevalence of these components has probably been overestimated, because DMC has been delineated by studying series of clinically diagnosed patients. Cases are often labeled as complete (with thymus aplasia) or partial DMC (thymus hypoplasia). This division does not correlate with presence or absence of other anomalies, save the fact that parathyroid gland aplasia is commonly associated with thymus aplasia (42). Patients with complete DMC die early (43). Reliable diagnosis of thymic aplasia can only be made at autopsy.

The overall prevalence of DMC has been estimated to be as high as 1 : 8000 live births. Deletions and translocations of chromosome 22q11 have been detected and can be screened in suspected cases. The chromosomal region most closely linked to hypoparathyroidism in this syndrome includes UFDIL, a protein linked to the ubiquitin proteosome degradation cascade. Hypocalcemic convulsions occurred in 61% and hypocalcemia in 85% of the reported cases (36). The hypocalcemia resolved in early childhood in a majority of patients. However, deficient function of the parathyroids may still manifest in adulthood during a hypocalcemic stress, such as an infusion of disodium edetate (44–46). It may even evolve again to frank symptomatic hypoparathyroidism (44). Parathyroid glands were searched for at autopsy in 85 patients; a hypoplastic glands were observed in 30, and none could be found in 41 even by careful serial sectioning (42). A malformation of the thyroid gland is also common and at least two of 44 patients were hypothyroid (36). Calcitonin-producing C cells of the thyroid gland, belonging to the derivatives of the third and fourth branchial pouches (cephalic neural crest cells) are deficient in numbers (47,48).

All patients have some facial dysmorphism, but the features that are most helpful for the diagnosis are ear shape, prominence of nasal root, and, in the younger child, small mouth (36). The ears are low set and posteriorly rotated with deficient upper helices and an increase in anteroposterior diameter, giving a relatively circular shape. At least one-fourth of the patients have a hearing deficit. The root and bridge of the nose are wide and prominent, and there is a marked indentation on either side of the nasal tip

above the midpoint of each nostril. Most patients have micrognathia, and about one-fourth have palatal clefts. The lips are often prominent and U-shaped, and the philtrum is short and poorly modeled. Lateral displacement of the inner canthi is frequent. Telecanthus with short palpebral fissures is common. The eyes may slant upward or downward.

A great number of variable other malformations have been reported. Among these, different renal and urinary tract anomalies are most common. Other frequent sites are the pharynx, gastrointestinal tract, lungs, spleen, skeleton, brain, and genitals. In the older child the features overlap with Shprintzen velocardiofacial syndrome (MIM 192430) with a bulbous nose, square nasal tip, and hypernasal speech associated with submucous or overt palatal clefting (49).

Approximately 90% of recorded patients have had a cardiac defect; half of them an anomaly of the aortic arch, most commonly type B interrupted arch or right aortic arch. One-fifth have truncus arteriosus communis. Hypoplastic left heart and coarctation of the aorta also occur. The right outflow tract is affected in some 12% of the patients, predominantly by obstructive anomalies, and a similar proportion has Fallot's tetralogy. An associated ventricular septal defect is present in a majority of the patients, and associated valve anomalies are common. In contrast, isolated septum defects and valve anomalies are infrequent, but ventricular septal defect alone may be present in late manifesting cases. One-fourth of the patients have an aberrant right subclavian artery that may cause dysphagia. The spectrum of circulatory anomalies ranges from left heart hypoplasia to a harmless abnormality of the subclavian artery.

Although infections are relatively rare causes of early death of patients with DMC, susceptibility to infections becomes more prominent with increasing age. Half of the 20 patients who died at the ages of 3 to 12 months succumbed in pneumonia or sepsis. Bacteria and ''Candida albicans'' were the common agents. However, patients have a predominantly mild cell-mediated immunodeficiency, usually associated with infections characteristic of humoral immunodeficiencies (50). Comparison between 19 newborns with chromosome 22q11.2 deletion detected because of a cardiac defect and comparable newborn cardiac patients without deletion showed that the deletion group had significantly lower peripheral blood T-cell numbers, although the function of their T-cells was largely preserved. A subgroup with markedly diminished T-cell numbers showed an increase in these cells over the first year of life (51). Only two cases of malignant neoplasm seem to be on record. Failure of the descent of thymus is very common, but immunodeficiency requiring correction occurs only in approximately 25% of the cases. Such patients can be identified by CD4 T cell enumeration, and by in vitro proliferation response to phytohemagglutinin (52,53). Approximately three-quarters of patients have frankly subnormal T-cell counts or evidence of thymus hypoplasia. Of 85 patients tested only five had a completely normal immune function. In the others findings varied greatly. The total blood lymphocyte count was normal in 71%. B lymphocyte counts were supranormal in half the patients, but antibody production capacity was subnormal in one-third. IgG responses to immunization with bacterial polysaccharides may be particularly impaired (54). Diversification of the immunoglobulin VH gene repertoire is restricted (55). Many patients have hypergammaglobulinemia. Transplantation with fetal thymus tissue or bone marrow has given promising results (56). There may be a specific predisposition to autoimmune diseases: several cases of Graves' disease and idiopathic thrombocytopenia have been reported, as well as two patients with juvenile rheumatoid arthritis (57).

Acquired forms of hypoparathyroidism often occur later in infancy and adolescence. Hypoparathyroidism has also been reported in a number of mitochondrial encephalomyopathies (i.e., Kearns–Sayre syndrome) where PTH secretion appears affected by the intracellular metabolic abnormality (58). Infiltrative processes such as excess deposition of iron (thalassemia and hemochromatosis), copper (Wilson's disease) or granulomatous processes in the parathyroid can impair the secretion of PTH. Exposure to radiation as part of therapy for hyperthyroidism or lymphoma has been linked to the onset of hypoparathyroidism as has the surgical removal or compromise of the vascular supply to the parathyroid glands.

The more common acquired form of hypoparathyroidism is the autoimmune destruction of the parathyroid gland. However, activating autoantibodies to CaR have been recently described in patients with autoimmune polyendocrinopathy without destruction of the parathyroid glands (59). It can be an isolated process or as part of a polyglandular autoimmune disease type 1, an autosomal recessive disorder also associated with hypoadrenocorticism, hypogonadism, thyroid disease, type I diabetes mellitus, pernicious anemia, chronic active hepatitis, malabsorption, and manifestations of ectodermal dysplasia such as alopecia, vitiligo, mucocutaneous candidiasis, keratopathy, and enamel hypoplasia (autoimmune polyendocrinopathy–candidiasis–ectodermal dysplasia [APECED]) (Vol. 2; Chap. 26) (60). In this disorder, chronic oral candidiasis is the first manifestation usually in early infancy. The average age of onset for mucocutaneous candidiasis, hypoparathyroidism and adrenal insufficiency is 5, 9, and 14 years of age, respectively. About half of affected children end up having at least these three manifestations. The presence of intestinal malabsorption complicates the treatment of hypocalcemia as calcium and vitamin D absorption is often impaired. This autosomal recessive syndrome has been linked to chromosome 21q22.3 and mutations in AIRE, a gene that encodes for a 545 amino acid transcription factor with zinc finger (61–63).

Several conditions are characterized by impaired PTH secretion despite the presence of viable parathyroid

tissue and PTH synthesis. PTH secretion can be impaired in the presence of severe hypomagnesemia. The etiology of hypomagnesemia can be secondary to intestinal malabsorption or excessive renal wasting as seen in Bartter syndrome and renal tubular acidosis. Forms of autosomal recessive primary hypomagnesemia have been described that are linked to loss-of-function mutations in PCLN1 in chromosome 3q. This gene codes for paracellin-1, a tight junction protein expressed in renal thick ascending loop of Henle and the distal convoluted tubule. Mutations in this protein are thought to block the paracellular reabsorption of magnesium and calcium (64,65). Another syndrome of hypomagnesemia is characterized by a primary defect in intestinal magnesium absorption and has been linked to chromosome 9q12-q22.2 (66). Finally, PTH secretion has been shown to be impaired in critical illness, perhaps mediated by an interleukin-mediated overexpression of CaR, but other various etiologies such hypoalbuminemia, hypercalcitonemia, alkalosis, and increased concentration of free fatty acids have been invoked.

Tissue insensitivity to PTH has a clinical presentation very similar to hypoparathyroidism, but the hypocalcemia is usually associated with very high levels of serum PTH. Blomstrand chondrodysplasia, a lethal autosomal recessive condition associated with loss-of-function mutations of PTHR1 is associated with short-limbed dwarfism, advanced skeletal maturation, decreased chondrocyte population, increased bone mineralization, and the biochemical triad consistent with a syndrome of PTH resistance (i.e., hypocalcemia, hyperphosphatemia and elevated PTH levels) (67–69). Mutations include a homozygous Pro132Leu substitution that prevents ligand binding to the receptor.

Pseudohypoparathyroidism

Pseudohypoparathyroidism (PHP) describes various familial disorders, often inherited as autosomal dominant trait, that are characterized by the peripheral resistance to PTH despite apparent normally functioning PTHR1 receptors. Hypocalcemia and hyperphosphatemia occurs in the presence of very elevated PTH levels but without a concomitant elevation of calcitriol levels or increased renal phosphaturia. Hypocalcemia is often not diagnosed until the mid childhood years.

In PHP, various steps in the signal transduction cascade of PTH can be affected to manifest different phenotypes. The primary basis for classification of different forms of PHP is location of the defect proximal or distal to the generation of cAMP. Proximal defects are identified by absence or markedly blunted response of plasma and urinary cAMP to exogenous PTH stimulation; this defines the criterion for PHP type I. In this type the renal mechanism of response to cAMP is intact as evidenced by responsiveness to injected (dibutyryl) cAMP (70). In patients with distal defects, plasma, and urinary cAMP responses to exogenous PTH are normal and basal urinary cAMP excretion may even be supranormal; such patients have type II PHP.

PHP type Ia syndromes, are due to inactivating mutations in the gene GNAS1 encoding the α subunit of the stimulatory G protein. Inactivating mutations in the GNAS1, the gene coding for the stimulatory protein $G_s\alpha$ involved in the PTHR1-coupled signal transduction pathway, are responsible for PTH resistance in this condition by preventing the G protein-mediated activation of adenylyl cyclase by the PTH receptor. All patients are heterozygous and have one normal GNAS1 allele and one defective allele. Although PTH shares the Gs protein complex and the adenylyl cyclase of the cellular signal transduction cascade with other hormones a part of the cascade is specific to PTH (and PTHrP) by structure (PTH receptor) or cell type (effector phosphoproteins of the cascade). These patients can show additional deficiencies due to the defective action of other peptide hormones that use the same stimulatory $G_s\alpha$ to enhance cAMP production. Defects of the shared components may cause resistance to several hormones. In particular, thyrotropin action is often affected, and occasionally hypothyroidism is diagnosed before the hypocalcemia is noted. The action of corticotropin, gonadotropin, glucagon, and GH-releasing hormone, among other hormones, have been shown to be affected because these hormones bind G protein-coupled receptors that activate intracellular pathways making use of the same $G_s\alpha$ (71).

Patients with PHP-Ia share an abnormal habitus called Albright's hereditary osteodystrophy. The biochemical abnormalities present in conjunction with a phenotype of short stature, stocky habitus, developmental delay, round face, short distal phalanx of the thumb, brachymetatarsals and brachymetacarpals, dental hypoplasia, and subcutaneous calcifications constitute the Albright hereditary osteodystrophy syndrome. Height SD score correlates with the activity of the Gs protein (72). Bone age is often advanced rather than retarded, unless hypothyroidism is present. A mild to moderate mental retardation occurs in 50% to 75% of patients; it is also associated with the deficiency of the Gs activity (73).

Dental abnormalities are common: enamel hypoplasia, small crowns, enlarged pulp chambers, root canals with open apices, pulp stones, blunted roots, delayed eruption of deciduous and permanent teeth, hypodentia, thickening of the lamina dura, and early tooth loss due to caries (74). Subnormal senses of smell (elevated detection and recognition thresholds for all vapors) and taste (detection and recognition thresholds supranormal for sour and bitter, normal for salt and sweet) are part of the picture (75). Olfaction is known to be mediated by G protein, which shows 88% amino acid identity to Gs (76). Hypothenar dermatoglyphic patterns and distally located triradii

are frequent (77). Degenerative changes occur in the hip joints, even necrosis of the femoral head (78). Spinal cord compression has occurred as a result of combination of abnormal vertebral fusion, shortened vertebral lamina, and soft tissue calcifications within the spinal canal (79). Hypertension is common in adult patients (80).

Pseudopseudohypoparathyroidism (pPHP) is used to described patients with the Albright osteodystrophy phenotype without the biochemical abnormalities and may represent the inheritance of the paternal defective gene, suggesting the presence of imprinting in the renal inheritance of this disorder. Paternal GNAS1 is weakly expressed in the renal cortex, thus a mutated gene inherited from the father should not manifest the renal PTH resistance phenotype (81). In kindreds with PHP-Ia and pPHP, at least 25 heterozygous nucleotide exchanges in GNAS1 gene have been identified causing variable mutations of Gs (82). Gs is ubiquitously expressed, but appears to have tissue-specific roles, and its heterozygous mutations may cause autosomal dominant diseases with resistance only to a few hormones. In some type I patients normal quantity of Gs has been observed; in one such patient a mutation of the adenylyl cyclase was identified (83).

PHP Type 1b resembles the biochemical phenotype of type 1a without the physical phenotype of Albright osteodystrophy. The G_sa subunit is normal despite the mapping of this form of PHP to chromosome 20q13.3, the site where GNAS1 is located. Since its inheritance is also parentally imprinted, a mutation in the promoter region of GNAS1 or an imprinting control element has been suggested (84). PHP type 1c has no physical phenotype but multiple hormone transduction pathways are affected without a described defect in any G protein (85). Type II PHP is another variant where the phenotypic features are absent and infusion of PTH induces the normal elevation of urinary cAMP but without the expected phosphaturia, suggesting a defect distal to cAMP production.

Vitamin D Deficiency or Resistance

If vitamin D stores are depleted, intestinal calcium absorption can decrease sufficiently to cause hypocalcemia. In growing children, the negative calcium balance affects bone deposition and results in rickets. The parathyroid response to hypocalcemia is intact, but the elevated levels of PTH cannot compensate for the absence of substrate necessary to produce $1,25(OH)_2D$. Inadequate sun exposure or lack of Vitamin D intake can cause a decrease in vitamin D levels. Children with liver disease or taking drugs that enhance the activation of liver hydroxylating enzymes (i.e., phenobarbital) may have impaired 25OHD production or increased turnover to inactive metabolites of 25OHD, respectively. In rare occasions, a deficiency in 1-α-hydroxylase activity in the kidney or the presence of abnormal receptors for $1,25(OH)_2D$, conventionally classified as vitamin D-dependent rickets (VDDR) I and II, respectively, can have the same biochemical consequences and clinical presentation as vitamin D deficiency, including hypocalcemia (86). Patients with VDDR-I do not respond to massive doses of vitamin D or 25OHD. Interestingly, alopecia is often seen in VDDR-II, suggesting a role of VDR in hair development and growth (Vol. 2; Chap. 23).

Alterations of Organs Involved in Calcium Homeostasis

When calcium handling by the gastrointestinal tract, bone or kidney is abnormal or not responsive to calciotropic hormones, hypocalcemia can persist despite an appropriate hormonal response (i.e., increased PTH secretion and calcitriol production). The hyperphosphatemia that ensues with renal failure causes hypocalcemia, as excess phosphate complexes with Ca^{2+}, reducing its serum concentration. The lack of calcitriol production in advanced renal failure further aggravates the risk for hypocalcemia by decreasing intestinal calcium absorption. In disorders that have intestinal malabsorption as one of their manifestations or in cases of short gut syndrome calcium absorption can diminish sufficiently to cause hypocalcemia. In conditions where calcium deposition in bone exceeds nutritional intake (i.e., hungry bone syndrome), as occasionally seen during the treatment phase of severe rickets or following parathyroid surgery for HPT, acute onset of hypocalcemia is not uncommon.

Other Causes

Hypocalcemia can occur in settings where there is a high influx of phosphate or another anion into the extracellular space to complex with Ca^{2+}. The release of high loads of phosphate in tumor lysis syndrome and rhabdomyolysis can cause severe hypocalcemia with deposition of calcium phosphate salts in various tissues. Likewise, an exogenous source of phosphate as in high phosphate content formula can have a similar effect in small infants. In acute pancreatitis, calcium are sequestered by free fatty acid complexes decreasing its effective concentration in serum, while the presence of citrate in exchange blood transfusions or alkalosis can decrease serum Ca^{2+} acutely. Drugs such as furosemide with its calciuric properties, calcitonin, and bisphosphonates with their inhibitory effect on calcium mobilization from bone and several antineoplastic agents have been associated with hypocalcemia that is usually reversible with cessation of therapy or evident only transiently during or shortly after their consumption.

Diagnosis and Evaluation of Hypocalcemia

Signs and Symptoms

Hypocalcemia can be asymptomatic in children and adolescents, especially when it is longstanding, and

is often diagnosed in the setting of a routine biochemical screen. Abrupt decreases in serum Ca^{2+}, on the other hand, predispose children to more severe symptoms, mostly neurological in nature, that require prompt medical attention. Early symptoms are usual neuromuscular in origin and include numbness around the mouth, tingling, paresthesias, muscular cramping (especially after vigorous exercise), and carpopedal spasm. More severe symptoms include seizures, tetany, laryngospasm, and mental status changes. In neonates, symptoms can be more subtle and the only manifestation may be poor feeding and vomiting; however, acute presentations are usually characterized by a history of recurrent seizures, twitching of the extremities, agitation, high-pitched voice, tachypnea, or apnea.

In older asymptomatic children, the physical exam usually reveals no striking abnormality other than hypereflexia, a positive Chvostek sign (twitching of facial muscles after tapping the facial nerve just in front of the ear) and/or a Trousseau sign (carpopedal spasm with hypoxia after maintaining a blood pressure cuff above the systolic blood pressure for three to five minutes). These findings are not exclusively present in hypocalcemic states; the Chvostek sign can be present in a significant fraction of adolescents and other ionic abnormalities such as hypokalemia, hyperkalemia, hypomagnesimia, and severe hypo- or hypernatremia can also cause tetany. Hypocalcemia affects cardiac function by impairing myocardial contractility and prolonging the QTc interval, increasing the predisposition to cardiac arrthymias. Ophthalmologic findings can include papilledema, optic neuritis, and subcapsular cataract formation. In some instances, neonates with acute hypocalcemia may present in cardiac failure. Infants with acute symptomatic hypocalcemia frequently show hypotonia, tachycardia, and a bulging fontanelle on physical examination.

Chronic hypocalcemia can be associated with calcium deposition in intracranial locations with a preference for basal ganglia. Other physical findings in chronic hypocalcemia include coarse hair, dry skin, brittle nails, and defective dentition, all the consequence of inadequate serum Ca^{2+} to support their structural integrity.

Some of the signs and symptoms point to the etiology of hypocalcemia. When hypocalcemia is accompanied by vitamin D deficiency and decreased intestinal calcium absorption, the bone abnormalities commonly seen in rickets are a prominent feature of the physical presentation. If the phenotypic features of type 1 PHP are present, PTH resistance should be suspected, whereas the presence of facial anomalies (i.e., mandibular hypoplasia, hypertelorism, short philtrum, and low set ears), a heart murmur or a history of recurrent infections suggests DiGeorge syndrome. The absence of a thymus shadow on a chest X-ray in a neonate with hypocalcemia should point to this syndrome as should a history of immunodeficiency or recurrent infections. A history of mucocutaneous candidiasis, vitiligo, or alopecia may suggest the presence of autoimmune polyendocrinopathy type 1.

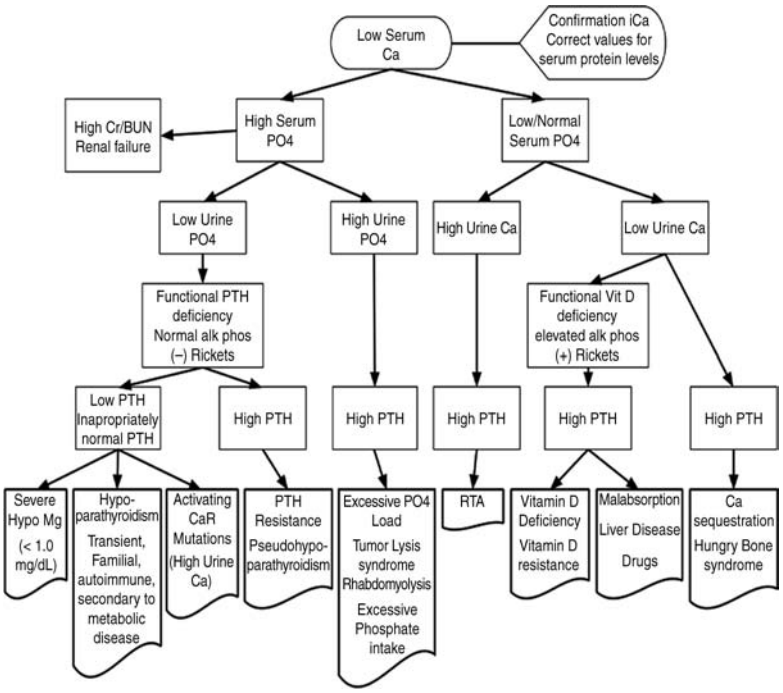

Figure 4 Diagnostic tree in the biochemical evaluation of hypocalcemia.

Biochemical Evaluation

A general biochemical evaluation guideline of hypocalcemia is included in Fig. 4. A close review of family history to search for familial forms of parathyroid disorders or some of its associated conditions can point to the appropriate etiology of hypocalcemia even before the biochemical evaluation. Serum calcium concentration should always be obtained and compared to normal values to confirm hypocalcemia. Since calcium is found in both protein bound and ionized forms in serum, conditions that alter protein content and binding affinity affect the Ca^{2+} concentration in serum. In acidic states, calcium is dissociated from albumin and the concentration of serum Ca^{2+} increases, while the reverse occurs in alkaline conditions. In the absence of ionized measurements of calcium, several conversion factors are available to help correct for protein content and pH in blood. Increases or decreases in pH by 0.1 units decreases and increases ionized Ca by 0.03 mmol/L, respectively.

Several equations are available to correct total calcium measurements for changes in albumin or protein concentration: Ca (mg/dL) = Ca (measured) + 0.8(4 − albumin (mg/dL)), Ca = Ca (measured)/(0.55 + total protein (g/L)/160). An ionized measurement is the more accurate assessment of serum Ca^{2+} concentration and has currently become more routinely available, especially in the hospital setting. Normal values often range 1.12 to 1.23 mmol/L in most laboratories. Adequate sampling is imperative to prevent excessive exposure to air or to high amounts of heparin because, in both circumstances, readings are artificially lower.

As part of a complete evaluation of mineral ion homeostasis, both serum phosphate and magnesium levels should be obtained. Vitamin D stores can be measured by obtaining 25OHD levels, while $1,25(OH)_2D$ levels provide a good measure of PTH activity. The serum alkaline phosphatase level is a measure of osteoblast activity and bone turnover. It is usually elevated in states of high bone turnover as seen in HPT and rickets. If there is evidence of liver dysfunction, the source of alkaline phosphatase can be distinguished by adequate fractionation of enzyme isoforms. Renal function can be adequately screened by measurement of total protein, electrolytes, bicarbonate, BUN, and creatinine. In addition, urine calcium, phosphate, and creatinine levels provide a measure of mineral ion handling by the kidney, especially when done in conjunction with serum measurements.

Several useful calculations provide a measure of calcium handling before and during therapy:

Ca × Phosphate: If more than 70 there is a high predisposition to insoluble mineral deposition in joints and tissues.

Urine Ca/Creatinine: If more than 0.2 calcium deposition in the urinary tract and nephrocalcinosis increases. In healthy neonates and infants, the median ratio is above 0.6 and only decreases after the first year of life. Spot measurements are usually adequate, especially if obtained early in the morning and fasting.

TRP (tubular reabsorption of phosphate) = 1 − (Urine phosphate × Serum Creatinine/Serum phosphate × Urine Creatinine): This measure provides a measure of phosphate retention by the kidney. TmP/GFR = TRP × Serum phosphate (normal range 2.5–4.2 mg/dL), TRP adjusted for glomerular filtration rate.

Phosphate levels should be compared to normal values adjusted for age and serve as a very good surrogate of PTH action. This is particularly useful since the PTH assay is not rapidly run in most hospital settings. A high serum phosphate is usually a sign of low PTH activity, but renal failure should always be ruled out first. A high urine phosphate associated with a high serum phosphate is seldom associated with a low PTH levels as is likely the consequence of a high phosphate load from tumor lysis syndrome or excessive phosphate intake. A low urine phosphate would be consistent with a low or inappropriately low PTH state that should be confirmed with actual PTH measurements. When PTH levels are low, severe hypomagnesemia, usually less than 1 mg/dL, should be ruled out and an exploration for causes of hypoprathyroidism should be initiated. Marked hypercalciuria should create suspicion for the possibility of autosomal dominant hypocalcemia. In cases of autoimmune hypoparathyroidism, antiparathyroid antibodies are seldom detected but the presence of antibodies to other endocrine glands (i.e., adrenal glands) or against aromatic L-amino acid decarboxylase, tyrosine hydroxylase and phenyl alanine hydroxylase can be sought. A very high PTH level in the setting of a high serum phosphate is consistent with a PTH resistance syndrome. Although the infusion of PTH has been useful to distinguish between PHP types 1a and 1b from other forms of PHP that are not expected to show a decrease in elevation of cAMP levels (<3 fold increase) (Elsworth–Howard test), the lack of biosynthetic PTH peptide availability until recently has limited its use. Treatment options are the same for all conditions of PHP.

In hypocalcemic states, PTH levels should be elevated when parathyroid function is normal. The normal range of serum intact PTH values usually falls between 10 and 65 pg/mL.

When the PTH level is appropriately elevated in the presence of hypocalcemia, the serum phosphate level provides a measure of PTH action in the kidney. An elevation in serum phosphate, in the setting of normal renal function, would indicate the absence of the expected phosphaturic effect of PTH on the kidney, making a form of PTH resistance or PHP the likely diagnosis. In PHP, PTH levels are frequently very elevated while calcitriol levels are generally in the normal range or even low despite normal vitamin D stores. To distinguish between different types of

PHP, in addition to careful description of the physical phenotype, a PTH infusion with concomitant measurement of urinary cAMP would be required; a test that is seldom performed because PTH is not readily available in most clinical centers. Fortunately, the treatment is currently similar for all forms of PHP and their clinical classification is less critical for adequate management.

If hypocalcemia is accompanied with normal or low serum phosphate levels, a form of vitamin D deficiency should be suspected if low urine Ca^{2+} is concomitantly observed, a diagnosis that would be supported by physical findings of rickets and an elevated alkaline phosphatase level. Low 25OHD levels would suggest a dietary deficiency, an intestinal malabsorptive process or improper processing by the liver. Normal 25OHD levels would point to a defect in calcitriol production or action. It is common to see very high levels of $1,25(OH)_2D$ in patients with vitamin D receptor defects or VDDR-II disorders. Hypocalcemia in conjunction with low/normal serum phosphate and a high urine calcium point to a defect in renal tubular function. PTH levels are not useful in distinguishing disorders of vitamin D or renal tubular function since they are commonly elevated in these conditions.

Management of Hypocalcemia

Acute Hypocalcemia

In a symptomatic patient the initial goal is to take the appropriate steps to eliminate symptoms associated with hypocalcemia. In patients whose acid–base status or the infusion of agents that may complex with calcium is responsible for the hypocalcemia, adequate steps to ameliorate these causes should be taken. In acute symptomatic cases or in neonatal hypocalcemia, an intravenous infusion of calcium is the most effective intervention. Calcium gluconate (10% Calcium Gluconate = 9.3 mg Ca/ml), 2 ml/kg can be administered slowly, over a 10 minute period to avoid cardiac conduction problems while monitoring the electrocardiogram (ECG). The dose can be repeated every six to eight hours. Phosphate and bicarbonate infusions should never be given concomitantly to prevent the precipitation of calcium salts. A central intravenous access is preferable, since inadvertent extravasation of calcium causes severe chemical burns and skin damage.

To maintain normocalcemia, a continuous intravenous infusion of calcium (20–80 mg Ca/kg/24 hours) is preferable over frequent boluses as long as there is good intravenous access since a large fraction of the calcium content in the bolus is lost in the urine during the infusion. The infusion rate should be titrated to achieve a low normal serum Ca^{2+} level. Hypomagnesemia should be corrected when present. $MgSO_4$ (50% solution) 25 to 50 mg Mg^{2+}/kg in intravenous or intramuscular form every four to six hours, 10 to 20 mg Mg^{2+}/kg for the neonate. A maintenance dose of 30 to 60 mg Mg^{2+}/kg/day as an oral or continuous intravenous infusion could also be given if necessary.

It is preferable to transition patients to oral therapy as soon as possible. In asymptomatic patients, it is likely that the hypocalcemia, even when very severe, has been longstanding and oral therapy should be the first line of therapy. Several forms of calcium supplements [calcium salts of carbonate (40% Ca), citrate (21% Ca), lactate (13% Ca), gluconate (9.4% Ca), and glubionate (6.6% Ca)] are available to be used for this purpose. The dose of oral calcium should provide 25 to 100 mg Ca/kg/day divided every four to six hours. Milk is also good source of calcium (119 mg Ca/100 mL) but not necessarily appropriate in hyperphosphatemic states since its phosphate content is high (93 mg/100 mL). If calcium losses from bone have been significant, as in untreated cases of nutritional rickets or following parathyroid surgery for HPT, much higher requirements for calcium are often required to sustain the remineralization of bone. In these instances, calcium intake should be increased to meet the additional requirements. Even under optimal conditions where calcium binders are avoided and there is adequate vitamin D supplementation, the intestinal absorption of calcium rarely exceeds 60% to 70%. Both forms of therapy should be adjusted as needed with monitoring, paying attention to serum Ca^{2+} levels, Ca $\times$ phosphate and urine Ca/urine creatinine to avoid the deposition of calcium salts in peripheral tissues and kidney.

Chronic Hypocalcemia

The overall goal in management of chronic hypocalcemia is to achieve a serum Ca^{2+} level that does not cause symptoms while avoiding hypercalcemia or excessive hypercalciuria (i.e., urine Ca/urine creatinine > 0.2), the latter being particularly difficult to achieve in hypoparathyroidism as the absence of PTH limits calcium absorption in the renal distal tubule. In hypoparathyroidism, serum Ca levels less than 9 mg/dL limits the degree of hypercalciuria; it is not unusual to maintain patients with hypoparathyroidism borderline hypocalcemic as long as they remain asymptomatic to avoid complications of hypercalciuria. In some patients that normocalcemia has been difficult to achieve without significant hypercalciuria, the addition of a thiazide diuretics has been shown to limit hypercalciuria while increasing serum Ca^{2+} significantly. Correction of hypocalcemia does not need to be so stringent in most forms of PHP since hypercalciuria is rarely seen even when calcium levels reach high normal values. It is not unusual to require relatively high doses of calcium to overcome longstanding hypocalcemia, especially in PHP; however, calcium requirements are frequently reduced once normocalcemia has been achieved and the degree of hyperphosphatemia has been reduced.

Patients with chronic hypomagnesemia may require daily parenteral injections of magnesium sulfate to prevent tetany, seizures, and other neurologic symptoms (speech slurriness, weakness, and

choreoathetoid movements). Vitamin D analogs are not useful to increase the absorption of magnesium. Alternative therapies to prevent the invasive intravenous or intramuscular treatments include overnight nasogastric infusions of magnesium. Mild forms of hypomagnesemia can be treated with oral regimens of magnesium gluconate or citrate.

In all forms of hypoparathyroidism, vitamin D administration is an integral part of the therapy once oral supplementation of calcium is initiated. Calcitriol, in most instances, is the adequate choice due to its short half-life and high activity, which limits its toxicity and increases efficacy, respectively. The standard dose of 10 to 50 ng/kg/day is usually sufficient to promote adequate calcium absorption, but the dose is often increased further if the hypocalcemia remains recalcitrant to oral therapy. Calcitriol is also the adequate choice in the treatment of hypocalcemia secondary to renal failure, liver disease or defects in 1-α-hydroxylase functions. In intestinal malabsorption syndromes where there is a deficiency in fat absorption, calcidiol (1–3 mcg/kg/d), the more polar vitamin D metabolite can be used. When hypocalcemia is caused by poor vitamin D stores, vitamin D, 1200 to 1600 U/day, or 50,000 U IM should be quite adequate since calcitriol production and action is not defective. Finally, patients with 1-α-hydroxylase deficiency or VDDR-I respond well to calcitriol therapy, while VDDR-II patients with an abnormal vitamin D receptor usually require an exceedingly high dose of calcitriol (up to 1000 mcg/day) or chronic parenteral calcium to maintain normal serum Ca^{2+}.

In general, phosphate binders are not required to manage hyperphosphatemia in all forms of hypoparathyroidism; moreover, the use of calcium alone limits intestinal phosphate absorption. The intake of phosphate-rich foods (i.e., dairy products) should not be encouraged. The use of an nonabsorbable antacid when serum phosphate levels remains greater than 6 mg/dL in the older child may be useful to prevent metastatic calcifications.

In chronic forms of hypoparathyroidism, frequent follow-up (i.e., every three to four months) to ensure adequate calcium balance may be adequate as is periodical screening of kidney function by urine analysis and ultrasound to rule out the presence of hematuria, kidney stones, and nephrocalcinosis.

HYPERCALCEMIA

Etiology

Hypercalcemia develops when either there is an increased influx of calcium from the gastrointestinal tract or bone into the extracellular space that exceeds the renal excretory capacity or when there is enhanced renal tubule absorption of calcium. Causes of hypercalcemia can also be divided into etiologies that involve abnormalities in calcium sensing, parathyroid function, calciotropic hormones, or defects in calcium handling by organs targeted by these hormones (Table 2).

Table 2 Causes of Hypercalcemia

- *Parathyroid dysfunction/hyperparathyroidism*
  - Altered Ca^{2+} sensing
    - Familial hypocalciuric hypercalcemia
    - Neonatal severe hyperparathyroidism
  - Excessive PTH production
    - Primary hyperparathyroidism
      - Multiple endocrine neoplasia (types I, IIa)
      - Sporadic forms
    - Secondary/tertiary hyperparathyroidism
      - Renal failure
      - Renal tubular acidosis
      - Treatment of hypophosphatemic rickets
    - Transient hyperparathyroidism
      - Neonatal hyperparathyroidism (secondary to maternal hypoparathyroidism)
  - Excessive PTH receptor activity
    - Jansen syndrome
- *Vitamin D excess*
  - Nutritional
  - Granulomatous disorders (sarcoidosis, tuberculosis, histoplasmosis)
  - Neoplasms and lymphomas
- *Immobilization*
- *Neoplasia*
  - Production of PTHrP
  - Production of cytokine and osteoclast activating factors
  - Osseous metastases
- *Other causes*
  - Hypophosphatemia
  - High calcium load (milk alkali syndrome)
  - Vitamin A intoxication
  - Drugs (e.g., thiazides, lithium)
  - Hypethyroidism
  - Adrenal insufficiency
  - Pheochromocytoma
  - Vasoactive intestinal peptide-secreting tumor
  - Hypophosphatasia
    - Juvenile rheumatoid arthritis
    - Neonates
      - William's syndrome
      - Subcutaneous fat necrosis

Familial Hypocalciuric Hypercalcemia

Familial hypocalciuric hypercalcemia (FHH) is an autosomal dominant disorder characterized by mild, asymptomatic hypercalcemia, increased tubular reabsorption of calcium, and inappropriately normal PTH values, all caused by the presence of an inactivation mutations in one of the alleles coding for the CaR (87). The set point for serum Ca^{2+} is increased in these patients (21). Many inactivating mutations are found in the receptor's extracellular domain (Fig. 3). Affected individuals often go undiagnosed until a laboratory screen reveals the hypercalcemia. They do not have the common skeletal and gastrointestinal manifestations seen in primary HPT and are not at risk to develop urinary calcium stones or pancreatitis unless there is a significant shift in the calcium set point. The parathyroid glands are normal in appearance and do not show significant hyperplasia in mild forms of the disorder. There is, nevertheless, a broad spectrum of the disorder ranging from mild hypercalcemia to severe, life-threatening hypercalcemia

that typically presents in the neonatal period. This severe form, classically described as neonatal severe HPT, is either homozygous for inactivation mutations of the CaR, or heterozygous for a very severe inactivation mutation aggravated by exposure to low Ca^{2+} in fetal development. These infants have very elevated PTH levels and all the manifestations of HPT including hyperplasia of the parathyroid glands; removal of most parathyroid tissue is often necessary.

The FHH phenotype has also been linked to an unidentified gene in chromosome 19q, suggesting that defects other than inactivating mutations of the CaR can cause a shift in serum Ca^{2+} set point (88). In addition, patients with hypercalcemia in the setting of other autoimmune disorders have been shown to have inactivating antibodies directed to the CaR (89).

Hyperparathyroidism

HPT is diagnosed when hypercalcemia is accompanied by elevated or inappropriately normal PTH levels. The hypercalcemia is the result of the increased PTH secretion from the overall increase in parathyroid total mass and/or the associated abnormalities in the set point for serum Ca^{2+} caused by a decrease in CaR expression. HPT is one of the most common causes of hypercalcemia in adults, but it is a relatively uncommon disorder in children and neonates. In one study only 2% of 414 cases were less than 19 years of age (90). Furthermore, less than 20% of pediatric cases are diagnosed in children younger than 10 years. Whereas, the incidence of HPT in adults shows that females outnumber males by almost 3 : 1, the incidence in children is almost reversed to a male : female ratio of 3 : 2. Most cases of HPT (80%) represent a sporadic adenomatous change in one of the parathyroid glands, but a subset of patients show generalized hyperplasia of all glands that can occur sporadically or as part of the multiple endocrine neoplasia (MEN) type 1 and 2A (Vol. 2; Chap. 27). Parathyroid carcinoma is an even less common but more indolent form of parathyroid cell neoplasia. Parathyroid adenomas show a marked decrease in sensitivity to elevations of serum Ca^{2+} likely secondary to reduced CaR (91). Hyperplastic glands remain more sensitive to Ca^{2+} but secrete more PTH by virtue of the increased cell number.

The underlying cause for sporadic primary HPT is not known, but most tumors are monoclonal in origin (92–94); the genetic defect in some of them has been allocated to translocation of cyclin D1 to the proximity of the PTH gene promoter inducing its overexpression (95). Cyclin D1 or PRAD1 is a 295 amino acid cyclin protein, which may be important in cell cycle regulation since it regulates protein kinases that act in the cell's mitotic cycle. It has been postulated that the overexpression of cyclin D1 plays a role in the development of parathyroid adenomas. Overexpression of this gene in parathyroid chief cells results from a chromosome inversion involving regions 11p15 and 11q13, repositioning the promoter for the PTH gene nearby the PRAD1 gene. This change leads to the overexpression of cyclin D1 increasing the rate of cell division. It should be noted that the activity of cyclin D1 has been noted to be increased in some parathyroid adenomas without this chromosomal rearrangement (96).

Abnormalities of the retinoblastoma tumor-supressor gene have been found in parathyroid carcinomas (97). In contrast to PRAD1, mutations in the tumor-suppressor genes RAD51 and RAD54 have not been detected in parathyroid adenomas. Likewise, mutations in the CaR gene have not been identified in sporadic adenomas. On the other hand, loss of heterozygosity in the MEN 1 region on chromosome 11q13 has been found in 30% of sporadic parathyroid tumors (98). This region codes for the tumor-suppressor gene menin, the affected gene in MEN type 1 patients.

In contrast to adenomas, the pathogenesis of parathyroid hyperplasia may involve different mechanisms. Diffuse hyperplasia of the parathyroid glands may occur following longstanding stimulation of parathyroid activity in response to hypocalcemia. A normal parathyroid is exposed to chronic hypocalcemia (e.g., renal failure, renal tubular acidosis, and therapy for hypophosphatemic rickets), may undergo hyperplastic changes with concomitant increases in PTH secretion that cause hypercalcemia and secondary HPT. Over time, the calcium concentration needed to suppress PTH secretion gradually increases, leading to a new steady state in which the serum calcium is maintained at a higher level (93,99). Ionized calcium concentrations of 1.1 to 1.4 mmol/l may be needed to achieve half-maximal suppression of PTH secretion (normal = 0.99 mmol/l). Eventually, PTH secretion may not be suppressible even by high circulating levels of calcium. Supporting this hypothesis, individuals with tertiary HPT may show a spectrum of evolving parathyroid dysfunction (99). In severe cases, often in the setting of renal failure, adenomatous changes can also occur (tertiary HPT). A similar but usually less severe and transient form of HPT has been observed in neonates born to mothers with hypoparathyroidism and exposed to low serum Ca^{2+} in utero.

Familial forms of HPT, accounting for about 10% of all cases and comprising most cases of hyperplasia, are usually transmitted in autosomal dominant fashion. In type 1 MEN, the affected gene, menin, has been mapped to chromosome 11q13 (100). HPT is associated with almost all affected members and is often the first manifestation of the disorder; pancreatic tumors, pituitary adenomas, and neuroendocrine tumors of the gastrointestinal tract are other common manifestations. MEN type 2A is also an autosomal dominant disorder in which HPT occurs in association with medullary carcinoma of the thyroid and pheochromocytoma. The incidence of HPT is only 10% to 30% and is rarely the first manifestation of the syndrome. The typical presentation is hyperplasia of all glands but adenomatous changes are not uncommon,

especially in type 2A. The affected gene is the RET proto oncogene in chromosome 10q11.2 (101).

Parathyroid hyperplasia, cystic adenomas, or carcinomas have been associated with a familial form of HPT and ossifying fibromas of the jaw (102,103). This disorder is linked to chromosome 1q21-q23 and affected members can have associated renal lesions (Wilms tumors or polycystic kidneys) and uterine lesions (adenomyomatous polyps). The gene linked to this from of HPT has been identified as HRPT2 coding for the protein parafibromin (104,105). Families with autosomal dominant (103) and recessive (106) forms of isolated HPT have also been described but the specific genetic defects associated with them have not been described yet.

Although most parathyroid-related causes of hypercalcemia are caused by an excess of chief cell activity, there are rare instances where the defect resides in the tissue sensitivity to normal levels of PTH. In Jansen syndrome, children present with hypercalcemia, a metaphyseal dysplasia and other skeletal findings consistent with HPT. Recently, the genetic defect has been identified as a mutation of the PTH receptor that renders it constitutively active (107). These children have undetectable PTH levels because their parathyroids respond appropriately to hypercalcemia.

Vitamin D Excess

Excessive exposure to vitamin D in the diet or for therapeutic reasons will cause an increase in intestinal calcium absorption and hypercalcemia. In this setting, phosphate absorption also is increased, and PTH levels are appropriately suppressed. Hypercalcemia is similarly present in a number of granulomatous disorders (i.e., sarcoidosis, tuberculosis, and leprosy), chronic collagen-vascular inflammatory disorders, and some neoplastic diseases (Hodgkin B cell lymphoma), where there is proliferation and activation of monocytic cells, production of $1,25(OH)_2D$ is increased due to the unregulated expression of 1-α-hydroxylase in these cells.

Other Causes of Hypercalcemia

As bone is the repository of greater than 98% of the body's calcium, increased or unregulated bone turnover can easily overcome the renal excretion capacity for calcium. Immobilization, particularly in adolescents and when prolonged for more than two weeks, results in decreased bone accretion and increased bone resorption that is initially noted as hypercalciuria, but when persistent, frank symptomatic hypercalcemia can occur requiring immediate treatment (108). Malignancy is a rare cause of hypercalcemia in children. When it occurs, it can be the result of metastases to bone with concomitant dissolution of mineral content or the production of lytic factors by the original tumor that promote the mobilization of calcium (i.e., PTHrP, IL-1, IL6, TNF, prostaglandins) (109).

Excess thyroid hormone can promote a disproportional stimulation of osteoclast function causing increased bone resorption and hypercalcemia (110,111). Increased prostaglandin E secretion by renal tubular cells in Bartter syndrome has been suggested to promote bone resorption. Drugs that are known to cause hypercalcemia include thiazide diuretics, which increase the renal tubular resorption of calcium while reducing the plasma volume. Vitamin A excess has been shown to cause hypercalcemia likely mediated by the activation of osteoclast-mediated bone resorption (112). Hypercalcemia has been observed in patients treated with lithium. PTH levels are elevated, suggesting a form of HPT. Lithium has been shown to decrease the sensitivity of the parathyroid cell to serum Ca^{2+}, by interfering with the signaling mechanisms utilized by the CaR (113).

Excessive intake of calcium in milk, calcium containing antacids and alkali can result in absorptive hypercalcemia. Conversely, severe hypophosphatemia associated with parenteral nutrition and prematurity is associated with a reciprocal increase in serum Ca^{2+} concentration, partly due to increased calcitriol levels and intestinal calcium absorption. Hypercalcemia has also been observed in adrenal insufficiency, pheochromocytoma and vasoactive polypeptide secreting tumors by mechanism(s) that have not been well defined. Patients with juvenile rheumatoid arthritis can have hypecalcemia caused by increased amounts of interleukin-1b, an osteoclast-activating cytokine (114).

Hypercalcemia is present transiently during infancy in 15% of children with Williams syndrome, a sporadic disorder linked to the loss of the elastin gene in chromosome 7 characterized by a defined facial features (e.g., dolichocephaly, periorbital prominence, bitemporal depression, long philtrum with prominent lips and nasal tip, full cheeks, epicanthal folds, and periorbital prominence) among other physical features. More prominently up to 30% of affected children have supravalvular aortic stenosis. The etiology of hypercalcemia is unknown; however, mildly elevated calcitriol and calcidiol levels have been reported (115,116). The hypercalcemia often resolves before the first year of life; however, hypercalciuria often persists.

Hypercalcemia, sometimes very severe and life-threatening, has been seen in infants with subcutaneous fat necrosis, a condition seen in neonates, often premature that have had traumatic births or a history of critical illness with significant poor peripheral perfusion. Subcutaneous fat undergoes necrosis, showing a significant infiltration by mononuclear cells. Although the etiology of hypercalcemia is not known, excessive prostaglandin E production and mononuclear-derived calcitriol, which in some cases have been mildly elevated, have been invoked as causes (117,118).

Diagnosis and Evaluation of Hypercalcemia

Signs and Symptoms

The degree of symptoms that children manifest depends on the elevation of serum calcium. Children

with mild (total calcium $<12\,mg/dL$) or chronic hypercalcemia frequently go undiagnosed unless a routine biochemical screen reveals the elevation of serum calcium. The predominant manifestation may be failure to thrive with arrest of weight gain and linear growth. In mild hypercalcemia (total calcium 12–13.5 mg/dL) generalized weakness, anorexia, constipation, and polyuria are usually present. In severe hypercalcemia (total calcium $>13.5\,mg/dL$), nausea, vomiting, dehydration and encephalopathic features including coma and seizures may occur. Neonates with severe hypercalcemia often present in respiratory distress and have hypotonia and apnea. It is not uncommon for relatives and patients to note significant psychological changes ranging from depression to paranoia and obsessive-compulsive behavior.

The physical examination is usually normal in hypercalcemic patients. In patients with MEN 2B, a marfanoid habitus is often present. A parathyroid mass is rarely palpable. When not dehydrated, hypertension may be noted, and a cardiac evaluation may show shortened QTc intervals in ECG tracings. In chronic hypercalcemia, a survey of soft tissues may reveal calcifications in kidney, skin, SQ tissues, cardiac arteries, and gastric mucosa. In untreated patients with prolonged HPT, and occasionally reported in untreated children where the diagnosis was never suspected, distinctive skeletal findings showing subperiostal resorption of the distal phalanges, tapering of the distal clavicles, salt and pepper appearance of the skull, bone cysts, and brown tumors (liquefied bone) are the constellation of findings that describe osteitis fibrosa cystica. These findings are readily visible by conventional radiography.

Biochemical Evaluation

The evaluation of hypercalcemia should include a thorough medical history searching for exposure to drugs, agents and conditions that can cause hypercalcemia, and a family history of hypercalcemia or other associated medical conditions. The approach to the biochemical evaluation is similar to the evaluation described for hypocalcemia and should initially include the measurement of serum intact PTH levels, phosphate, and magnesium together with measurements of urine calcium excretion. Renal function should also be assessed to rule out renal insufficiency, and a urine analysis is useful to look for the presence of hematuria or calcium salt residue.

It is sometimes useful to structure the evaluation as outlined in the diagnostic tree in Fig. 5. Since PTH levels are often not available on the day of the evaluation, a measurement of urinary calcium can help distinguish HPT and other forms of hypercalcemia from FHH in cases of mild hypercalcemia. It should be noted that the hypocalciuria is not always detected in well described cases of FHH and repeat screening is recommended in suspected cases. At any rate, marked hypercalciuria would not be expected in

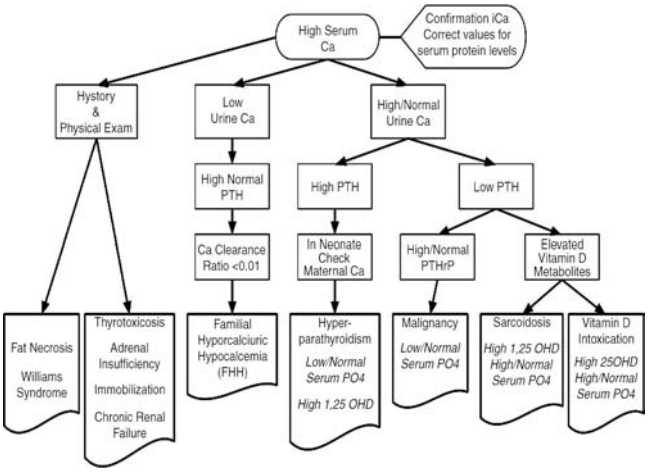

Figure 5 Diagnostic tree in the biochemical evaluation of hypercalcemia.

FHH. HPT is diagnosed when hypercalcemia is noted in conjunction with elevated PTH levels. In the absence of secondary causes of HPT, the presence of hypercalciuria is consistent with primary HPT. Hypercalciuria is usually present in HPT, since the PTH-mediated increase in tubular calcium resorption does not fully compensate for the increase in calcium concentration in the glomerular filtrate. The degree of hypercalciuria has significant diagnostic value, especially when trying to distinguish mild HPT from FHH, since mild elevations of PTH are often seen in both cases. The calculation of 24-hour urinary calcium clearance provides a measure of calcium handling by the kidney. Decreased urinary calcium excretion in the presence of mild hypercalcemia should raise the possibility of inactivating mutation of the CaR and FHH. A better measure of hypercalciuria that takes into account changes in glomerular filtration is the calcium clearance ratio [(Urine Ca $\times$ Serum creatinine)/(Urine creatinine $\times$ Serum Ca)]. The clearance ratio in FHH is one-third of that in typical primary HPT, and a value less than 0.01 is virtually diagnostic of FHH. Unfortunately, as noted earlier, FHH patients do not always show significant hypocalciuria. Mild elevations of magnesium can sometime distinguish FHH from HPT, since serum magnesium is usually in the low normal range in HPT. A family history of asymptomatic hypercalcemia would provide further support for a diagnosis of FHH. Both parents should be evaluated when the diagnosis is suspected in a child. Adequate distinction between HPT and FHH is not trivial since hypercalcemia in FHH has not been associated with any long-term adverse outcome and requires no treatment. Furthermore, the surgical removal of parathyroid tissue in FHH, in cases that were thought to represent HPT, does not correct the hypercalcemia.

In cases where the MEN syndrome is suspected, an adequate screen for other hormonal abnormalities in MEN 1 is warranted. Isolated HPT as the first manifestation in MEN 2B is rare, but a genetic screen is currently available to rule mutation in the RET gene in suspected cases. In high probability cases, the presence of a pheochromocytoma should be ruled out prior to a parathyroidectomy.

When PTH levels are adequately suppressed in the presence of hypercalcemia, elevated 25OHD levels would suggest vitamin D intoxication. Elevated $1,25(OH)_2D$ without a concomitant elevation of 25OHD points to an ectopic source of 1-α-hydroxylase. In both settings, hyperphosphatemia and marked hypercalciuria are usually present greatly increasing the predisposition to calcium toxicity. In the absence of elevated PTH and vitamin D metabolites, hypercalcemic patients that have not been exposed to high calcium ingestion or prolonged immobilization should be screened for the secretion of other hypercalcemic factors (i.e., PTHrP, prostaglandin E).

Management of Hypercalcemia

The management of hypercalcemia depends on the severity and cause of the elevation of serum Ca^{2+}. When hypercalcemia is mild and the patient is asymptomatic, no initial treatment may be necessary and medical efforts to reach a diagnosis should be given preference. When hypercalcemia is severe (total serum calcium >14 mg/dL) or when there are symptoms and signs of cardiac, gastrointestinal and central nervous system dysfunction, prompt intervention is appropriate. Since patients are usually dehydrated because of the polyuria and anorexia associated with severe hypercalcemia, the initial step is to provide adequate hydration, preferably in the form of isotonic saline at 3000 cc/M^2 for the first 24 to 48 hours, to restore vascular volume, increase glomerular filtration rate and dilute serum Ca^{2+}. After hydration, the loop diuretic furosemide (1 mg/kg every six hours) can further inhibit the reabsorption of calcium, especially in the presence of sodium, further promoting calciuresis. In comatose patients, hemodialysis should be considered as a means to decrease serum Ca^{2+} more aggressively.

If hypercalcemia does not respond to these initial measures, agents that block bone-resorption may be useful as adjuvant therapy. Calcitonin 4 U/kg SQ q 12 hours is commonly used for this purpose; however, its efficacy diminishes with continuous administration due to tachyphylaxis. Bisphosphonates, analogs of pyrophosphate that inhibit osteoclast action, have been used, specially when hypercalcemia is primarily driven by the mobilization of calcium from bone as in cases of tumor induced hypercalcemia, severe HPT or immobilization. Both etidronate (7.5 mg/kg/day) and parmidronate (0.5–1.0 mg/kg/dose) could be used, the latter given as a single-dose intravenous infusion. The lowering of serum calcium levels can be of days to weeks duration. There should be some caution about its repeated use, since there is still limited experience with the use of these pharmacological agents in growing children. Agents such as plicamycin and gallium nitrate have similar effects in bone, but their use is associated with significant toxicity and should be avoided if possible.

Additional steps in the management will be partially determined by the etiology of hypercalcemia. When hypercalcemia is due to excess vitamin D ingestion or activity, glucocorticoids (prednisone 1 mg/kg/day) can be very effective since they inhibit both 1-α-hydroxylase activity and intestinal calcium absorption. Glucocorticoids are also effective in the treatment of hypercalcemia in juvenile rheumatoid arthritis since it reduces interleukin-1-β production Ketoconazole (3 mg/kg/day divided in three doses) is also a very effective inhibitor of 1-α-hydroxylase activity, but its use is associated with significant gastrointestinal side effects and can cause adrenal insufficiency.

Pharmacological agents (i.e., calcimimetics) that can activate the CaR and suppress PTH secretion in affected glands are already in the market for the treatment of secondary HPT from renal failure and parathyroid carcinoma (119). Their use in children has not been approved yet. Adolescents and young patients with well described HPT, preferably confirmed by several measurements of serum calcium and PTH, the surgical removal of the affected gland is ultimately required to control hypercalcemia, especially since this step may be curative. A number of imaging techniques (i.e., neck ultrasound, computed tomography, magnetic resonance imaging, and radionuclide scanning) have been used to detect a hyperfunctioning gland; however, the reported sensitivities have ranged between 40% to 90% and may be more informative when used in combination. More recently, ^{99m}Tc-sestamibi scanning has shown some promise, especially in the visualization of adenomas (120). Intraoperative radionuclide imaging and measurements of PTH are now feasible, aiding the surgeon in his search for hyperplastic or adenomatous tissue since successful removal would be reflected by an adequate rapid drop of PTH levels (121). In cases of an isolated adenoma, its resection is usually curative. In cases of isolated hyperplasia or secondary HPT, removal of three- and one-half glands is recommended. Total parathyroidectomy is recommended with autotransplantation of minced parathyroid tissue in the forearm for patients with MEN or other forms of general hyperplasia, where it could easily be removed in cases of recurring hypercalcemia. Postsurgical hypocalcemia is common and easily treated with calcium supplements. It is becoming common practice to supplement patients with calcium and vitamin D immediately in the postsurgical period to avoid symptoms of hypocalcemia. Inadequate temporary dysfunction of the remaining parathyroid tissue due to an impaired vascular supply caused by local inflammation is not uncommon. Pre- or peri-surgical localization of an adenoma can prevent an unnecessary

exploration of both sides of the neck and limit the risk of postoperative hypocalcemia. In cases of severe HPT, hypocalcemia can be more severe and prolonged due to hungry bone syndrome. These patients have severe phosphate and calcium deficits as mineral bone deposition takes place. The use of both calcium and phosphate supplements together with calcitriol is recommended. In some instances, permanent hypoparathyroidism ensues, requiring lifelong therapy. Thus, although the initial treatment of symptomatic hypercalcemia is similar regardless of etiology, the appropriate diagnosis, the cause that has lead to the clinical presentation is essential to implement the adequate acute and long-term therapeutic intervention.

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Neonatal Calcium, Magnesium, and Phosphorus Disorders

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INTRODUCTION

Calcium (Ca) is the most abundant mineral in the body and, together with phosphorus (P), forms the major inorganic constituent of bone. Magnesium (Mg) is the fourth most abundant cation and is the second most common intracellular electrolyte in the body. The maintenance of mineral homeostasis requires a complex interaction of hormonal and nonhormonal factors; adequate functioning of various body systems, in particular, the renal, gastrointestinal, and skeletal systems; and adequate dietary intake. From a clinical perspective, mineral homeostasis is reflected in the maintenance of circulating concentrations of Ca, Mg, and P in the normal range, and integrity of the skeleton.

In the circulation, the amount of Ca, Mg, and P is less than 1% of their respective total body content; however, disturbances in serum concentrations of these minerals are associated with disturbances of physiologic function manifested by numerous clinical symptoms and signs. Chronic and severely lowered serum concentrations of these minerals also may reflect the presence of a deficiency state.

Skeleton has the dual function of providing both structural and mechanical support as well as being a reservoir for mineral homeostasis. At all ages, the total body content of Ca, Mg, and P in the skeleton are about 99%, 60%, and 89%, respectively, and disturbances in mineral homeostasis can result in osteopenia and rickets in infants and children, and osteomalacia and osteoporosis in adults.

The mechanisms to maintain mineral homeostasis in neonates are the same as for children and adults. However, the newborn infant has a number of unique challenges to maintain mineral homeostasis during adaptation to extrauterine life and to continue the rapid rate of growth. These include an abrupt discontinuation of high rate of intrauterine accretion of Ca (approximately 120 mg/kg/day), Mg (approximately 4 mg/kg/day), and P (approximately 70 mg/kg/day) during the third trimester (1,2), a smaller skeletal reservoir available for mineral homeostasis, a delay in establishment of adequate nutrient intake for at least a few days or longer, particularly in the sick and preterm infants, and high requirement for these minerals for the most rapid period of postnatal skeletal growth, with an average gain in length of more than 25 cm during the first year. There also may be diminished end-organ responsiveness to hormonal regulation of mineral homeostasis, although the functional capacity of the gut and kidney improves rapidly within days after birth. The effects of these issues are exaggerated in infants with heritable disorders of mineral metabolism such as extracellular calcium-sensing receptor (CaR) mutations, and in infants who have experienced adverse antenatal events such as maternal diabetes, intrapartum problems such as perinatal asphyxia or maternal Mg therapy, or postnatal problems such as immature function of multiple organs from premature birth.

Increased understanding of the physiology and molecular basis of mineral metabolism allows a better understanding of the pathophysiology of the resultant clinical disorder. This in turn allows a more rational management to minimize the adverse impact from disturbed mineral homeostasis and to prevent iatrogenic causes precipitating or prolonging these problems.

NORMAL CIRCULATING CONCENTRATION

Calcium (1 mmol/L 4 mg/dL)

Serum Ca occurs in three forms: approximately 40% is bound, predominantly to albumin; approximately 10% is chelated and complexed to small molecules such as bicarbonate, phosphate, or citrate; and approximately 50% is ionized. Complexed and ionized Ca (iCa) are ultrafilterable.

Total Ca concentrations (tCa) in cord sera increase with increasing gestational age and may be as high as 3 mmol/L at term, and are significantly higher than paired maternal values at delivery. Serum tCa reaches a nadir during the first two days after

birth; thereafter, concentrations increase and stabilize at a level generally above 2 mmol/L. In infants exclusively fed human milk, the mean serum tCa increases from 2.3 to 2.7 mmol/L over the first six months postnatally. Normally, serum tCa in children and adults remains stable, with a diurnal range of less than 0.13 mmol/L. During the third trimester of pregnancy a modest reduction in maternal serum tCa concentration (average 0.1 mmol/L) is associated with a decrease in serum albumin concentration.

Serum iCa concentration is the best indicator of physiologic blood Ca activity. Extracellular Ca concentration particularly iCa is normally maintained within a narrow range and is critical to many important biological functions. The Ca ion serves as an intracellular second messenger, but also functions as an extracellular messenger through the actions of extracellular calcium sensing receptor (CaR). Ca homeostasis also involves the maintenance of an extremely large Ca concentration gradient across the cellular plasma membrane.

Measurement of serum iCa is firmly established in clinical medicine, and simple, rapid, and direct determination of iCa from whole blood, plasma, and serum by ion-selective electrodes are freely available. Whole blood iCa analyzers are gaining popularity because they are adaptable for ''point of care'' testing. However, some differences exist in values from different iCa analyzers particularly for whole blood iCa values (3), as a result of differences in the design of the reference electrode, formulation of calibrating solutions, and the lack of a reference system for iCa. Thus, normative data should be generated according to the subject's age, the instrument, and type of sample used for iCa measurement.

One report showed a wide range of cord whole blood iCa of 0.4 to 1.85 mmol/L from apparently normal pregnancies (4). This is a much wider range compared from multiple reports based on cord sera, although the range for whole blood iCa becomes much narrower within hours after birth and similar to serum iCa values. Cord serum iCa increases with increasing gestational age and is higher than values in paired maternal sera. In healthy term neonates, serum iCa averages 1.25 mmol/L with 95% confidence limits of 1.1 to 1.4 mmol/L (4.4–5.6 mg/dL) and there is a decline in serum iCa in the first 48 hours of life with a nadir at 24 hours (5). Serum iCa generally changes in parallel with tCa in healthy humans. However, serum iCa is stable and normal during pregnancy in contrast to a slight reduction in tCa. Serum tCa and iCa are correlated in infants and adults but is inadequate to predict one from the other with sufficient accuracy. Serum iCa decreases in the presence of high serum albumin, P, bicarbonate, and heparin. Serum iCa increases with increased Mg, and is inversely related to blood pH. The effect of the latter may be minimized by the immediate analysis of serum samples for iCa.

Magnesium (1 mmol/L 2.4 mg/dL)

Approximately 30% of serum Mg is in the protein-bound form, with the remainder in the ultrafilterable portion. Seventy to 80% of ultrafilterable Mg is in ionic form, the remainder being complexed to anions, particularly phosphate, citrate, and oxalate. Cord serum total Mg (tMg) is higher than paired maternal values (6–8) and remains slightly higher in infants and young children (0.92 ± 0.13 mmol/L, mean $\pm$ 2 SD) compared to adults values of 0.88 ± 0.13 mmol/L. Ion-selective electrodes are being used in the measurement of ionized Mg (iMg) in whole blood and sera. iMg concentrations average 62% to 70% of the tMg concentration in cord and postnatal sera. Cord serum iMg is also higher than that in maternal serum. The clinical role of iMg (vs. tMg) in a number of disease states appears limited (9).

Cellular Mg content of most tissues is 6 to 9 mmol/kg wet weight, and most of this Mg is localized in membrane structures (e.g., microsome, mitochondria, and plasma membrane). The much smaller pool of free Mg in the cell is maintained at about 1 mmol/L and is in an exchanging equilibrium with membrane-bound Mg. This unbound intracellular Mg has a critical role in cellular physiology and catalyzes enzymatic processes concerned with the transfer, storage, and use of energy. Intracellular Mg usually remains stable despite wide fluctuations in serum Mg. In Mg-deficient states, however, the intracellular content of Mg can be low despite normal serum concentrations.

Phosphorus (1 mmol/L 3.1 mg/dL)

The total P in serum can be divided into an acid-insoluble fraction, comprising mainly phospholipids, and an acid-soluble fraction, comprising a small amount of organic ester phosphate, and all of inorganic phosphate. Normally, more than 90% of the inorganic phosphate is diffusible.

Cord serum P concentrations at term ranges from 1.8 to 2.3 mmol/L (conversion, 1 mmol/L = 3.1 mg/dL) and are higher than maternal values. In contrast to serum Ca and Mg concentrations, there are large variations in postnatal serum P concentrations. In most newborn infants there is a rise in serum P over the first 48 hours after birth, probably unrelated to intestinal absorption of P because dietary P intake is limited at this age. Renal excretion of P is low and contributes to the maintenance of high serum P. Serum P concentrations are high during infancy (1.25–2.60 mmol/L and 3.9–8.0 mg/dL) compared with those in adults (0.9–1.5 mmol/L and 2.8–4.6 mg/dL), and there is a rough correlation between the rate of skeletal growth and the serum P concentration. In adults, serum P has a biphasic diurnal rhythm, with peaks in the afternoon and at 3:00 AM; maximal change between peak and trough values is less than 0.4 mmol/L (1.2 mg/dL) (10). Serum P concentrations also fall by about 0.1 mmol/L (0.3 mg/dL) after a

meal (11). During pregnancy, maternal serum P concentration remains stable.

In the cell, phosphate is the principal intracellular anion and is mostly in the form of organic phosphate. The intracellular inorganic phosphate is normally in equilibrium with both extracellular phosphate and intracellular glyceraldehyde-3-phosphate, an intermediate compound in the regeneration of ATP. The cellular phosphorus/nitrogen ratio is relatively constant. For example, it is 0.07 (by weight) in muscle, and gains or losses of nitrogen by the body are usually accompanied by corresponding gains or losses of extraosseous P. The relationship between potassium, the major intracellular cation, and P is more variable.

PHYSIOLOGIC CONTROL OF MINERAL HOMEOSTASIS

Calciotropic hormones, including parathyroid hormone (PTH) and 1,25 dihydroxyvitamin D [1,25$(OH)_2$D], and possibly calcitonin (CT), appear to maintain Ca and P homeostasis by intermodulation of their physiologic effects on each other and on the classic target organs: kidney, intestine, and bone. Dietary intake of Ca, Mg, P, and other nutrients including sodium, glucose, and protein also may significantly contribute to the regulation of mineral homeostasis. PTH serves as the major component of the rapid response to hypocalcemia, whereas 1,25$(OH)_2$D, with its major effect on elevating intestinal absorption of Ca, is responsible for a slower but more sustained contribution to the maintenance of normocalcemia. CT, on the other hand, appears to function in the opposite role to PTH but with the capacity to stimulate the production of 1,25$(OH)_2$D, which in theory may serve an additional regulatory role in the maintenance of Ca homeostasis. The direct action of PTH and CT results in a net decrease in serum P, whereas, 1,25$(OH)_2$D increases serum P. Maintenance of serum Ca and P in turn are critical to the growing skeleton.

In contrast, the control of Mg homeostasis by calciotropic hormones under physiologic conditions appears to be limited. However, Mg is critical to the maintenance of Ca homeostasis because Mg regulates the production and secretion of PTH, acts as a cofactor for the 25 hydroxyvitamin D (25 OHD) -1α-hydroxylase enzyme in the production of 1,25$(OH)_2$D, and maintains adequate sensitivity of target tissues to PTH. Furthermore, Mg is considered as mimic/antagonist of Ca as it often functions synergistically with Ca, yet competes with Ca in the gut and kidney for transport and other metabolic pathways.

Parathyroid Hormone

In humans, parathyroid glands are derived from the third and fourth pharyngeal pouch. The PTH gene, along with the genes for insulin, β-globulin, and CT, is located on chromosome 11p15. The initial translational product of the mRNA is a 115 amino-acid prepro-PTH. Prepro-PTH then undergoes proteolytic cleavage in endoplasmic reticulum to remove a 25 residual amino-terminal signal sequence to form pro-PTH. The prohormone-specific region is cleaved further during subsequent intracellular processing to generate the 84 amino-acid secreted form of the intact hormone with a relative molecular mass (M_r) of 9500. PTH is synthesized by the chief cells and stored in secretory granules. It is colocated and secreted with chromogranin A, a protein that may act in autocrine- or paracrine-regulated release of PTH.

About 50% of the newly generated PTH is proteolytically degraded intracellularly and some of the inactive fragments are also secreted. After release into the circulation, the intact PTH (IPTH) molecule has a serum half-life of five to eight minutes and undergoes a series of cleavages by endopeptidases in the liver and kidney. The amino-terminal fragments contain the biologically active fractions, with the 1 to 34 fragment having the most calcemic activity; modifications at the amino terminal, particularly at the first two residuals, can abolish its biologic activity. Large fragments of the carboxyl terminal are increasingly reported to have some in vitro biological activity.

Circulating immunoreactive PTH is a complex mixture of intact 1–84 PTH, multiple peptide fragments from the amino and carboxyl terminals, and mid-molecular regions. Normally, there are greater amounts of middle and carboxyl fragments than intact hormone in the circulation because of metabolic breakdown of the short-lived, intact hormone, coupled with glandular secretion of PTH fragments. The fragments are cleared from the blood virtually exclusively by glomerular filtration. IPTH and amino-terminal fragments constitute less than 20% of PTH immunoreactivity in the peripheral circulation. PTH molecules reactive in the widely used commercial immunoradiometric assays (IRMA) designed to detect both amino- and carboxy-terminal epitopes of the peptide have been considered as IPTH assays. However, there have been reports that the large 7 to 84 fragment of PTH is also detected by these assays. This large fragment is biologically inactive and present in greater concentrations in uremic states or hyperparathyroidism. The conventional IPTH technique increases the PTH concentration by 30% to 50%, compared to the latest chemiluminescence or IRMA techniques that measure the ''whole'' or ''bio-intact'' PTH. Therefore, the treatment of secondary hyperparathyroidism based on data from conventional IPTH assays theoretically may lead to overtreatment and oversuppression of biologically active PTH, although the clinical significance of this possibility remains to be defined. In any case, consistency of the PTH assay methodology, and serial measurements are critical to the interpretation and management of pathologic states.

PTH concentrations in cord blood frequently are low and do not correlate with PTH concentrations in maternal sera (12,13). Earlier studies of higher levels of bioactive PTH in cord sera from cytochemical assay was probably related to elevated concentrations of PTH-related protein (PTHrP). Small amounts (about 5%) of perfused fragments (35–84,44–68, and 65–84 amino acids), but probably not the whole PTH molecule, are reported to cross the human placenta. Serum PTH concentrations increase postnatally coincident with the fall in serum Ca in both term and preterm infants (12–16). The rise in serum IPTH is greater for preterm infants with hypocalcemia compared to term infants reflecting appropriate PTH response. Serum PTH concentrations are similar for children and adults but are increased in the elderly. Serum concentrations of IPTH as measured by IRMA showed no change during normal pregnancy. In adults, serum IPTH is present in picomolar concentrations. It has a significant circadian periodicity, spontaneous episodic pulsatility with distinct peak property, and a significant temporal coupling with serum iCa and phosphorus concentrations and prolactin secretion.

PTH effects on end-organ systems appear to be mediated through its binding to specific receptors. The type 1 PTH/PTHrP (PTH1R) receptor binds equally to PTH and PTHrP at the N-terminus. It has been identified in bone, cartilage, kidney, intestine, aorta, urinary bladder, adrenal gland, brain, and skeletal muscle, and belongs to a superfamily of guanine-nucleotide-binding (G) protein-coupled cell membrane receptors (GPCR), a family of proteins that transduce an extracellular signal to an intracellular response via a seven helical transmembrane domain. GPCR also include those for CT, secretin, growth hormone-releasing hormone, corticotrophin-releasing hormone, glucagon, vasoactive intestinal polypeptide, and others. The gene for PTH1R is located on chromosome 3p21.1–p24.2. It contains 17 exons and encodes a mature glycoprotein of 593 amino acids and consists of extended extracellular, ligand-binding amino terminal and intracellular G protein-associated carboxyl-terminal domains.

Signal transduction mediated by G proteins results in multiple second messenger pathways to effect both stimulatory and inhibitory end-organ responses via stimulating G protein (Gs) -activated adenylyl cyclase with cyclic AMP (cAMP) production and protein kinase A activation, and Gq-activated phospholipase C that generates inositol phosphate and diacylglycerol, followed by activation of protein kinase C. PTH also increases Ca transport channels and tyrosine phosphorylation of a number of intracellular proteins.

Other receptors including PTH2R and a receptor that binds to the large fragment of the carboxy-terminal fragment of PTH (CPTHR) exist but their physiologic significance remains ill defined.

In physiologic terms, PTH acts synergistically with $1,25(OH)_2D$ and is the most important regulator of extracellular Ca concentration. PTH acts directly on bone and kidney, and indirectly on intestine. Immediate control of blood Ca is probably due to PTH-induced mobilization of Ca from bone, and increased renal distal tubular reabsorption of Ca. PTH also decreases proximal tubular and thick ascending limb reabsorption of sodium, Ca, phosphate, and bicarbonate. PTH effects on kidney are mediated primarily through stimulation of sodium/calcium exchange, calcium transport proteins and renal 25 OHD-1α-hydroxylase, but a decrease in sodium dependent phosphate co-transporter, NPT-2. Maintenance of steady-state calcium balance is probably from increased intestinal Ca absorption secondary to increased $1,25(OH)_2D$ production. PTH increases acutely within minutes the rate of Ca release from bone into blood. Chronic effects of PTH are to increase the numbers of osteoblasts and osteoclasts and to increase the remodeling of bone. Continuous exposure to elevated concentrations of PTH leads to suppressed osteoblastic and increased osteoclastic activity. In contrast to its classic action on Ca mobilization from bone, the amino-terminal fragments of PTH and PTHrP, and small pulses of PTH have an anabolic effect on bone, independent of its resorptive action. Other PTH effects on bone include enhanced collagen synthesis, activities of alkaline phosphatase, ornithine and citrate decarboxylases, and glucose-6-phosphate dehydrogenase; DNA, protein and phospholipid synthesis.

Extracellular Ca is the most potent regulator of PTH secretion and is mediated by the cell-surface CaR, which detects minute perturbations in the extracellular iCa concentration and responds with alterations in cellular function that normalize iCa. Thus, iCa functions as extracellular as well as intracellular messenger. The human CaR gene is located on chromosome 3q13.3–q21 and encodes a cell-surface protein of 1078 amino acids. The CaR gene is developmentally upregulated, and CaR transcripts are present in numerous tissues including chief cells of the parathyroid glands, kidneys (in particular the thick ascending limb), brain and nerve terminals, breast, lung, intestine, adrenal and skin, and also the precursor and mature osteoblasts and osteoclasts. CaR is also a member of the GPCR superfamily. It contains at least seven exons, of which six encode the large (600 amino acid) amino-terminal extracellular domain and/or its upstream untranslated regions, while a single exon codes for the remainder of the receptor including a cytoplasmic carboxy-terminal intracellular domain. Signal transduction mediated by G proteins results in activation of phospholipase C that generates IP_3 and DAG, and subsequent stimulation of protein kinase C and Ca transport channels.

Low or falling serum Ca concentrations result in active secretion of preformed PTH within seconds. There is a sigmoidal type of PTH secretion in response to decreased serum Ca, which is most pronounced when serum Ca is in the mildly hypocalcemic range.

PTH secretion is 50% of maximal at a serum iCa of about 1 mmol/L (4 mg/dL); this is considered as the calcium set point for PTH secretion (17). Sustained hypocalcemia increases PTH mRNA within hours. Protracted hypocalcemia leads within days to cellular replication and increase gland mass. High serum Ca suppresses PTH secretion via activation of CaR, and probably increases the proteolytic destruction of preformed PTH.

In the kidney, CaR decreases the basal and PTH-stimulated paracellular reabsorption of Ca, Mg, and sodium via multiple mechanisms including inhibition of cAMP accumulation; stimulation of phospholipase A2 activity, thereby promoting the release of free arachidonic acid that is metabolized via the lipooxygenase pathway to P450 metabolites that inhibit the activities of Na-K-2Cl cotransporter and the K^+ channel; and may affect renal water regulation by inhibition of vasopressin-abated water flow. In chronic renal failure, downregulation in the expression of renal CaR may account for the development of secondary hyperparathyroidism (18), and downregulation of PTH receptors may account for the skeletal resistance to the calcemic effect of PTH (19). Extracellular Ca exerts numerous other actions on parathyroid function, including modulation of the intracellular degradation of PTH, cellular respiration, membrane voltage, and the hexose monophosphate shunt.

Maintenance of Ca homeostasis through other organs also may be possible, for example, through the presence of CaR in intestinal cells, and probable modulation of CT secretion from changes in intracellular Ca. Furthermore, expression of the CaR in gastrin-secreting G-cells and acid-secreting parietal cells, together with data indicating that the CaR exhibits selectivity for l-aromatic amino acids, would appear to provide a molecular explanation for amino-acid sensing in the gastrointestinal tract, regulation of PTH secretion and urinary Ca excretion, and the physiologic interaction between Ca and protein metabolism.

Decrease in serum Mg concentration stimulates PTH secretion (20,21), although chronic hypomagnesemia inhibits secretion of PTH (21,22). Hypomagnesemia is also associated with an increased target tissue resistance to PTH probably from inactivity of adenylate cyclase, a Mg-requiring enzyme. Hypermagnesemia rapidly decreases the secretion of PTH in vivo in human subjects, and PTH concentration remains depressed despite concomitant hypocalcemia, presumably in part due to stimulation of CaR by other divalent cations such as Mg.

Hyperphosphatemia stimulates PTH synthesis, secretion, and parathyroid gland hyperplasia by lowering the serum Ca concentration (23) and also independent of calcium and calcitriol, by posttranscriptional mechanisms (24).

Vitamin D and its metabolites 25 OHD and $1,25(OH)_2D$, acting through vitamin D receptors (VDRs), decrease the level of PTH mRNA. Additional systemic factors (growth hormone, insulin-like growth factor-I, estrogen, progesterone, CT, cortisol, catecholamines, prostaglandins, and somatostatin) and local factors [interleukin-l (IL-1)] modulate PTH secretion and function, although their role in the regulation of Ca and Mg metabolism under physiological conditions is not clear.

Calcitonin

CT is secreted primarily from the thyroid C cells and also from many extrathyroidal tissues including placenta, brain, pituitary, mammary gland, and other tissues. Developmentally, CT-containing cells and parathyroid gland cells are thought to be derived from the same tissue source as the neural crest. In the rat, the number of thyroid C cells and secretion of CT increase from fetal life to suckling, a period of rapid growth. There is probably no placental crossover of CT; the human placental tissue is able to produce CT in response to the presence of Ca in the culture medium. In human neonates, the CT content in crude tissue preparations of thyroid is larger than that of the adult thyroid.

There are two CT genes, α and β, located on chromosome 11p15.2 near the genes for β-globulin and PTH. Two different RNA molecules are transcribed from the α gene. It comprises of six exons with the fourth exon translated into the precursor for CT, and fifth is translated into the precursor for CT gene-related peptide-I (CGRP-I). The initial translational product of the mRNA is prepro-CT, a 141 amino-acid peptide. It is cleaved by endopeptidase at the endoplasmic reticulum to form pro-CT, a 13 kD 116 amino-acid peptide. CT (between 60th and 91st position of the pro-CT peptide) and equimolar amounts of non-CT secretory peptides, corresponding to the flanking peptides linked to the amino and carboxy terminals of the prohormone, are generated during precursor processing. Further structural modifications to the CT molecule occur intracellularly prior to release into the circulation. These include formation of a disulfide bridge between two cysteine remnants in positions 1 and 7, and hydroxylation of the C terminal proline residue; both are essential for binding of CT to its receptor. The CT monomer is a 32 amino-acid peptide (M_r, 3400). CGRP-I is synthesized wherever the CT mRNA is expressed, e.g., in medullary carcinoma of thyroid, although there is no translational product from CGRP-I sequence.

The β or CGRP-II gene is transcribed into the mRNA for CGRP predominantly in nerve fibers in the central and peripheral nervous system, blood vessels, thyroid and parathyroid glands, liver, spleen, heart, lung, and possibly bone marrow. CGRP, a 37 amino-acid peptide (M_r, 4000), is also generated from the larger precursor molecule pro-CGRP, a 103 amino-acid peptide. Seventy-five amino-terminal residues of each preprohormone for CT and CGRP are predicted to be identical.

Classic bioactivity of human CT (hCT) is present in the full 32 amino-acid structure or its smaller fragments, such as hCT 8–32 and hCT 9–32; the ring structure of CT enhances, but is not essential for, hormone action. Basic amino-acid substitutions confer a helical structure in this region as found in salmon and other nonmammalian CT result in greater potency in lowering serum Ca, and probably longer circulating half-life. The kidney appears to be the dominant organ in the metabolism of hCT. A small percentage of the metabolic clearance rate of CT in humans may be accounted for by enzymatic degradation in blood. Injected hCT monomer disappears from the blood in vivo with a $t_{1/2}$ of approximately 10 minutes; in contrast, the $t_{1/2}$ of hCT in plasma incubated in vitro at 37°C may be longer than 20 hours (25). Depending on the animal species, other sites such as liver, intestine, and bone may be involved in the metabolism of CT.

Circulating immunoreactive CT and CGRP are a heterogeneous mixture of different molecular forms and are recognized as long as the antigenic epitopes recognized by the antiserum are expressed. Immunoreactive CT or CGRP concentration is expressed in gravimetric or molar equivalents of synthetic CT or CGRP. Sample preparation with initial extraction, gel chromatography, and high-performance liquid chromatography separation, and the use of two-site immunoassay can improve the sensitivity and specificity of CT measurements. Serum CT concentrations during pregnancy are variable. Cord sera CT concentrations are high compared to paired maternal CT concentrations (26). Serum CT further increases during the first few days after birth and may reach levels 5- to 10-fold higher than adult CT concentrations. Serum CT concentrations decrease progressively during infancy; however, in preterm infants up to three months after birth, the mean serum CT concentrations may remain twice the adult value. There is also a small peak of serum CT concentration during late childhood. In human adults, the basal serum CT concentration may be lower in women than in men, but the concentration is not affected by old age. The CT secretory response to Ca infusion is lower in women, and with old age. In human adults, serum CT and CGRP concentrations are found in the picomolar range. Diurnal variability has been reported for serum CGRP but not for serum CT. In normal individuals, larger precursor molecules of CT such as procalcitonin are not detected.

CT function is mediated by binding to receptors linked to G proteins, a member of the GPCR superfamily, and activation of adenylate cyclase and phospholipase C. CT receptors (CTR) have been identified in the central nervous system, testes, skeletal muscle, lymphocytes, placenta, and other tissues. The function of CTR can be influenced by accessory proteins, receptor isoforms, genetic polymorphisms, developmental and/or transcriptional regulation, feedback inhibition, and the specific cellular or tissue background. The CTR gene is located on chromosome 7q2l.2–q21.3 and encodes a 490 amino-acid G protein-linked receptor with seven transmembrane domains. Two isoforms of human CTR arise by alternative splicing of an exon of 48 nucleotides that encodes a l6 amino-acid insertion within the first intracellular loop. The isoform with the insertion (hCTR-l) activates only adenylate cyclase, whereas the other isoform (hCTR-2) activates both adenylate cyclase and phospholipase C. CGRP functions are also mediated by receptors.

Presence of receptor-activity-modifying proteins can posttranslationally modify the initially orphan CT receptor-like (CL) receptor and CTR to exhibit different receptor function, i.e., functional modification of G protein-coupled receptors is possible.

Secretion of CT is stimulated by an increase in serum Ca and Mg concentrations and by gastrin, glucagon, and cholecystokinin, along with several other structural analogs of these hormones (e.g., pentagastrin and prostaglandin E2), glucocorticoid, norepinephrine, and CGRP; and suppressed by hypocalcemia, propranolol and other adrenergic antagonists, somatostatin, chromogranin A, and vitamin D. CT gene transcription is positively regulated by glucocorticoid and negatively regulated by protein kinase C, Ca, and vitamin D. CT may activate the l-hydroxylase system independent of PTH, whereas 1,25$(OH)_2$D decreases CT gene expression in adult rats but is ineffective in 13-day-old suckling rats. The latter observation may be related to fewer 1,25$(OH)_2$D receptors in C cells of immature rats. CT induces refractoriness to its own actions from downregulation in the number, and functional reduction of receptor mRNA is a well-known phenomenon. Clinically, it is manifested as the ''escape'' phenomenon during therapy with CT.

In humans, changes in Ca and P metabolism are not seen despite extreme variations in CT production. In the neonate, there is neither an identifiable hypocalcemic response to the postnatal surge in serum CT nor a blunting of CT secretion in the presence of hypocalcemia. In adults, there are no definite effects attributable to CT deficiency, for example, totally thyroidectomized patients receiving only replacement thyroxin; or CT excess, for example, patients with medullary carcinoma of thyroid, except for the chronic suppression of bone remodeling. The clinical significance of CT is related to its use as a tumor marker in the management of medullary carcinoma of the thyroid, and its pharmacological effect to inhibit osteoclast-mediated bone resorption and to increase renal Ca clearance. The pharmacological activities of CT are useful for the suppression of bone resorption in Paget disease, for limited use in the treatment of osteoporosis, and for early-phase treatment of severe hypercalcemia. In addition, CT also increases renal clearance of Mg, P, sodium, and water. The net effect of CT is a lowering of serum Ca and P concentrations. Thus, the bioactivity of CT on calcium metabolism

frequently is opposite that of PTH; CT probably modulates the effect of PTH on target organs.

The noncalcium-related actions of CT and associated molecules are increasingly being expanded. For example, CT and CTR may play an important role in a variety of processes as wide ranging as embryonic development and sperm function/physiology. In addition, pro-CT detectable in the plasma is not produced in C-cells of the thyroid and is being explored as a marker of bacterial-induced inflammation/sepsis. Production of pro-CT after exposure to bacterial endotoxin and inflammatory cytokines tumor necrosis factor (TNF) and IL-6 appears to be primarily from neuroendocrine cells in the lung and intestine. Cells of neuroendocrine origin express all proteins related to CT (CGRP-I and -II and amylin) derived from the same family of genes and it is speculated that ''inflammatory'' pro-CT may be coded by the same gene family. There are no enzymes in the plasma that could break down pro-CT, and when it is secreted into the circulation, it has a $t_{1/2}$ of 25 to 30 hours, thus increasing serum pro-CT. After administration of endotoxin, the peak circulating concentrations of TNF, IL-6, pro-CT and C reactive protein occur at about 90 minutes, 180 minutes, six to eight hours, and 24 hours, respectively.

CGRP primarily affects catecholamine release, vascular tone and blood pressure, and cardiac contractility. Its clinical role probably also lies in its potential pharmacological effect. The influence of CGRP on Ca and P homeostasis is minor compared to that of CT. However, amylin, a pancreatic islet-derived or synthetic 37 amino-acid peptide, is a member of the CGRP family with a potent hypocalcemic effect despite sharing only 15% of its aminoacid sequence with hCT. The hypocalcemic effect of amylin is probably mediated by the CTR on osteoclasts, and it is 100-fold more potent than CGRP. Both CT and CGRP inhibit gastric acid secretion and food intake.

Vitamin D

Vitamin D (M_r, 384) can be obtained from diet or synthesized endogenously. It must undergo several metabolic transformations primarily in the liver and kidney to form the physiologically most important metabolite, 1,25$(OH)_2$D, which functions as a hormone in the maintenance of mineral homeostasis. Under in vivo conditions, there are over 30 other vitamin D metabolites, with and without putative functions.

Dietary vitamin D (1 μg = 40 IU) is derived from plants as ergocalciferol (vitamin D_2) and from animals as cholecalciferol (vitamin D_3). Dietary vitamin D is absorbed from the duodenum and jejunum into lymphatics, and about 50% of the vitamin D in chylomicron is transferred to vitamin D-binding protein (DBP) in blood before uptake by the liver.

In animals, vitamin D_3 can be synthesized endogenously in the skin. During exposure to sunlight, the high-energy ultraviolet (UV) photons (290–315 nm) penetrate the epidermis and photochemically cleave the bond between carbons 9 and 10 of the sterol B ring of 7-dehydrocholesterol (7 DHC or provitamin D_3) to produce previtamin D_3. It then undergoes a thermally induced isomerization to vitamin D_3 that takes two to three days to reach completion. Thus, cutaneous synthesis of vitamin D_3 continues for many hours after a single sun exposure. Previtamin D_3 is photolabile; continued exposure to sunlight causes the isomerization of previtamin D_3 to biologically inert products, principally to lumisterol. No more than 10% to 20% of the initial provitamin D_3 concentrations ultimately end up as previtamin D_3, thus preventing excessive production of previtamin D_3 and vitamin D_3.

Vitamin D_3 synthesis in the skin is directly dependent on the amount of skin surface exposure, type and pigmentation of skin, duration of sunlight exposure and affected by time of day, season, latitude, and altitude. Other environmental factors affecting cutaneous vitamin D_3 synthesis include total ozone, ground cover, aerosol and cloud thickness, and the use of UV-B sunscreen. Melanin in the skin competes with 7 DHC for UV photons, but the production of vitamin D_3 can be compensated by increasing the duration of sunlight exposure; use of topical sunscreen blocks UV photons; and aging decreases the capacity for cutaneous synthesis of vitamin D_3.

The term ''vitamin D'' is frequently used generically to describe vitamins D_2 and D_3 and, correspondingly, their metabolites. In mammals, vitamins D_2 and D_3 appear to metabolize along the same pathway, and there is little functional difference between their metabolites. However, differences in affinity to DBP and receptors between the parent vitamins D_2 and D_3 and their metabolites, support the contention that vitamin D_3 is more bioavailable than D_2.

In the circulation, vitamin D and its metabolites are protein bound, mainly to DBP (about 85%) and to albumin (about 15%). The DBP gene is located on chromosome 4q11–13. It is a member of the albumin multigene family of proteins that includes albumin and α-fetoprotein. DBP is an approximately 53 kD globulin in the human and its X-ray crystallographic structure has been determined. Plasma DBP concentration (4–8 μM) is approximately 20-folds higher than that of the total circulating vitamin D metabolites (approximately 10^{-7} M), i.e., it is normally less than 5% saturated with vitamin D metabolite. The amount of unbound or free 25 OHD and 1,25$(OH)_2$D, important in determining bioactivity, is less than 1% of the total concentration.

Vitamin D must undergo two additional metabolic steps to produce the physiologically most active vitamin D metabolite, 1,25$(OH)_2$D. Vitamin D is hydroxylated at C25 position by cytochrome P450 isoforms of 25 hydroxylase to 25 OHD in the liver and

transported to the kidney in the circulation to the kidney for hydroxylation at C1 position by 25 OHD-1α-hydroxylase (CYP 1α) to $1,25(OH)_2D$.

Regulation of 25 hydroxylase activity is limited and there are few limitations to the production of 25 OHD. However, in vivo administration of $1,25(OH)_2D$ (27) inhibits hepatic production of 25 OHD and Ca deficiency (28) increases the metabolic clearance of 25 OHD with subsequently decreased circulating 25 OHD. Quantitatively, 25 OHD (1 nmol/L = 0.4 ng/mL) is the most abundant vitamin D metabolite in the circulation and is a useful index of vitamin D reserve.

The activity of 1α-hydroxylase and therefore production of $1,25(OH)_2D$ are tightly regulated. It is the rate-limiting hormonally regulated step in the bioactivation of vitamin D. PTH increases transcriptional activity of the CYP 1α gene promoter and increases mRNA for $1,25(OH)_2D$. Decrease in serum or dietary Ca or P increases mRNA for and serum concentration of $1,25(OH)_2D$ independent of PTH (14,29,30). However, hypophosphatemia in renal wasting disorders does not elicit appropriate phosphate conservation or an increase in $1,25(OH)_2D$ production. These disorders include X-linked hypophosphatemic (XLH) rickets, autosomal-dominant hypophosphatemic rickets (ADHR), and tumor-induced osteomalacia. They have similar phenotypic manifestations characterized by hypophosphatemia, decreased renal phosphate reabsorption, normal (and thus inappropriately low) or low serum calcitriol concentrations, normal serum Ca and PTH, and defective skeletal mineralization.

XLH results from mutations in the PHEX (phosphate-regulating gene with homologies to endopeptidases on the X chromosome, Xp22.1) gene, encoding a membrane-bound endopeptidase (31), whereas ADHR is associated with mutations of the gene encoding fibroblast growth factor (FGF) -23 and linked to chromosome 12p13.3 (32). FGF-23 is a small heat-sensitive molecule of less than 25 kD that inhibits sodium-dependent phosphate wasting and probably inhibits CYP 1α. The endopeptidase, PHEX, degrades native FGF-23 that provides the biochemical link among these clinical syndromes. XLH rickets also has been associated with mutations in CLCN5, a voltage-gated chloride channel gene located on Xp11.22.

Other factors that enhance $1,25(OH)_2D$ production include estrogen, prolactin, growth hormone, insulin-like growth factor-I, and PTHrP. $1,25(OH)_2D$ production is feedback regulated and is inhibited by chronic deficiency or low circulating Mg. Mg deficiency also lowers serum $1,25(OH)_2D$ response to low-Ca diet but does not appear to limit $1,25(OH)_2D$ production in animals. The effect of Mg on $1,25(OH)_2D$ metabolism is presumably related in part to its role as a cofactor of the 1α-hydroxylase enzyme. In contrast to the rapid increase within minutes in the secretion and serum concentration of PTH, measurable alteration in serum $1,25(OH)_2D$ concentrations usually occurs only hours after exposure to an appropriate stimulus.

There are increasing reports of other sources of 25 OHD and $1,25(OH)_2D$. Extrarenal production of $1,25(OH)_2D$ at extrarenal sites may serve intracrine, autocrine, and paracrine functions and its production from sources such as macrophages, particularly in granulomatous disease states, may not be tightly regulated. The $1,25(OH)_2D$ production is stimulated by interferon (INF)-γ and is not responsive to changes in dietary calcium intake, and the metabolic degradation of $1,25(OH)_2D$ also may be impaired thereby increasing the extracellular accumulation of $1,25(OH)_2D$ and may lead to hypercalcemic state.

The degradation of $1,25(OH)_2D$ is tightly regulated. $1,25(OH)_2D$ strongly induces the enzyme 25 OHD-24 hydroxylase (CYP 24) in all target cells for vitamin D. CYP 24 catalyzes several steps of $1,25(OH)_2D$ degradation, collectively known as the C24 oxidation pathway, which starts with 24-hydroxylation and culminates in the formation of the biliary excretory form, calcitroic acid. CYP 24 expression is inhibited by PTH and by dietary phosphate restriction. In kidney and intestine in particular, upregulation of the 24 hydroxylase enzyme in response to $1,25(OH)_2D$ treatment is rapid and occurs within 4 hours (33). Physiologic production of $24R,25(OH)_2D$ is therefore an important means to regulate the circulating concentration of $1,25(OH)_2D$ and catabolism of vitamin D, although it also may have a role in bone integrity and fracture healing in the chick model. Most of the other vitamin D metabolites are derived primarily from further metabolic alterations to 25 OHD and $1,25(OH)_2D$ through oxidation or side chain cleavage and have poorly defined physiologic function. However, many analogs of vitamin D metabolites and inhibitors of CYP24 have been studied for the numerous potential pharmacological actions that involve less calcemic inducing and greater maturation and differentiation effects.

$1,25(OH)_2D$ function, like other steroid hormones, is mediated primarily through modulation of the cellular genome by binding to specific nuclear receptors (VDR), a 424 amino-acid phosphoprotein (34). The VDR gene contains nine exons and is located on chromosome 12q13–14 near the site of the gene for 25 OHD-lα-hydroxylase (35). VDR is a member of the subfamily of nuclear receptors with a ligand-binding domain that binds classic hormones that include thyroid hormone, androgens, estrogens, progesterone, glucocorticoids, aldosterone, hormonal forms of vitamin A, and $1,25(OH)_2D$. It has several functional domains including a 110 residual N-terminal DNA-binding domain with two zinc fingers, a C-terminal hormone-binding domain, and a hinge region important for nuclear localization. The VDR interacts with the 9-*cis* retinoic acid nuclear receptor retinoid-X-receptor (RXR) to form a heterodimeric RXR–VDR complex that binds to specific DNA sequences, termed vitamin D-responsive elements. After $1,25(OH)_2D$

binds to the receptor, it induces conformational changes (36) that result in the recruitment of a multitude of transcriptional coactivators that stimulate the transcription of target genes. VDR also can adopt a dual role of acting as a repressor in the absence of ligand and then subsequently as a coactivator when ligand binds. VDR is upregulated by $1,25(OH)_2D$ at both the mRNA and the protein levels and is increased during growth, gestation, and lactation but shows an age-dependent decrease in mature animals and humans, supporting the notion that VDR may be up- or downregulated, depending on Ca needs.

Although $1,25(OH)_2D$ regulates over 60 genes whose actions include those associated with calcium homeostasis and immune responses, as well as cellular growth, differentiation, and apotosis involving wide range of biological processes including calcium and bone health, diabetes and autoimmune disorders, and neoplastic disorders. However, two basic clinical functions define the major classic actions of vitamin D. The first is that vitamin D is required to prevent rickets in children and osteomalacia in adults. The second is the prevention of hypocalcemic tetany. These functions are maintained by $1,25(OH)_2D$ through its effect on a number of target tissues, primarily intestine, kidney, and bone, with modulating effects from other hormones including PTH and CT.

The genomic action of $1,25(OH)_2D$ can be preceded by more rapid nongenomic actions that occurs in minutes and involve plasma membrane receptor and second messengers such as cAMP, increased Ca transport, and protein kinase C and mitogen-activated protein kinase activation. This nongenomic rapid increase in cytosolic Ca is reported to occur in various cell types from intestine, parathyroid, osteoblast, pancreas, vascular smooth muscle, myocytes, monocytes, and leukemic cells.

Quantification of vitamin D and its metabolites has been achieved by several different methods including high-performance liquid chromatography, with detection by UV absorbance or binding assays, and immunoassays based on antibodies raised to vitamin D metabolite conjugates. Values from different laboratories cannot be compared without making direct comparison of their assay procedures. Interlaboratory coefficients of variation for the measurement of 25 OHD, $24,25(OH)_2D$, and $1,25(OH)_2D$ may range between 35% and 52%. Furthermore, differences between vitamins D_2 and D_3 in their affinity to the vitamin D-binding protein and receptors, and different chromatographic behavior on various preparative chromatographic systems demand that great care be taken with assay techniques when dealing with patients who have significant vitamin D_2 intake. To ensure reliable results, appropriate vitamin D standards must be used for standard curve generation in performing competitive protein-binding assays of these compounds.

Maternofetal transfer of vitamin D and its metabolites varies, depending on the species. In humans, the cord serum vitamin D concentration is very low and may be undetectable; the 25 OHD concentration is directly correlated with, but is lower than, maternal values, consistent with placental crossover of this metabolite; $1,25(OH)_2D$ concentrations also are lower than maternal values, but there is no agreement on the maternofetal relationship of this and other dihydroxylated vitamin D metabolites (3,37–39). However, the placenta, like the kidney, produces $1,25(OH)_2D$, making it difficult to ascertain just how much fetal $1,25(OH)_2D$ results from placental crossover versus placental synthesis. $24,25(OH)_2D$ also crosses the placenta and its concentration in the sera of mothers and newborns varies with the seasons, being highest in autumn. It appears that the human fetus receives the bulk of its vitamin D already metabolized to 25 OHD.

Seasonal and racial variations in serum 25 OHD concentrations occur, presumably from variations in endogenous production. Serum 25 OHD as with $24,25(OH)_2D$ is lower in winter. These changes may be reflected in cord serum values. In normal adults, serum $1,25(OH)_2D$ concentrations are relatively constant and maintained within approximately 20% of the overall 24-hour mean, and show no seasonal variation consistent with the tightly regulated lα-hydroxylase activity. African-American mothers, infants, and young children tend to have lower 25 OHD and higher $1,25(OH)_2D$ concentrations than whites. Serum $1,25(OH)_2D$ in the newborn become elevated within 24 hours after delivery and appears to vary according to Ca and P intake.

The circulating $t_{1/2}$ of vitamin D is about 24 hours and for 25 OHD is two to three weeks, although the latter $t_{1/2}$ is decreased in vitamin D-deficient individuals. $1,25(OH)_2D$ has a much shorter $t_{1/2}$ of three to six hours. Metabolites of 25 OHD and $1,25(OH)_2D$ may undergo enterohepatic circulation after exposure to intestinal β-glucuronidase. The physiological role of enterohepatic circulation of vitamin D metabolites has not been precisely quantitated.

Nonclassical Control of Mineral Homeostasis

Factors other than the classic calciotropic hormones: PTH, CT, $1,25(OH)_2D$, whether acting systemically or locally on multiple effector organs are increasingly being recognized as important to the maintenance of mineral homeostasis in certain circumstances. The ultimate effect on mineral homeostasis often involves bone formation and/or bone resorption, and flux of Ca and Mg between extracellular fluid and bone, with or without direct involvement by calciotropic hormones. Skeletal health, particularly in the growing skeleton, requires the integrated actions of classic calciotropic hormones, endocrine modulators of growth, numerous cytokines and growth factors and their receptors, as well as their endogenous modulators.

Many factors such as growth hormone, insulin-like growth factor-I, estrogen, progesterone, cortisol, and TNF, can affect the secretion or function of one

or more of the calciotropic hormones. In turn, many factors such as insulin-like growth factor-I, transforming growth factor-β1, IL-1, -2, -4, and -6, TNF-α, and INF-γ can be modulated by calciotropic hormones.

Local factors such as transforming growth factor-β_1, lymphotoxin, TNF-α, INF-α, -β, and -γ and IL-1 and -6 act in a paracrine (i.e., cell-to-cell) or autocrine (i.e., cell-to-own cell) fashion may influence Ca flux of bone cells. The effects on Ca flux based on these interactions are probably more important under pathologic situations. INF-γ from activated macrophages (40,41) stimulates 25 OHD-1α-hydroxylase mRNA and enzyme production, with little or no feedback inhibition by $1,25(OH)_2D$, which potentially may compromise Ca homeostasis.

Interaction between systemic and local factors can occur, and some factors such as PTHrP may act both systemically and locally (42). PTHrP, is also known as PTH-like peptide, PTH-like protein, or human humoral hypercalcemic factor. PTHrP and PTH genes appear to be members of the same gene family. PTHrP cDNA encodes a 177 amino-acid protein consisting of a 36 amino-acid precursor segment and a 141 amino-acid mature peptide. The mature PTHrP contains several structural or functional domains. The N-terminal 1–13 region has eight of 13 residues similar to PTH. The amino acids 34–111 segment is highly conserved among species while amino acid 118 to the C-terminus is poorly conserved. PTHrP gene expression is found in an extensive variety of endocrine and nonendocrine tissues. PTHrP biological activity and immunoreactivity for PTHrP mRNA have been found in many tissues, by as early as seven weeks of gestation, including the fetus, placenta, lactating breasts, and milk in human and in various tissues in the sheep and pig. Both PTH and PTHrP appear to bind to the same G protein-linked receptor. Synthetic and recombinant PTHrPs can mimic the effects of PTH on the classic PTH target organs, involving activation of adenylate cyclase and other second messenger systems.

Several PTHrP assays with varying sensitivities and specificities have been developed that account for the variability reported between assays (43). The stability of PTHrP in plasma samples may be enhanced if sample collection is done in the presence of protease inhibitors. Circulating immunoreactive PTHrP concentrations are low or undetectable in normal subjects. Serum PTHrP is increased during pregnancy (44,45) and is similar to or lower than umbilical cord PTHrP concentrations. In cord sera, PTHrP concentrations are 10- to 15-fold higher than that of PTH. Amniotic fluid PTHrP concentrations at mid-gestation and at term are 13- to 16-fold higher than the cord or maternal levels, and the concentration of PTHrP in milk is 100-fold higher. PTHrP concentrations correlate positively with total milk calcium.

PTHrP concentrations in the circulation of individuals with humoral hypercalcemia of malignancy (HHM) are elevated. The amino-terminal fragment PTHrP 1–74 appears to be specific for HHM, whereas the carboxy-terminal fragment PTHrP 109–138 is elevated in the serum of patients with HHM or renal failure. The levels of PTHrP in these patients are similar to the concentration of PTH (10^{-12} to 10^{-11} mol/L).

Clinically, PTHrP is the humoral mediator secreted by tumors that results in the syndrome of HHM and the measurement of PTHrP is of clinical utility primarily as a tumor marker in HHM. Physiologically, PTHrP is an important paracrine regulator of several tissue-specific functions that may directly or indirectly affect fetal and neonatal mineral homeostasis, probably through its effect on smooth muscle relaxation, placental calcium transport, lactation, fetal bone development, and in the control of cellular growth and differentiation.

DISTURBANCES IN SERUM MINERAL CONCENTRATIONS

Hypocalcemia

Neonatal hypocalcemia may be defined as a serum tCa concentration less than 2 mmol/L (8 mg/dL) in term infants and 1.75 mmol/L (7 mg/dL) in preterm infants with iCa below 1.0 to 1.1 mmol/L (4.0–4.4 mg/dL), depending on the particular ion-selective electrode used. Whole blood iCa show similar values to serum iCa and are often used to determine hypocalcemia. However, the appropriate range used is also subject to the type of instrument used (3).

The definition of hypocalcemia is based on the clinical perspective, because serum Ca concentrations are maintained within narrow ranges under normal circumstances, and the potential risk for disturbances of physiologic function increases as the serum Ca concentration decreases below the normal range. Furthermore, improvements in physiologic function, e.g., changes in cardiac contractility, blood pressure, and heart rate, are reported in hypocalcemic infants undergoing Ca therapy (46–48), and higher mortality rate has been reported for children with hypocalcemia in pediatric intensive care settings (49).

Clinically, there are two peaks in the occurrence of neonatal hypocalcemia. An early form typically occurs during the first few days after birth, with the lowest concentrations of serum Ca being reached at 24 to 48 hours of age; late neonatal hypocalcemia occurs toward the end of the first week. These findings reflect in part the traditional clinical practice of screening for biochemical abnormalities in small or sick hospitalized infants during the first few days, and in symptomatic infants during hospitalization and after hospital discharge. However, the nadir of the serum Ca concentration may occur at less than 12 hours (50–53) or not until some weeks after birth (34,54), and that many neonates, particularly those with genetic defects in Ca metabolism, may be hypocalcemic but remain asymptomatic and undetected

Table 1 Risk Factors for Neonatal Hypocalcemia

Maternal
Insulin-dependent diabetes
Hyperparathyroidism
Vitamin D or magnesium deficiency
Medication: calcium antacid and anticonvulsant (?)
Narcotic use (?)
Peripartum
Birth asphyxia
Infant
Intrinsic
Prematurity
Malabsorption
Malignant infantile osteopetrosis
Parathyroid hormone: impaired synthesis, secretion, regulation, or responsiveness
Mitochondrial fatty acid disorder (like infant)
Extrinsic
Diet: Inadequate calcium, excess phosphorus
Enema: phosphate
Exchange transfusion with citrated blood
Infectious diarrhea (like diet)
Clinical therapy (like diet): phototherapy, alkali, high rate of intravenous lipid

during the early neonatal period. This also may contribute to the less frequent diagnosis of late neonatal hypocalcemia compared to early neonatal hypocalcemia. Additionally, increased understanding of the mechanisms of disturbed Ca metabolism would support the approach to neonatal hypocalcemia based on risk factors and pathophysiologic basis rather than the traditional ''early'' or ''late'' onset.

Pathophysiology

Multiple risk factors for neonatal hypocalcemia (Table 1) support the existence of varied and frequently interrelated pathophysiologic mechanisms (Table 2). However, the pathophysiologic mechanisms are not fully defined for all cases of hypocalcemia. In most cases of neonatal hypocalcemia, there is a decrease in both tCa and iCa, although iCa may be decreased without lowering tCa.

There are common bases for the occurrence of hypocalcemia particularly for ''early''-onset hypocalcemia. These include the abrupt discontinuation of placental Ca supply at birth, limited or no dietary calcium, transient limited increase in the serum PTH concentration, possibly end-organ resistance to PTH and $1,25(OH)_2D$, and elevated serum CT concentration. Many illnesses may preclude early enteral feeding but many clinicians do not use parenteral nutrition that contains Ca for one or more days after birth, thus increasing the risk for hypocalcemia. Even in healthy term infants, the amount of calcium retention from milk feeds probably is less than 20 mg/kg body weight on the first day, rising to about 45 to 60 mg or more/kg on the third day; these amounts are significantly lower than the daily in utero Ca accretion of over 100 mg/kg during the third trimester.

Hypocalcemia (in varying degree of severity) may occur in association with ''transient'' congenital hypoparathyroidism (TCHP), i.e., suppression of fetal and neonatal parathyroid function from maternal hyperparathyroidism (34,54) and maternal use of high doses of calcium carbonate (29) as antacid, and thus, impaired PTH response to the interruption of placental supply of Ca at birth. Neonatal hypocalcemia is often the first manifestation that leads to the diagnosis of maternal hyperparathyroidism.

In the neonate, hypocalcemia frequently occurs in the presence of rising concentration of PTH in the circulation. It is possible that this represents either a relative inadequate response of the parathyroid gland or end-organ resistance to PTH. Resistance to pharmacological doses of $1,25(OH)_2D$ demonstrated in vitro (30) and in vivo in infants (52,53) also might contribute to hypocalcemia.

Despite the hypocalcemic effects of CT, the role of CT in the development of neonatal hypocalcemia remains uncertain. Serum CT concentrations continued to increase after birth in neonates of normal and diabetic pregnancies (15,50) irrespective of the variation in serum Ca; in neonates with birth asphyxia (50); and in preterm infants (14). The stimulus for the postnatal rise in serum CT, despite falling serum Ca, is unknown. There are conflicting reports on the effect of Ca supplementation to suppress the postnatal surge in CT secretion. However, serum CT is increased after an intravenous bolus of Ca during exchange blood transfusion (31).

The above problems are exaggerated in the preterm infant and accounts for the inverse relationship between the frequency of hypocalcemia versus birth weight and gestational age; over 50% of preterm very-low-birth-weight neonates may have hypocalcemia (51–53). Infants with intrauterine growth retardation may have hypocalcemia if they are also preterm or have birth asphyxia; otherwise, there is apparently no increased incidence of hypocalcemia related to growth retardation per se (32).

Hypomagnesemia may be contributory to hypocalcemia in infants of mothers with insulin-dependent diabetes (55), although gestational diabetes may (56) or may not (57) have disturbed mineral metabolism. Both hypocalcemia and hypomagnesemia may be the result of a common insult from the diabetic pregnancy, and rigid control of maternal glucose levels during pregnancy may significantly diminish these complications (35). Severe and persistent cause of hypomagnesemia from any cause can result in hypocalcemia (see hypomagnesemia section). Deficiency of various minerals including Ca and Mg, and trace minerals such as zinc can result from chronic intestinal malabsorption and fistula or enterostomy loss. During infancy, congenital or acquired short bowel syndrome or any chronic diarrheal condition, especially if

Table 2 Pathophysiology of Neonatal Hypocalcemia

Physiologic basis	Mechanism	Clinical association
Calcium (Ca)	Decreased reserves	Prematurity
	Decreased intake or absorption	Prematurity, malabsorption syndrome
	Increased Ca complex	Chelating agent (e.g., citrated blood for exchange transfusion, long-chain free fatty acid)
Magnesium (Mg)	Decreased tissue store	IDM, maternal hypomagnesemia
	Decreased intake or absorption	Prematurity, malabsorption syndrome, specific Mg malabsorption (rare)
	Increased loss	Intestinal fistula, enterostomy, or renal (primary or secondary)
Phosphorus (P)	Increased load	Exogenous (e.g., dietary, enema) phosphate loading
pH	Increased	Respiratory or metabolic alkalosis (e.g., shifts Ca from ionized to protein-bound fraction)
PTH	Inadequate or defective synthesis or secretion	Maternal hypercalcemia; DiGeorge association, hypoparathyroidism, hypomagnesemia, PTH gene mutations
	Impaired regulation	
	Impaired responsiveness	CaR-activating mutations: autosomal-dominant or sporadic hypocalcemia with hypercalciuria
		Chronic hypomagnesemia, type 1 PTH receptor-inactivating mutation (?), pseudohypoparathyroidism
Calcitonin	Increased	IDM, birth asphyxia, prematurity
Vitamin D	Deficiency	Severe maternal deficiency;
	Decreased response to 1,25 $(OH)_2D$	Prematurity
Osteoclast activity	Absent	Malignant infantile osteopetrosis
Miscellaneous	Increased anabolism	Hungry bone/refeeding syndrome
	Others?	Mitochondrial fatty acid disorder, rotavirus diarrhea, phototherapy, narcotic withdrawal

Abbreviations: 1,25$(OH)_2D$, 1,25 dihydroxyvitamin D; IDM, Infant of insulin-dependent diabetic mother; CaR, Calcium-sensing receptor; PTH, parathyroid hormone.

associated with steatorrhea are leading causes of malabsorption and possibly impaired enterohepatic circulation of vitamin D and vitamin D metabolites.

Excessive P load can result in hypocalcemia. Cow-milk ingestion and even with ''humanized'' cow-milk–derived formulas (36,58) with ''lower'' P content compared with cow milk, but higher compared with human milk; and cereals that typically have high P content are typical sources of dietary P load. Accidental overdose of oral phosphate supplement (59) or phosphate-containing enema (60) are less frequent causes of excessive P load.

Neonatal hypocalcemia from impaired synthesis or secretion of PTH in the newborn may be secondary to maternal hypercalcemia or to developmental defects of parathyroid gland. A variety of mutations of PTH or CaR genes, some with Mendelian modes of inheritance, can affect the synthesis, metabolism, and function of PTH and result in hypocalcemia.

Relative inadequacy or transient nature of the PTH response to the abrupt withdrawal of the placental transfer of Ca contributes to the fall in serum Ca after birth. This also may be responsible for the hypocalcemia induced from exchange transfusion using citrated blood (31,61), or feeding of the relatively high P content of cow-milk formula (39,58). The ability of the neonatal parathyroids to respond to hypocalcemic stress increases with postnatal age. Neonates with TCHP may have prolonged hypocalcemia that requires treatment until late infancy or early childhood, and hypoparathyroidism may recur in later childhood (62–64).

Hypoparathyroidism in the infant is a heterogeneous group of disorders and may occur sporadically or with differing Mendelian modes of inheritance (65–67). Synthesis of defective PTH can occur in the autosomal-dominant form with a point mutation in the signal peptide-encoding region for the prepro-PTH. The autosomal-recessive form is associated with a mutation in the donor splice site leading to transcriptional loss of the second axon and prevention of translation. The X-linked–recessive form is associated with embryonic dysgenesis of parathyroid glands.

Deletion of chromosome 22q11.2 is associated with varied phenotypic manifestation including DiGeorge and velocardiofacial/Shprintzen syndromes. Both syndromes may represent different degrees of the same disorder with partial or complete absence of derivatives of the third and fourth pharyngeal pouches, and possibly the fifth pouch, and are often associated with defective development of the third, fourth, and sixth aortic arches. It is estimated that up to 30% of these patients may have hypoparathyroidism although far fewer patients develop hypocalcemia (68). Delayed motor development, cognition and neurodevelopment, and behavior and temperament problems are frequently reported in more than 50% of affected patients (69,70). Early screening and intervention for these problems are advised. Multiple other organ system (68,71) may be involved and include some combination of congenital heart disease, primarily involving the aortic arch, decreased T-cell number or function, and possibly thyroid C-cell deficiency. DiGeorge association may be inherited in an autosomal-dominant fashion (72).

Dysregulation of PTH can result from activating mutations of CaR with reduction in EC50 (concentration of extracellular Ca required to elicit half of the maximal increase in intracellular inositol phosphate) to suppress PTH synthesis. It is manifested as autosomal-dominant or sporadic cases of hypocalcemia with hypercalciuria (73,74). The latter is an effect of the mutated CaR in the kidneys. Hypocalcemia is usually mild and asymptomatic, and diagnosis is often delayed beyond the neonatal period, although hypocalcemia was likely present during the immediate newborn period.

Relative defective response to PTH can result in neonatal hypocalcemia. Inactivating mutation of the type 1 PTH receptor gene, as documented in Blomstrand's chondrodystrophy, is present in the prenatally lethal form of short limb dwarfism (75). Theoretically, this defective response to PTH may result in hypocalcemia but the regulation of serum Ca has not been evaluated in vivo.

Impaired end-organ response to PTH occurs with chronic hypomagnesemia and may involve simultaneous impairment in both PTH and $1,25(OH)_2D$ pathways (22). End-organ unresponsiveness to PTH associated with genetic defect is classically manifested as pseudohypoparathyroidism type 1a (PHP-1a) or Albright's hereditary osteodystrophy. The biochemical basis of the defect is proximal to cAMP production (76). It is inherited in an autosomal-dominant fashion with heterozygous inactivating mutations in the maternal GNAS1 exons that encode the α-subunit of the Gsα.

Multiple mutations that result in diminished amount and activity of the G proteins have been reported and include abnormalities in splice junctions associated with deficient mRNA production and point mutations. The inactivating mutation of the gene impairs the production of the adenylate cyclase second messenger system, leading to resistance to multiple hormones (including PTH, vasopressin, and thyrotropin) that activate Gsα. Clinical manifestations include short stature, round face, brachymetacarpals and brachymetatarsals, dental dysplasia, subcutaneous calcifications, abnormalities in taste, smell, hearing, and vision, and developmental delay. Biochemical abnormalities include hypocalcemia, hyperphosphatemia, increased circulating PTH, and insensitivity to the administration of exogenous PTH (unaltered urinary Ca, P, and cAMP) in the absence of compromised renal function. The extent of resistance to other hormones is variable and the complete biochemical picture is usually not evident until two to three years after birth.

Different phenotypic expression can result from parent-specific methylation with parental imprinting of the GNAS1 gene, involving selective inactivation of either the maternal or paternal allele. In the case of the Gsα gene, it is paternally imprinted (silenced) so that the disease PHP-1a is not inherited from the father carrying the defective allele but only from the mother (77). However, the defective allele is not imprinted or silenced in all tissues and reflects haplotype insufficiency. For example, PHP type 1b is characterized by isolated resistance to PTH without the accompanying skeletal manifestations. Paternal isodisomy of chromosome 20q in patients that lack the maternal-specific methylation pattern within GNAS1 results in normal Gsα protein and activity in the fibroblast but not in the renal proximal tubules (78). There is a third type, PHP-1c reported in a few patients that differs from PHP-1a only in having normal erythrocyte levels of Gsα; presumably there is a post-Gsα defect in adenyl cyclase stimulation. All type 1 PHP individuals show a deficient urinary cAMP response to the administration of exogenous PTH. Whereas, individuals with pseudo-pseudo-hypoparathyroidism have typical clinical manifestation of PHP-1a but have normal serum Ca and normal response of urinary cAMP to exogenous PTH. The mutated GNAS1 gene is inherited from the father, with suppression of the mutant copy in selected tissues and there is a 50% reduction but not absent Gsα subunit.

Infants with neonatal hypocalcemia seizures and ''transient'' biochemical features of PHP have been reported (79). These infants have elevated serum PTH and P with hypocalcemia at diagnosis. Administration of exogenous human PTH (1–28,33,37–41) showed little phosphaturic effect although there was brisk response in plasma and urine cAMP and alkaline phosphatase. After initial treatment for hypocalcemia, the serum Ca and PTH spontaneously normalized before six months of age.

Maternal anticonvulsant therapy with phenytoin and phenobarbital also may result in neonatal hypocalcemia presumably from increased clearance of vitamin D secondary to the induction of hepatic cytochrome P450 enzyme system. However, other maternal factors including seasonal variation in sunlight exposure, increased maternal age and parity, and poor socioeconomic status, may contribute to development of neonatal hypocalcemia, presumably in part from varied and probably deficient maternal vitamin D. Furthermore, there is no seasonal variation in the rate of early neonatal hypocalcemia (80) despite seasonal variation in maternal and fetal vitamin D status, as indicated by maternal and cord 25 OHD concentrations. Thus, maternal vitamin D or Mg deficiency probably predisposes to but is not the primary cause of hypocalcemia in the neonate.

Malignant infantile osteopetrosis may present with neonatal hypocalcemia presumably reflecting continued Ca uptake from unopposed bone formation (81). Rapid replenishment of nutrients in severe deficiency, including after prolonged starvation, often leads to disturbed blood biochemistries including hypokalemia, hypophosphatemia, - hypomagnesemia, and hypocalcemia. This is known as ''the refeeding syndrome'' or ''hungry bone syndrome'' with excessively rapid shift of electrolytes and minerals

Table 3 Diagnostic Workup for Neonatal Hypocalcemia

History
Screen for risk factors (Table 1)
Physical examination
General examination with focus on peripheral and central nervous and cardiovascular systems
Associated features, e.g., infant of a diabetic mother, prematurity, birth asphyxia, congenital heart disease, pseudohypoparathyroidism, etc.
Investigations[a,b,c]
tCa and iCa, magnesium (Mg) and phosphorus (P), total protein and albumin, and simultaneous "intact" or "whole" PTH
Acid–base status
Complete blood count (lymphocyte count)
Electrocardiogram (Q-Tc $>$ 0.4 sec or Q_o—Tc $>$ 0.2 sec)
Chest X ray (thymic shadow, aortic arch)
Urine Ca, P, Mg, and creatinine
Meconium and urine screen for narcotics
Maternal serum tCa, iCa, Mg, P, urine Ca, and P, if suspect maternal or heritable Ca disorder, particularly in persistent neonatal hypocalcemia
Additional workup as indicated: vitamin D metabolites, T-cell number and function, malabsorption studies, response to exogenous PTH, molecular genetic studies (deletion of 22q11.2, PTH receptor and end-organ responsiveness abnormalities, and calcium-sensing receptor defects, etc.), and family screening

[a]If serum tCa and iCa are normal, diagnostic workup should focus on noncalcium-related causes of clinical symptomatology, e.g., serum glucose, sepsis workup, screen for excretion of illicit drugs, neuroimaging studies, etc.
[b]Resolution of clinical symptomatology when serum tCa or iCa has been normalized confirms the role of hypocalcemia.
[c]Maternal and family screening for calcium disorders is indicated in the absence of specific diagnosis for the neonatal hypocalcemia.
Abbreviations: tCa, total Ca concentrations; iCa, serum ionized Ca; PTH, parathyroid hormone.

intracellularly in various tissues, in particular, muscle and bone (82,83).

The pathophysiology in some situations with hypocalcemia remains ill defined. About 40% of infants with severe diarrhea from rotavirus have hypocalcemia and it resolves with symptomatic support and improvement in diarrhea (84). Mitochondrial fatty acid disorders have been associated with severe metabolic anomalies including hypoglycemia, hypocalcemia, hyperkalemia, and metabolic acidosis, and organ dysfunction including hepatic and cardiac failure (85).

Decreases in serum iCa can occur without decreases in serum tCa. Agents that complex Ca in the blood would be expected to decrease iCa. Such agents include citrate, which is used as an anticoagulant for blood storage. During "exchange blood transfusion," iCa can decrease to 0.5 mmol/L in spite of administration of conventional amounts of Ca (i.e., 0.5–1 mL of 10% Ca gluconate for each 100 mL of blood exchanged) during the transfusion. Increased levels of long-chain free fatty acids from intravenous lipid emulsion can complex Ca and reduce iCa in vitro; thus hypocalcemia potentially can occur with excessive rate of intravenous lipid infusion. Alkalosis can result in shifts of Ca from the ionized state to the protein-bound fraction. Because alkalosis per se increases neuromuscular hyperirritability, the combination of decreased serum iCa and alkalosis may precipitate clinical tetany in an infant with borderline serum Ca status. In clinical practice, administration of sodium bicarbonate in the therapy of metabolic acidosis often occurs in situations with high risk of hypocalcemia such as prematurity or perinatal asphyxia, but whether it has an independent role in the development of hypocalcemia is not known. The mechanisms for hypocalcemia in some situations are not known. For example, neonates with severe hyperbilirubinemia tend to have lower iCa (86), the use of phototherapy may be associated with hypocalcemia (87), and infants born to narcotic-using mothers are reported to have a lower serum iCa if they manifest withdrawal symptoms (88).

Diagnosis

Suspicion of hypocalcemia must be confirmed by measurement of serum tCa and iCa because clinical manifestations are many and varied and may be indistinguishable from other common neonatal diseases (Table 3). Confirmation of hypocalcemia as the cause of clinical manifestations is its reversibility when serum tCa or iCa has been normalized.

The less mature the infant, the more subtle and varied are the clinical manifestations and the infant is frequently asymptomatic. Clinical manifestations may include irritability, jitteriness or lethargy, feeding poorly with and without feeding intolerance, abdominal distension, apnea, cyanosis, and seizures, which may be confused with manifestations of hypoglycemia, sepsis, meningitis, anoxia, intracranial bleeding, and narcotic withdrawal. The degree of irritability of the infants does not appear to correlate with serum Ca values. Frank convulsions are seen more commonly with "late" neonatal hypocalcemia. In newborn infants, the classic signs of tetany from peripheral hyperexcitability of motor nerves including carpopedal spasm (spasm of the wrists and ankles, Trousseau sign), facial spasm (Chvostek sign), and laryngospasm (spasm of the vocal cords) are uncommon.

The level of iCa that determines which feature of tetany will be manifested varies among individuals and will be affected by other components of the extracellular fluid, e.g., hypomagnesemia and alkalosis

lower, whereas hypokalemia and acidosis raise, the threshold for tetany. At physiologic concentrations of hydrogen and potassium ion, tetany may develop in older infants at an iCa less than 0.8 mmol/L (3.2 mg/dL); and will almost always be manifested (with the possible exception of preterm infants), at an iCa less than 0.6 mmol/L (2.4 mg/dL). If serum albumin concentrations are normal, the corresponding serum tCa concentrations usually are less than 1.8 mmol/L (7.2 mg/dL). In the preterm infant, serum iCa may not decrease to the same extent as tCa, presumably in part because of the sparing effect of lower serum albumin and acidosis found frequently in these infants, which tend to increase iCa. This also may partially explain the frequent lack of clinical signs of hypocalcemia in preterm infants. The measurement of electrocardiographic QT intervals, corrected for heart rate, and standard nomogram relating serum tCa and total protein to iCa, have little value for the prediction of neonatal serum iCa. Serum tCa is correlated with iCa but is also inadequate for the prediction of one from the other.

Management

Symptomatic hypocalcemia, manifested as seizures, for example, should be treated promptly with parenteral Ca. It is possible that neonatal hypocalcemia may resolve spontaneously (Table 4).

However, asymptomatic hypocalcemia probably also should be corrected, as Ca potentially can alter important cellular functions where Ca serves either as a first or second messenger in cellular activity.

Any neonate with seizures should have blood drawn for diagnostic tests before therapy. Intravenous administration of Ca salts is the most effective and most rapid means of elevating serum Ca concentrations. Gradual or abrupt decrease in heart rate during the infusion is an indication to slow or stop the infusion. In neonates, 10% Ca gluconate [0.45 mmol (18 mg) elemental Ca/kg] can effectively increase serum iCa, heart rate, cardiac contractility, and blood pressure (46–48). In children, small equimolar doses [0.07 mmol (2.8 mg) elemental Ca/kg] of 10% Ca chloride compared to 10% Ca gluconate may result in higher mean arterial blood pressure with a slightly greater mean increase (0.06 mmol/L and 0.2 mg/dL) in the measured serum iCa (89). Prolonged use of Ca chloride in high doses may be associated with acidosis and probably should be avoided. With intravenous Ca therapy, bolus infusion may be associated with a transient slight decrease in blood pH and serum P, and with hypercalcemia. Continuous infusion probably is more efficacious than intermittent therapy, because renal loss of Ca may be greater with the latter method; a dose of 1.25 to 2.0 mmol (50–80 mg) elemental Ca/kg/day has been used successfully in the treatment and prevention of neonatal hypocalcemia. Intravenous Ca supplement should be rapidly weaned, or replaced with Ca-containing parenteral nutrition if the infant is not expected to tolerate enteral feeding.

Arterial infusion of Ca in high concentrations potentially is fraught with many dangers and should be avoided. Massive sloughing of soft tissue may occur in the distribution of the arterial supply; for example, inadvertent administration into a mesenteric artery theoretically can lead to necrosis of intestinal tissues. If an umbilical venous catheter is used, the tip should be in the inferior vena cava and not intracardiac, as administration of Ca directly into the heart may result in arrhythmia. Parenteral nutrition solutions containing standard mineral (including calcium) content can be safely infused through appropriately positioned umbilical venous or umbilical arterial catheters. Direct admixture of Ca preparation with bicarbonate or phosphate solution will result in precipitation and must be avoided.

Oral Ca supplement at the similar dosage as parenteral Ca [1.87 mmol (75 mg) elemental Ca/kg/day in four to six divided doses] should be started if the infant is expected to tolerate it, and the serum Ca is normalizing after the initial intravenous Ca therapy. Oral Ca preparations generally contains higher

Table 4 Management of Neonatal Hypocalcemia

Acute-phase therapy
Correction of hypomagnesemia, acid–base problem, etc., if possible
Intravenous 10–20 mg elemental Ca/kg as 10% Ca gluconate or 10% Ca chloride (provides 9 mg elemental Ca/mL or 27.2 mg/mL, respectively) with dextrose water or normal saline infused over 5 to 10 min under constant ECG monitoring; repeat as necessary until resolution of severe symptomatology such as seizures
In infants that are not fed enterally, this is followed by intravenous continuous infusion at 50–75 mg elemental Ca/kg/day. Alternately, parenteral nutrition containing 50 mg elemental Ca/100 mL is preferred and continued until feeding
In asymptomatic infants, oral 50–75 mg elemental Ca/kg/day in 4–6 divided doses. 1 mL of calcium carbonate, glubionate, gluceptate, gluconate, lactate, or chloride contains 40, 23, 18, 9, 13, and 27 mg elemental Ca, respectively
Once serum tCa normalized, half the Ca supplement daily for 2 days, then discontinue
Serial serum tCa (+/− iCa) q 12–24 hr until clinically stable, q 24 hr until normalized, and at 24 hr after Ca supplement discontinued
Maintenance therapy: treat underlying disorder if possible
Low phosphorus formula (PM 60/40®, Abbott Laboratories, Columbus, OH, U.S.) if serum P is high ($>$ 2.6 mmol/L or 8 mg/dL) until serum Ca and P normalized
Prolonged and higher Ca doses, and $1,25(OH)_2D$ may be needed, e.g., hypoparathyroidism

Abbreviations: ECG, electrocardiography; iCa, serum ionized Ca; $1,25(OH)_2D$, 1,25 dihydroxyvitamin D; tCa, total Ca concentrations.

Ca concentration than intravenous preparations, for example, Ca glubionate, gluceptate, and carbonate have respectively 2.88, 2.25, and 2.5 mmol (115, 90, and 200 mg) elemental Ca per 5 mL, and are useful for infants, particularly those requiring fluid restriction. All oral Ca preparations are hypertonic. This effect can be lowered by administrating oral Ca supplement with feeds. Syrup base oral Ca preparations also have high sucrose content that may constitute a significant carbohydrate load for small preterm infants and may be associated with an increase in frequency of bowel movements. Alternately, an intravenous preparation can be used orally if the fluid volume is tolerated. Treatment of asymptomatic hypocalcemia can be instituted with oral Ca supplement in the same dosage.

Duration of supplemental Ca therapy depends on the underlying cause of hypocalcemia and usually lasts several days for most cases of neonatal hypocalcemia, or may be prolonged as in the case of hypocalcemia caused by malabsorption. Hypoparathyroidism from fetal parathyroid hypoplasia or dysgenesis usually requires life long treatment to prevent hypocalcemia.

Serum Ca concentrations should be measured daily during the first few days of treatment and for one or two days after discontinuation, until serum tCa and iCa concentrations are stabilized. Persistently low serum Ca concentrations should prompt further investigations even in the absence of suspicious history or physical features associated with pathologic causes of hypocalcemia.

For severe persistent hypocalcemia, vitamin D or one of its analogs is often used in addition to Ca supplementation. The use of $1,25(OH)_2D$ is preferred because it can raise serum Ca within one to two days after initiation of therapy and leaves no residual effects within several days of its discontinuation. Vitamin D has slower onset of action of two to four weeks and the residual effect also lasts several weeks after its discontinuation, thus making dosage adjustment more difficult.

Successful management of neonatal hypocalcemia also depends on the resolution, if possible, of the primary cause of hypocalcemia. For example, a poor response to Ca therapy often may result from concurrent Mg deficiency. Hypomagnesemia, if present, must be treated to obtain maximal response to Ca therapy. In phosphate-induced hypocalcemia, high-phosphate formulas and solids should be discontinued, and human milk or a low-phosphate formula should be substituted. Use of aluminum hydroxide gel to bind intestinal phosphate should be avoided because of potential risk for aluminum toxicity (90).

Early milk feeding and the use of calcium-containing parenteral nutrition within hours after birth are the best means to minimize the development and recurrence of hypocalcemia, and may negate the need for use of Ca supplementation. Delaying premature delivery and minimize perinatal asphyxia, judicious use of bicarbonate therapy, and mechanical ventilation to avoid alkalosis also are important. Maintenance of normal maternal vitamin D status with exogenous vitamin D supplement, if needed, in theory may be helpful in maintaining normal fetal vitamin D status and may secondarily prevent hypocalcemia in some neonates. Early feeding and provision of Ca to the gut in the neonate enhances the ability of vitamin D metabolites to prevent hypocalcemia.

Pharmacological prevention of neonatal hypocalcemia has focused primarily on the prophylactic use of Ca salts or vitamin D metabolites. In newborn infants, Ca supplementation results in sustained lowering of serum IPTH concentrations compared to unsupplemented controls (16). Theoretically, Ca supplementation may decrease the metabolic stress from hypocalcemia and minimize the potential for depletion of tissue Ca stores. Early studies used up to 1.8 to 2.0 mmol (72–80 mg)/kg/day of oral Ca supplement and about half this amount intravenously to prevent hypocalcemia. However, it should be noted that a similar amount of Ca can be provided from an intake of 150 to 200 mL/kg/day of standard term infant formula or human milk. Standard preterm infant formula can provide almost 5 mmol (200 mg) of Ca/kg/day and parenteral nutrition with 1.25 to 1.5 mmol (50–60 mg) Ca/100 mL can easily provide 1.5 mmol (60 mg) of Ca/kg/day. These nutritional sources of Ca have been the standard practice in most neonatal nurseries for over a decade and are well tolerated. Early feeding or parenteral nutrition must be considered as the best means to prevent neonatal hypocalcemia, particularly for the preterm infant. Vitamin D_3 and its metabolites have been used in attempts to prevent neonatal hypocalcemia with variable degrees of success. In small preterm infants, serum Ca was normalized only at pharmacological doses of $1,25(OH)_2D$ (53).

Complications of hypocalcemia vary with the clinical manifestations, and may be related to the therapy and underlying pathophysiology. Acute complications are associated with clinical manifestations including seizure, apnea, cyanosis and hypoxia, bradycardia, and hypotension. Therapy-related complications such as cardiac arrhythmia, arterial spasm and tissue necrosis, extravasation of Ca solution, can be avoided by continuous electrocardiography (ECG) monitoring during Ca infusion, avoiding infusion of Ca into arterial line and checking for venous patency before Ca infusion. There is also a risk for metastatic calcification from aggressive Ca treatment in the presence of hyperphosphatemia. In situations where PTH is absent or nonfunctional, its protective hypocalciuric action cannot occur; therefore raising markedly the serum Ca concentration may cause hypercalciuria, renal stones, nephrocalcinosis, and possible renal damage. These complications have been reported during therapy in patients with activating CaR mutation, even while the patients are

Table 5 Pathophysiology of Neonatal Hypercalcemia

Phosphate deficiency
Calcium-containing parenteral intake with inadequate or no phosphorus
Very-low-birth-weight infants fed human milk or, less commonly, standard formula
Parathyroid related
Hereditary primary hyperparathyroidism
Calcium-sensing receptor-inactivating mutations: familial hypocalciuric hypercalcemia, neonatal severe hyperparathyroidism
Parathyroid hormone receptor-activating mutation
Secondary hyperparathyroidism
Maternal: hypocalcemia, renal tubular acidosis
Neonatal: renal tubular acidosis
Parathyroid hormone-related protein-secreting tumors
Vitamin D
Excessive intake
Mother: high-dose vitamin D
Neonate: high-dose vitamin D prophylaxis, overfortification of milk
Increased 1,25 dihydroxyvitamin D
Subcutaneous fat necrosis
Histiocytic disorders, disseminated tuberculosis with septic shock and hemophagocytic syndrome (?)
Calcitonin response impairment in congenital hypothyroidism
Vitamin A excess
Uncertain pathophysiologic mechanism
Chromosomal/gene abnormalities
Idiopathic infantile hypercalcemia/Williams syndrome
Severe infantile hypophosphatasia
Microdeletion of 4q
Heritable metabolic defect
Blue diaper syndrome
Glycogen storage disease type 1a, congenital lactase, or sucrase-isomaltase deficiency, disaccharidase deficiency
Extracorporeal membrane oxygenation therapy

normocalcemic (74). Long-term outcome of neonates with history of hypocalcemia generally depends on the underlying cause, for example, patients with 22q11.2 deletion syndromes frequently have defects of multiple-organ systems and neurodevelopmental delay unrelated to hypocalcemia (68–71). Isolated transient hypocalcemia even in symptomatic cases have not been associated with long-term sequelae although recurrent prolonged seizures from poorly controlled hypocalcemia potentially may be associated with neurodevelopment delay.

Regular clinical follow-up and laboratory monitoring such as for serum Ca and IPTH, are necessary in infants with ''transient'' hypoparathyroidism because some of these infants are at risk for ''recurrence'' of hypoparathyroidism and hypocalcemia as late as adolescence (62–64).

Hypercalcemia

Hypercalcemia in infants occurs much less frequently than hypocalcemia. However, it is increasingly being diagnosed because serum Ca is usually part of a panel of chemistry tests, and because of increasing knowledge of its pathogenesis. Hypercalcemia is present when serum tCa more than 2.75 mmol/L (11 mg/dL) or when iCa is greater than 1.4 mmol/L (5.6 mg/dL), depending on the particular ion-selective electrode used. In pathologic hypercalcemia, elevation of serum iCa usually occurs simultaneously with elevation of tCa; however, elevated tCa may occur without elevation of iCa. Elevation of protein available to bind Ca (e.g., prolonged application of tourniquet before venipuncture, with resultant transudation of plasma water into tissues, shown in adult patients with multiple myeloma, and possibly adrenal insufficiency) may result in elevation of serum tCa. A change in serum albumin of 1 g/dL generally results in a parallel change in tCa of about 0.2 mmol/L. Conversely, reduced albumin binding of Ca may result in normal serum tCa in the presence of elevated iCa.

Pathophysiology

Hypercalcemia may occur within hours after birth or delayed for weeks or months (Table 5). It may result from increased intestinal or renal Ca absorption, increased bone turnover, or from iatrogenic causes.

In neonatal intensive care setting, hypercalcemia is often iatrogenic from inadequate provision of dietary phosphate during and after hospitalization, as with the use of low or no phosphate parenteral nutrition or feeding human milk without fortifier in very-low-birth-weight infants (91–95). Phosphate deficiency or hypophosphatemia stimulates 1α-hydroxylase and synthesis of $1,25(OH)_2D$, which enhances intestinal absorption and renal reabsorption of Ca and P. Increased Ca absorbed in the presence of increased $1,25(OH)_2D$ cannot be deposited in bone in the absence of phosphate and contributes to hypercalcemia. Simliarly, the continued use of high-dose Ca prophylaxis in normocalcemic infants with

inadequate or no P intake also can result in hypercalcemia. Decreased renal Ca excretion or relative PTH resistance in the neonate also may exaggerate the extent of hypercalcemia.

Neonatal hyperparathyroidism frequently results in marked hypercalcemia. It may be a sporadic congenital occurrence or show a Mendelian inheritance, or it may be secondary to maternal hypocalcemia.

Hereditary primary hyperparathyroidism manifested in neonates is associated with inactivating mutations of CaR. The severity of hypercalcemia is related to the extent of CaR mutation. Mild hypercalcemia (serum tCa < 3.0 mmol/L and 12 mg/dL) associated with heterozygous mutated CaR is manifested clinically in most patients with familial hypocalciuric hypercalcemia (FHH). The normal urinary Ca excretion despite hypercalcemia is an effect of the mutated CaR in the kidneys. Serum PTH is usually within the normal range but is higher than expected for the degree of hypercalcemia. FHH has been reported in patients from two hours to 82 years of age and is usually diagnosed in infants as part of a screening procedure after diagnosis of a family member with hypercalcemia or familial multiple endocrine neoplasia. It is inherited as an autosomal-dominant trait with a high degree of penetrance (96). There usually is significant hypophosphatemia and a modest increase in serum Mg concentration, and functional parathyroid glands are needed for full expression. Neonatal hyperparathyroidism associated with FHH that resolves spontaneously over several months has been reported (97). More severe hypercalcemia with serum tCa of 3 to 3.3 mmol/L (12–13 mg/dL) has been attributed to coexpression of the normal and mutated CaR, with the latter having a functional equivalent of a "dominant-negative" effect. The most marked hypercalcemia (serum Ca > 4 mmol/L and 16 mg/dL) occurs in neonatal severe hyperparathyroidism with homozygous inactivating germ-line mutations of the CaR gene. This severe disorder can be lethal within the first few weeks after birth (98,99).

Activating mutations of the PTH/PTHrP receptor gene in Jansen metaphyseal dysplasia presumably have the receptor defects in the kidney, bone, and chondrocytes at the growth plate. The clinical manifestations include postnatal-onset short limb dwarfism with radiographic rachitic changes, and mild hypercalcemia occurs in about 50% of the affected patients (100).

Neonatal hyperparathyroidism may be secondary to various causes of maternal hypocalcemia including maternal hypoparathyroidism (101), maternal (102), or neonatal (103) renal tubular acidosis. Presence of metabolic acidosis independently increases bone resorption, enhances the renal effects of hyperparathyroidism, and the hypercalcemic effects are augmented by decreased renal excretory capacity of the neonate.

Elevated serum PTHrP and hypercalcemia are found in increasing number of infants with a variety of tumors (104–107) including malignant hepatic sarcoma, infantile fibrosarcoma, renal adenoma, and rhabdoid tumors. There is also associated mortality in some cases although the relative contribution to death from hypercalcemia versus the underlying disease is not clear.

Hypercalcemia was reported in 34% of neonates and infants from intermittent high-dose vitamin D (600,000 IU each three to five months) prophylaxis (108). Hypercalcemia also has been reported in infants given human milk with very high vitamin D content (7000 IU/L), from high-dose vitamin D therapy for maternal hypoparathyroidism, from milks with excessive vitamin D fortification from errors during processing, and in preterm infants given chronic vitamin D supplementation in addition to high-Ca and high-P milk formula. Neonates with extensive subcutaneous fat necrosis often have a history of perinatal asphyxia and may develop hypercalcemia after a period of low or normal serum Ca concentrations (109). There is an anecdotal report that body cooling for the treatment of birth asphyxia could augment the development of subcutaneous fat necrosis. Hypercalcemia is reported to occur between 2 and 16 weeks, most commonly at six to seven weeks after the development of subcutaneous fat necrosis. Increased prostaglandin E activity, increased release of Ca from fat and other tissues, and unregulated production of $1,25(OH)_2D$ from macrophages infiltrating fat necrotic lesions, have been postulated to be responsible for the hypercalcemia in this condition. Histiocytic disorders and disseminated tuberculosis with septic shock and hemophagocytic syndrome may be complicated with hypercalcemia in infants; whether this is also related to nonrenal production of $1,25(OH)_2D$ is not known. Vitamin A toxicity is associated with hypercalcemia presumably secondary to the retinoic acid stimulation of osteoclastic activity and bone resorption. In infants, vitamin A toxicity in infants may occur at intakes as low as 2100 IU/100 kcal and can be fatal (110).

Hypercalcemia may develop before and during thyroxine therapy of infants with congenital agoitrous hypothyroidism (111). In theory, deficient CT response to Ca loading, or an increased degradation of CT, may be responsible for the hypercalcemia.

Neonatal hypercalcemia is reported in other situations in which the pathophysiology remains uncertain. Idiopathic infantile hypercalcemia, often considered as part of Williams syndrome, is associated with varying manifestations including hypercalcemia, mental retardation, elfin facies, and supravalvular aortic stenosis. There also may be prenatal and postnatal growth failure. The presence of hypercalcemia in infants with Williams syndrome is variable, and serum Ca may be normal, but the presence of nephrocalcinosis and soft-tissue calcifications in some of these infants suggests that hypercalcemia may have occurred previously. An exaggerated response to pharmacological doses of vitamin D_2 and a blunted CT response to Ca loading and PTH infusion may

Table 6 Diagnostic Workup for Neonatal Hypercalcemia

History
Familial or maternal disturbances in calcium (Ca) or phosphorus (P) metabolism
Gestational age, difficult labor, ECMO therapy, and pre-ECMO therapy
Intake of calcium, phosphorus, vitamins D and A for mother and infant
Physical examination
General examination with focus on growth parameters, hydration status, heart rate, blood pressure, cornea for band keratopathy (rare)
Associated features (e.g., subcutaneous fat necrosis, elfin facies, congenital heart disease, developmental delay)
Investigations
Serum total and ionized Ca, magnesium, P, creatinine (Cr), total protein and albumin, alkaline phosphatase (total and bone specific), simultaneous "intact" or "whole" PTH, 25 hydroxyvitamin D and 1,25 dihydroxyvitamin D
Acid–base status
Urine Ca, P, Cr, amino acids
X ray of chest, hands, and long bones
Ultrasound of kidneys and abdomen, ophthalmologic examination, electrocardiogram (shortened QT interval, bradycardia) for complications
If above do not yield diagnosis, other tests depend on associated history and symptomatology
Parental (both parents) serum and urine Ca, P, Cr
Molecular studies
Family screening depends on the primary diagnosis
Serum PTH-related protein and screen for occult tumor
Screen for metabolic defects, unusual dietary supplement

Abbreviations: ECMO, extracorporeal membrane oxygenation; PTH, parathyroid hormone.

contribute to the pathogenesis of hypercalcemia in idiopathic infantile hypercalcemia. Several genetic defects in idiopathic infantile hypercalcemia, including hemizygosity at the elastin gene on the long arm of chromosome 7 have been reported (112,113). No mutation of the CT/CGRP gene has been detected. However, the cellular mechanism that leads to the phenotypic expression remains unknown.

Severe infantile hypophosphatasia is associated with hypercalcemia. It is a rare autosomal-recessive disorder associated with decreased synthesis of tissue nonspecific alkaline phosphatase from a deletion or point mutation in its gene located on chromosome 1. These patients have severe bone demineralization, low serum alkaline phosphatase, and elevated urinary pyrophosphate and phosphoethanolamine. The condition may be lethal in utero or shortly after birth because of inadequate bony support of the thorax and skull, although milder phenotypes are compatible with survival to adulthood (114).

Microdeletion of long arm of chromosome 4 has been associated with hypercalcemia and cardiac failure (115).

Blue diaper syndrome is a rare familial disorder with impaired intestinal transport of tryptophan. The blue discoloration of the urine results from the hydrolysis and oxidation of urinary indican, an end product of intestinal degradation of unabsorbed tryptophan and hepatic metabolism of its intermediate metabolites. Blue discoloration of the urine has been reported within weeks after birth, although hypercalcemia and nephrocalcinosis are not reported until some months after birth. Glycogen storage disease type 1a and congenital lactase deficiency and congenital sucrase-isomaltase deficiency with chronic diarrhea have been associated with hypercalcemia and nephrocalcinosis. Hypercalcemia apparently resolves without specific treatment following treatment for disaccharidase deficiency.

Transient hypercalcemia occurs in infants during extracorporeal membrane oxygenation (ECMO) therapy varying in frequency from less than 5% to about 30%, depending on whether the cutoff point used is above 2.5 or above 2.25 mmol (12 mg or 11 mg/dL), respectively (116,117).

Diagnosis

Neonates with hypercalcemia may be asymptomatic despite the onset of hypercalcemia at birth (Table 6). In these cases, there are often delays of weeks or months before diagnosis is made, coincidental to a chemistry panel screening during the course of other illness or because of hypercalcemia in another family member.

Presence of family history of Ca disorder or anatomic anomalies (e.g., elfin facies, evidence of congenital heart disease, and subcutaneous fat necrosis) on physical examination of the infant may be helpful in arriving at the diagnosis.

Symptoms and signs frequently are nonspecific and include lethargy, irritability, poor feeding with or without feeding intolerance, constipation, polyuria, dehydration, and failure to thrive. Hypertension associated with hypercalcemia in adults also may occur in infants, although it may be in part related to treatment-related relative fluid overload as in many infants who require ECMO therapy.

Management

Therapy depends on the extent of elevation of serum Ca and whether the infant is symptomatic (Table 7). For mildly elevated serum tCa (< 12 mg/dL) in

Table 7 Management of Neonatal Hypercalcemia

Acute
Remove etiologic factor, if possible, e.g., discontinue vitamin D and Ca supplement
Intravenous normal saline (20 mL/kg) and loop diuretic (furosemide 2 mg/kg). Reassess and repeat q 4–6 hr as necessary. Monitor fluid balance and serum calcium, magnesium, sodium, potassium, phosphorus, and osmolality q 6–12 hr. Prolonged diuresis may require Mg and potassium replacement
Use lower Ca content milk or parenteral nutrition if possible to maintain nutrition
In neonates with low serum P ($<$ 1.3 mmol/L; 4 mg/dL), oral phosphate supplement at 0.5–1 mmol (15–30 mg) elemental P/kg/day in 4 divided doses may normalize serum P and Ca. In infants not being fed, can use parenteral nutrition containing usual amount of phosphate [1–1.5 mmol (31–46 mg)/100 mL] but no calcium until serum Ca returned to normal
Minimal data on the use of hormone, e.g., subcutaneous or intramuscular recombinant human calcitonin (4–8 IU/kg q 6 hr), $+/-$ oral glucocorticoid (prednisone 0.5–1 mg/kg/day). Other drugs, e.g., bisphosphonates (oral etiodronate 25 mg bid, intravenous pamidronate 0.5 mg/kg) are experimental
Peritoneal dialysis or hemodialysis with a low calcium dialysate may be considered in severely symptomatic patient refractory to medical therapy
Parathyroidectomy may be needed when clinically stabilized
Maintenance
Depends on underlying cause
Additional general therapy may be needed: low Ca, no vitamin D infant formula (Calcilo XD, Ross products Division, Abbott Laboratories, Columbus, OH, U.S.); minimize sunlight exposure to lower endogenous synthesis of vitamin D

the presence of iatrogenic cause, e.g., phosphate-free parenteral nutrition or the use of Ca supplement without any dietary phosphate intake, resolution of the underlying cause should resolve the problem. Dietary P deficiency-induced hypercalcemia is becoming less common with the increasing use of commercial fortifier for human-milk–fed preterm infants, and the use of high Ca- and high P-containing infant formula and parenteral nutrition for the preterm infant. In patients with low serum P concentrations, large amounts of phosphate supplement may cause hypocalcemia and the possibility of metastatic calcification. Phosphate supplement given orally may result in diarrhea.

With moderate-to-severe hypercalcemia, the initial treatment is nonspecific with expansion of extracellular fluid compartment (10–20 mL/kg of 0.9% sodium chloride intravenously) and furosemide (2 mg/kg) -induced diuresis. Care should be taken to avoid fluid and electrolyte imbalance with careful monitoring of fluid balance and serum Ca, Mg, sodium, potassium, and osmolality at 6- to 12-hour intervals. Furosemide therapy may be repeated at four- to six-hour intervals. Prolonged diuresis also requires replacement of Mg losses.

Minimal information is available on the use of hormonal and other drug therapy for neonatal hypercalcemia. Nonmammalian source of CT, e.g., salmon CT (4–8 IU/kg every 12 hours, subcutaneously or intramuscularly), has greater hypocalcemic effect and longer duration of saction, compared with recombinant hCT. However, salmon CT has greater potential for allergic reaction and induction of antibody formation. The hypocalcemic effect decreases after a few days of any CT treatment. Steroid (prednisone 0.5–1 mg/kg/day) therapy may result in significant problems including hypertension, hyperglycemia, and gastrointestinal hemorrhage, and thus are not recommended for long-term therapy. Bisphosphonates, oral etidronate (25 mg bid), and intravenous pamidronate (0.5 mg/kg) have been used for hypercalcemia in the mother and neonate. Long-term use of pamidronate in infants and children with osteogenesis imperfecta decreases serum iCa with compensatory increase in PTH (118). The effects on growth plate, bone production, and mineralization remains unknown and its use should be restricted to acute short-term therapy. Dialysis in the neonate is not without technical or metabolic complications. Rarely, parathyroidectomy may be necessary, although it is not always effective. Development of calcimimetic agents are able to amplify the sensitivity of the CaR to iCa and suppress PTH levels, with a resulting decrease in blood iCa, offer potential for noninvasive therapy of hypercalcemia.

Treatment for chronic conditions also includes restriction of dietary intake of vitamin D and Ca, and minimizing exposure to sunlight to decrease endogenous vitamin D production. A low-Ca, low-vitamin D_3, low-iron infant formula is available for the management of hypercalcemia in infants (Calcilo XD, Abbott Laboratories, Columbus, OH, U.S.). This formula contains only trace amounts of Ca less than 10 mg/100 kcal and no vitamin D. Long-term use of this formula alone will lead to calcium depletion; iatrogenic vitamin D deficiency is also a concern in this situation, and both can result in deleterious consequences.

Complications of hypercalcemia vary with the clinical manifestations at presentation. Persistent hypercalcemia may result in ectopic calcification, which involves the ectopic deposition of a solid phase of calcium and phosphate in walls of blood vessels, and in connective tissue about the joints, gastric mucosa, renal parenchyma, and cornea, especially when accompanied by normal or elevated levels of serum P. Prolonged therapy, such as severe limitation of Ca and vitamin D intake, may be associated with hypocalcemia and bone demineralization (119). Severe hypercalcemia can be fatal, although some of the infants have other potentially lethal underlying conditions. Long-term complications usually depend on the underlying cause of hypercalcemia, including failure to thrive and nephrocalcinosis.

Neonatal hypercalcemia may not develop until some weeks after the onset of the insult and may

Table 8 Pathophysiology of Neonatal Hypomagnesemia

Decreased tissue accretion
Infants of mothers with insulin-dependent diabetes or hyperparathyroidism
Small-for-gestational-age infants
Chronic maternal magnesium deficiency
Decreased absorption
Extensive small intestine resection
Specific intestinal magnesium malabsorption
Increased loss
Intestinal fistula or diarrhea
Hepatobiliary disorders
Decreased renal tubular reabsorption
Primary: potassium channel and Na-K-2Cl cotransporter gene mutations, renal tubulopathies with hypo- or hypercalciuria
Secondary: extracellular fluid compartment expansion, osmotic diuresis, drugs (e.g., loop diuretic, aminoglycoside, ibuprofen overdose)
Others
Increased phosphate intake
Exchange transfusion with citrated blood

Source: From Ref. 120.

resolve spontaneously, as in subcutaneous fat necrosis. Therefore, serum Ca should be monitored at regular intervals in certain situations to determine the onset of hypercalcemia and to determine the continue need for treatment. Family screening for hypercalcemia should be done unless a specific nonfamilial cause for hypercalcemia is established in the index case.

Hypomagnesemia

Hypomagnesemia is present when serum tMg is less than 0.6 mmol/L (1.5 mg/dL). There are no data on the level of iMg during hypomagnesemia. Tissue Mg deficiency, however, may be present despite normal serum Mg concentrations.

Pathophysiology

Decreased tissue accretion of Mg is a major cause of hypomagnesemia (Table 8). The compensatory response at birth to abrupt termination of placental transfer of Mg will be impaired if there is reduced tissue Mg. The severity and prevalence of hypomagnesemia in infants of insulin-dependent diabetic mothers are directly related to the severity of maternal diabetes, which is thought to reflect the severity of maternal Mg deficiency (121). Mg infusion in infants results in greater increases in serum Ca and PTH in those with initially low serum Mg concentrations, and in children with insulin-dependent diabetes, compared to normal control subjects.

Maternal hyperparathyroidism has been associated with neonatal hypomagnesemia (122). Negative Mg balances may occur with hyperparathyroidism that may account for neonatal hypomagnesemia. Alternately, neonatal hypoparathyroidism in this situation may lead to hypomagnesemia from reduced PTH mobilization of bone Mg to extracellular fluid.

In theory, chronic maternal Mg deficiency from any cause may result in decreased tissue Mg accretion for the fetus. Hypomagnesemia occurs more frequently in infants with intrauterine growth retardation compared to infants with appropriate weight-for-gestation.

Intestinal resection, particularly of the jejunum and ileum, the major sites of Mg absorption, with malabsorption and rapid intestinal transit time may lead to Mg deficiency and hypomagnesemia. Mg content in bile, gastric fluid, and pancreatic secretion varies from 0.2 to 5.0 mmol/L (0.5–12 mg/dL). Diarrheal Mg content may be as high as 7.1 mmol/L (17 mg/dL). Thus, chronic losses from diarrhea, intestinal fistula, or enterostomy may be associated with significant Mg loss.

Infants with congenital biliary atresia and neonatal hepatitis may have low serum Mg concentrations. This is thought to be partly related to increase aldosterone-related renal Mg losses.

Hypomagnesemia can occur as primary defect in Mg transport in the intestine or kidney or in conjunction with a variety of inherited hypokalemic salt-losing tubulopathies. Familial hypomagnesemia with secondary hypocalcemia can result from gene mutation in a member of the long transient receptor potential channel protein (TRPM6), a bifunctional protein that combines Ca- and Mg-permeable cation channel properties with protein kinase activity, expressed in intestinal epithelia and kidney tubules (123). Convulsions and persistent tetany, associated with hypomagnesemia and hypocalcemia can manifest in young infants. It exhibits autosomal-recessive inheritance with the gene map locus at 9q22 (124). Familial hypomagnesemia may occur concurrently with acromesomelic dysplasia, Maroteaux type (AMDM; 602875). The AMDM gene has been mapped to the pericentric region of chromosome 9 (9p13–q12).

Renal tubulopathies with Bartter-like syndromes may be subclassified to hypo- or hypercalciuric groups and without or without hypomagnesemia. Multiple mutations of the genes associated with potassium channel, Na-K-2Cl cotransporters have been reported. In some cases, antenatal manifestation of polyhydramnios with high amniotic fluid chloride concentrations have been reported. Postnatal manifestation may occur in the neonate with dehydration, severe salt wasting and hyposthenuria, moderate hypokalemia, metabolic alkalosis, hyperprostaglandinuria, and failure to thrive, and potentially may be life threatening. Hypomagnesemia and hypocalcemia may occur. Hypercalciuria, nephrocalcinosis, and osteopenia can occur in infants.

Mutations in PCLN-1, which encodes the renal tight junction protein paracellin-1 (claudin-16), resulting in impaired reabsorption of Mg and Ca in the thick ascending limb of Henle's loop have been reported (125). These patients typically present with urinary tract infection, polyuria, hematuria, hypomagnesemia, hypercalciuria, nephrocalcinosis, and progressive renal failure. A variant syndrome

with hypocalciuria is thought to present later with short stature, substantially lower serum Mg, and more episodes of tetany.

Secondary defects in renal tubular reabsorption of Mg may result from extracellular fluid expansion caused by excessive glucose, sodium, or fluid intake, or from osmotic diuresis, diuretics such as furosemide, high doses of aminoglycosides such as gentamicin, and ibuprofen overdose.

Elevation of serum phosphate concentrations decreases serum Mg and infants on high-phosphate milk preparations have lowered serum Mg concentrations. Whether these changes are related to decreased Mg absorption or through the shift of Mg from extracellular to intracellular compartments are not well defined. In infants with uremia, serum Mg concentrations may be decreased, possibly in relation to higher blood phosphate concentrations (126). Patients with renal failure, however, become hypermagnesemic at Mg loads that do not affect people with normal renal function.

Exchange blood transfusions using citrate as anticoagulant result in complexing of citrate with Mg, which leads to hypomagnesemia, especially after multiple exchanges (62,127).

Diagnosis

Suspicion of hypomagnesemia must be confirmed by measurement of serum tMg and iMg if available because clinical manifestations are many and varied and may be indistinguishable from other common neonatal diseases. The less mature the infant, the more subtle and varied are the clinical manifestations, and the infants frequently are asymptomatic.

The typical deficit required to produce symptomatic hypomagnesemia is approximately 0.5 to 1.0 mmol (12–24 mg)/kg of body weight. However, critical assessment of Mg deficiency is difficult because more than 99% of total body Mg is found in intracellular fluids or is complexed in the skeleton. It has been proposed that high Mg retention after a Mg load may be a test to reflect Mg deficiency. Infants generally retain large amounts of infused Mg, however, and there are large variations in response; the clinical utility of this test thus appears limited in infancy. Confirmation of hypomagnesemia as the cause of clinical manifestations is its reversibility when serum tMg or iMg has been normalized.

Hypomagnesemia associated with malabsorption, or increased losses from the gut or kidney, also are at risk for concurrent hypocalcemia, hypokalemia, and possible disturbance of acid–base status. The loss of other nutrients such as zinc also may be considerable. Symptoms and signs of hypomagnesemia, which often coexists with hypocalcemia, may be indistinguishable. Thus, simultaneous measurement of serum Ca (total and ionized if available), phosphorus, potassium, sodium, chloride and bicarbonate, urea nitrogen and creatinine, and zinc status may be indicated. Measurement of urine and intestinal fluid content of Mg also may be helpful in diagnosis and management. Additional investigations would depend on the underlying etiology, and the status of other nutrients also may need to be considered.

Typically, hypomagnesemia is associated with decreased circulating PTH concentrations, decreased production of active vitamin D metabolites, in particular $1,25(OH)_2D$, and resistance to PTH and $1,25(OH)_2D$. When hypomagnesemia coexists with hypocalcemia, a trial infusion of 6 mg elemental Mg/kg over one hour with pre- and postinfusion measurement of total and iCa and PTH may be helpful in the diagnosis of the primary defect. An increase in serum PTH after Mg infusion is indicative of hypoparathyroidism and hypocalcemia secondary to Mg deficiency, whereas no change or a decrease in serum PTH supports the diagnosis of hypocalcemia unrelated to Mg deficiency.

Management

Clinical manifestations of symptomatic hypomagnesemia such as seizures should be treated promptly with parenteral Mg. Asymptomatic hypomagnesemia probably also should be corrected, as Mg potentially can alter important cellular functions and may lead secondarily, to hypocalcemia with its attendant complications. Hypocalcemia occurring under this circumstance is corrected only when the Mg disturbance is corrected.

Any neonate with seizures should have blood drawn for diagnostic tests before therapy. The treatment of choice for acute hypomagnesemic seizures is 50% Mg sulfate ($MgSO_4 \cdot 7H_2O$), 0.05 to 0.1 mL/kg (0.1–0.2 mmol/kg or 2.5–5.0 mg/kg elemental Mg) given by slow intravenous infusion over 15 to 20 minutes, or by intramuscular route. The frequency of Mg administration depends on the clinical response and the rate of increase in serum Mg. Repeat doses may be given at 2- to 12-hour intervals. Infants receiving parenteral Mg therapy should receive continuous cardiorespiratory monitoring. Serum Mg concentrations should be measured daily or more frequently as clinically indicated to evaluate efficacy and safety and until values are stable.

Concomitantly, oral Mg supplements can be started if oral fluids are tolerated. Fifty percent Mg sulfate can be given at a dose of 0.2 mL/kg/day. In specific Mg malabsorption, daily oral doses of 1 mL/kg/day may be required. Oral Mg salts are not well absorbed, and large doses may cause diarrhea. The maintenance Mg supplement should be diluted five- to six-fold to allow for more frequent administration, maximizing gut absorption, and minimizing side effects. Some oral preparations of Mg (e.g., Mg l-lactate dihydrate), especially those in a sustained-release form, may have greater bioavailability than other sources of Mg (e.g., Mg oxide, hydroxide, and citrate). Practical experience with the use of Mg salts other than Mg sulfate in infancy is limited, however.

Table 9 Pathophysiology of Neonatal Hypermagnesemia

Increased load
Maternal magnesium sulfate administration
Neonatal magnesium therapy
Parenteral nutrition
Antacid
Enema
Decreased excretion
Prematurity and asphyxia

Source: From Ref. 120.

Potassium and zinc deficiency frequently coexists with Mg-deficient states, especially when there are abnormal gastrointestinal losses or malabsorption. Appropriate replacement therapy is needed. Treatment of underlying disorders (e.g., closure of gastrointestinal fistula) should be pursued actively. Life long Mg therapy is needed if the underlying cause persists, such as genetic defect in Mg transport.

Complications of hypomagnesemia vary with the clinical manifestations, and may be related to therapy and underlying pathophysiology. Prolonged dietary Mg deprivation in human adults leads to personality change, tremor, muscle fasciculations, spontaneous carpopedal spasm, and generalized spasticity as well as hypomagnesemia, hypocalcemia, and hypokalemia. Mg depletion in pregnant rats results in fetal mortality, malformations, hypomagnesemia, decreased skeletal Mg content, hemolytic anemia, hypoproteinemia, and edema.

In infants, acute complications associated with clinical manifestations including seizure, apnea, cyanosis and hypoxia, bradycardia, and hypotension. Possible complications of intravenous infusion include systemic hypotension, and prolongation or even blockade of sinoauricular or atrioventricular conduction. Isolated transient hypomagnesemia even in symptomatic cases has not been associated with long-term sequelae. Long-term outcome of neonatal hypomagnesemia depends on the underlying cause and adequacy of therapy. Neurodevelopmental status has been reported as normal (128) or with severe deficit presumably from suboptimal therapy and recurrent seizures (129).

Hypermagnesemia

Hypermagnesemia is present when serum Mg is more than 1.04 mmol/L (>2.5 mg/dL). There are insufficient data to define hypermagnesemia based on the measurement of serum iMg alone.

Pathophysiology

Hypermagnesemia may result from a combination of excessive Mg load and a relatively low capacity for renal excretion of Mg (Table 9). Neonatal hypermagnesemia most commonly occurs after maternal Mg sulfate administration for preeclampsia. In mothers given Mg sulfate, serum Mg concentrations have been reported from 1.1 to 5.8 mmol/L (2.6–14.0 mg/dL), with umbilical cord serum Mg concentrations from 0.8 to 4.8 mmol/L (2.0–11.5 mg/dL) (130,131); concomitant maternal hypocalcemia also may occur secondary to decreased serum PTH concentrations. Variations in parenteral Mg intake (93,94,132) resulting from high Mg content or high rate of infusion of parenteral nutrition fluids may result in hypermagnesemia, particularly in critically ill neonates with impaired renal function. The use of Mg-containing antacids or enemas can cause hypermagnesemia. Prematurity and perinatal asphyxia may aggravate hypermagnesemia, presumably because of decreased renal Mg excretion.

Diagnosis

Most neonates with hypermagnesemia, particularly preterm infants, are asymptomatic, even at serum Mg concentrations of more than 1.25 mmol/L (3 mg/dL) (93,94,130–132). Clinical signs may not correlate with serum Mg concentrations, although there does appear to be a correlation with the duration of maternal Mg sulfate therapy, possibly representing tissue Mg content. With judicious use of Mg sulfate in the mother, however, signs of Mg intoxication should be uncommon in the infant. In adults with hypermagnesemia, hypotension and urinary retention occur at serum Mg concentrations of 1.67 to 2.5 mmol/L (4.0–6.0 mg/dL); central nervous system depression, hyporeflexia, and electrocardiographic abnormalities (i.e., increased atrioventricular and ventricular conduction time) at 2.5 to 5.0 mmol/L (6.0–12.0 mg/dL); and respiratory depression, coma, and cardiac arrest above 5.0 mmol/L (12.0 mg/dL). Clinical signs of neuromuscular depression with floppiness and lethargy, and respiratory depression are frequent manifestations of severe neonatal hypermagnesemia. Acute hypotonia, apnea, hypotension, and refractory bradycardia mimicking septic shock syndrome has been reported in premature infants accidentally overdosed with Mg in parenteral nutrition (133).

Serum Ca concentrations may be normal, increased, or decreased and should be measured in all infants with suspected hypermagnesemia. Hypermagnesemia might in theory displace bound Ca in the circulation and lead to elevation of serum iCa concentration. Hypermagnesemia may suppress PTH and $1,25(OH)_2D$ production and may result in lower serum Ca concentrations (134,135). Rickets have been reported when maternal Mg therapy is prolonged (e.g., in tocolysis to prevent preterm delivery). It is speculated that excess Mg interferes with normal mineralization of fetal bone.

In newborn infants, a delay in passage of meconium (i.e., meconium plug syndrome) has been thought to be related to neonatal hypermagnesemia. In pregnant and newborn rats and dogs, however, hypermagnesemia does not have an effect on intestinal motility or the consistency of meconium.

Management

In asymptomatic infants with normal renal function, serum Mg generally return to normal within several days after adequate hydration and nutritional support, and elimination of further Mg intake. These infants should be cared for in a facility that can provide cardiorespiratory support in case additional complications develop.

For symptomatic infants, intravenous Ca given in the same dosage as for treatment of hypocalcemia may be useful for acute therapy, because Ca is a direct antagonist of Mg. Loop diuretics (e.g., furosemide) with adequate fluid intake may hasten Mg excretion. Exchange blood transfusion with citrated blood is an effective treatment for severely depressed hypermagnesemic infants. Citrated donor blood is particularly useful because the complexing action of citrate will expedite removal of Mg from the infant. Peritoneal dialysis and hemodialysis may be considered in refractory patients.

In infants, acute complications are associated with clinical manifestations including respiratory depression and hypoxia, bradycardia, and hypotension; and potential complications associated with therapy such as exchange transfusion. Isolated transient hypermagnesemia even in symptomatic cases has not been associated with long-term sequelae.

Hypophosphatemia

An infant can be considered to be hypophosphatemic when serum P concentration is less than 1.3 mmol/L (conversion, 1 mmol/L = 3.1 mg/dL).

Pathophysiology

Hypophosphatemia in infants is frequently iatrogenic and associated with disturbances in the concentration of other electrolytes and minerals (Table 10). Acute hypophosphatemia does not necessarily indicate phosphate depletion and it usually results from redistribution of P from the extracellular to intracellular space and may occur in septicemia, alkalosis, and glucose loading. It may be accentuated during starvation and refeeding. The ''refeeding syndrome'' from overzealous increase in the delivery of nutrients particularly calories, can be associated with multiple electrolyte abnormalities, including hypophosphatemia, hypokalemia, hypomagnesemia and hypocalcemia, and life-threatening complications (83,84). Glucose and P moved from extracellular to intracellular compartments to meet the metabolic needs leads to lowering of serum P, and insulin facilitates these processes. Respiratory alkalosis, liver disease, and hypokalemia also may contribute to ''shift hypophosphatemia.'' In infants receiving parenteral nutrition, hypophosphatemia is exaggerated if there is an associated rapid increase in delivery of carbohydrates (glucose), probably the result of transcellular shift of P in addition to a relative or absolute deficiency in P intake.

Table 10 Pathophysiology of Neonatal Hypophosphatemia

Nutritional
Transcellular shift
Glucose load
Refeeding syndrome
Low phosphate intake
Low or no phosphate parenteral nutrition
Human milk or standard milk formula for preterm infants
Low phosphorus absorption
Vitamin D deficiency
Malabsorption
Elevated renal phosphorus loss
Vitamin D deficiency with secondary hyperparathyroidism
Excessive nutrient intake: sodium, glucose, amino acids
Non-nutritional
Hyperparathyroidism
Hypophosphatemic rickets (X-linked and autosomal dominant)
Fanconi syndrome (idiopathic or secondary to inborn errors of metabolism such as cystinosis and tyrosinosis)
Chronic diuretic therapy

Hypophosphatemia with a ''nutritional'' basis occurs most frequently as a result of inadequate intake of P. For example, in infants receiving low P intake from parenteral nutrition (93,94) and in preterm infants fed unfortified human milk (92,95,136). Prolonged inadequate dietary P intake can result in tissue P deficiency with multiple clinical consequences including skeletal (osteopenia, fractures, and rickets in infants and children or osteomalacia in adults) and extraskeletal (myopathy, cardiomyopathy, and other systems) manifestations.

Hypophosphatemia from decreased intestinal P absorption can be an early manifestation of vitamin D deficiency (137,138). Theoretically, any severe and prolonged malabsorption syndrome may be associated with hypophosphatemia. Excessive nutrient intake, including sodium, glucose, amino acids, particularly if they are delivered intravenously, may exceed the renal reabsorptive capacity or lead to extracellular fluid compartment expansion; both can result in increased renal loss of water and the infused nutrients including P, because renal excretory mechanisms for most nutrients (including P and water) are interdependent and are generally effected by sodium-dependent cotransport systems. Thus, increase in renal excretion of any of these nutrients potentially can increase the renal P excretion (139).

Non-nutritional causes of neonatal hypophosphatemia are much less frequent than nutritional causes. In the latter situations, the primary mechanism for developing hypophosphatemia is probably decreased renal P reabsorption, which may or may not be secondary to elevated PTH.

Diagnosis

Serum P is usually normal at birth. Hypophosphatemia may occur within days of birth or from the onset of the etiologic factor such as the use of phosphate-free parenteral nutrition (Table 11). Acute

Table 11 Diagnostic Workup for Neonatal Hypophosphatemia

History
Family history of mineral metabolism disorders
Gestational age
Dietary history
Chronic diuretic therapy
Physical examination
General examination with focus on growth parameters, muscle tone, and skeletal abnormalities
Associated features, e.g., inborn errors of metabolism
Investigations
Serum phosphorus, total and ionized calcium, magnesium, glucose, creatinine, alkaline phosphatase
Urine phosphorus, glucose, calcium, creatinine
Other tests if above do not yield diagnosis
Serum "intact" or "whole" parathyroid hormone, 25 hydroxyvitamin D, 1,25 dihydroxyvitamin D, amino acid
Urine amino acids
X rays of hands and long bones (rachitic and hyperparathyroid changes)

hypophosphatemia in neonates is usually asymptomatic and may be diagnosed from routine screening because of a family history of disturbances in mineral metabolism. Hypophosphatemia may be an early manifestation of P deficiency. Chronic hypophosphatemia is indicative of P deficiency and may have multiple clinical consequences affecting hematological, immunological, cardiorespiratory, neuromuscular, skeletal, and peripheral and central nervous systems. These include impairment of oxygen release from hemoglobin, neutrophil dysfunction, muscle weakness and respiratory failure, and skeletal demineralization and deformity. Clinical signs such as muscle weakness attributed to hypophosphatemia are usually noted when the serum P concentration is less than 0.7 mmol/L (2.2 mg/dL). However, many of the clinical signs of P deficiency are masked by the underlying illness or by the therapy administered to the infant; for example, bronchopulmonary dysplasia and hypophosphatemia may occur simultaneously, and it may be difficult to distinguish the relative contribution of each to the respiratory failure in an affected infant; multiple blood transfusion may mask the effect of decreased oxygen delivery associated with hypophosphatemia.

More dramatic skeletal manifestations of prolonged and severe hypophosphatemia, such as rickets and osteomalacia, usually are not present until after the neonatal period (2,94,137,138,140–142). Infants with XLH vitamin D-resistant rickets, a dominantly inherited disorder of renal P wasting, usually do not have hypophosphatemia at birth. However, serial monitoring of infants in these families show hypophosphatemia, decreased percentage renal tubular reabsorption of P (100%×P clearance/creatinine clearance), increased bone turnover as indicated by increased serum alkaline phosphatase and increased urinary hydroxyproline, occur in affected infants as early as three weeks after birth (143) and in most affected infants by three months (143,144). Onset of growth delay and radiographic rachitic changes depend on the success of therapy and may occur during infancy. In other situations, such as neonatal primary hyperparathyroidism and Fanconi syndrome, the presenting symptoms and signs may be unrelated to hypophosphatemia.

In most cases of neonatal hypophosphatemia, the diagnosis can be made with a careful review of the history and a few laboratory investigations. Severe hypophosphatemia (serum $P < 0.7$ mmol/L) can develop from nutritional causes and is typically associated with hypercalcemia, almost total absence of P in the urine and urine Ca may be elevated, i.e., renal tubules that are resistant to the PTH effects (92).

Management

Nutritional hypophosphatemia may be treated with P supplementation at 0.5 to 1.5 mmol/kg of elemental P per day. Calcium supplementation (see Hypocalcemia section above) is useful because it may minimize the fall in serum Ca concentration during P treatment, and it would alleviate Ca deficiency, which also occurs frequently in these infants. However, the best means to deliver the appropriate amount of P and Ca is by providing a balanced intake of all nutrients with high P and high Ca milk or parenteral nutrition (2). Standard vitamin D supplementation of 400 IU/day is adequate therapy for vitamin D-deficient rickets in infancy (138). Therapy of non-nutritional hypophosphatemia varies with underlying disorder. For example, early administration of $1,25(OH)_2D$ at 30 to 40 ng/kg/day and phosphate at 40 to 50 mg/kg/day in addition to adequate general nutritional intake can improve mineral metabolism and may be useful to obviate severe growth delay and leg deformities (141–144). Ca supplement also may be needed.

Hyperphosphatemia

An infant can be considered to be hyperphosphatemic when the serum P concentration is above 2.6 mmol/L (conversion, 1 mmol/L = 3.1 mg/dL).

Pathophysiology

Nutritional causes usually occur with the infusion of excessive P content (93) or alternating delivery of Ca and P in parenteral nutrition solution (Table 12) (96). Ingestion of cows' milk-type formulas (36,58) and early introduction of high-P–containing cereals (145) may lead to neonatal hyperphosphatemia. Accidental overdose of oral phosphate supplement can be fatal (59). Vitamin D (146,147) and vitamin A (148,149) toxicity may result in hyperphosphatemia, in addition to hypercalcemia, from increased bone turnover. Non-nutritional causes include an excess phosphate load from the use of P-containing enemas (61,149). Neonates with asphyxia may have increased release of intracellular P to the extracellular compartment in addition to a low renal glomerular filtration. Decreased P excretion and hyperphosphatemia in the

Table 12 Pathophysiology of Neonatal Hyperphosphatemia

Nutritional
High phosphate load
Cow-milk–type formula
Parenteral nutrition
Cereal
Accidental oversupplementation
Hypervitaminosis D and A
Non-nutritional
High phosphate load from phosphate enema
Diminished phosphorus excretion
Perinatal asphyxia
Renal failure
Hypoparathyroidism
Pseudohypoparathyroidism

neonate can result from intrinsic renal failure, such as congenitally dysplastic kidneys, and from absent or nonfunctional PTH, such as hypoparathyroid and pseudohypoparathyroid states.

Diagnosis

History and physical examination consistent with clinical situations (Table 12) are useful for the diagnosis. Clinical features associated with underlying disorder such as Albright's hereditary osteodystrophy may be present. Serum P is usually normal at birth even in neonates with intrinsic renal failure or PTH disorders and increases during the first few days. There is usually concurrent hypocalcemia. Hyperphosphatemia, and hypocalcemia, may occur within hours after a phosphate load (61,149). Hyperphosphatemia may be asymptomatic, or it may be manifested because of its associated hypocalcemic effects (see Hypocalcemia section above).

Management

Removal of excessive P load if possible, for example, eliminating or minimizing the P load with the use of "humanized" formulas with low P content and a "high" Ca/P ratio of about 2:1, and temporarily discontinuing the use of cereals. A brief period of Ca supplementation is necessary if there is associated hypocalcemia.

Nonspecific lowering of serum P for the acute management of hyperphosphatemia may be achieved with normal saline infusion and forced natriuresis as for hypercalcemia [see section on hypercalcemia]. The use of Ca or aluminum salts as chelating agent for intestinal phosphate in the presence of hyperphosphatemia may predispose to metastatic calcification (150) and potential for aluminum toxicity (91) respectively and are not recommended.

For conditions that cause long-term hyperphosphatemia, the treatment is aimed at the underlying cause.

SKELETAL MANIFESTATIONS OF DISTURBED MINERAL HOMEOSTASIS

Pathophysiology

Skeletal manifestations of disturbed mineral metabolism in infants usually present as osteopenia or rachitic changes on standard radiograph (Table 13). True fetal or congenital rickets is rare. It may result from severe maternal nutritional osteomalacia associated with Ca and vitamin D deficiency, maternal hypo- or hyperparathyroidism, or prolonged maternal treatment with Mg sulfate or phosphate-containing enemas.

The most frequent cause of skeletal abnormalities in infancy is nutritional deficiency although it may occur secondary to disorders of metabolism of multiple organs including gut, pancreas, liver, and kidney. In the Western world, rickets and osteopenia presenting during infancy occur most frequently in small preterm infants, and may occur in more than 30% of extremely low-birth-weight (<1 kg) infants. The rate of occurrence depends on the nutrient intake and is associated most frequently with prolonged low-Ca and/or low-P parenteral nutrition and prolonged intake of soy formula or unfortified human milk (2). The primary underlying cause in preterm infants appears to be mineral deficiency, particularly Ca and P, which was demonstrated over two decades ago (151) and confirmed by many investigators (2); vitamin D deficiency is of secondary importance. Preterm infants fed unfortified human milk or limited preterm infant formula from fluid restriction may have low serum concentrations of 25 OHD. The major reason for the low serum 25 OHD in these situations is the increased metabolism of 25 OHD with mineral deficiency. Unfortunately, vitamin D deficiency secondary to inadequate mineral deficiency is still frequently

Table 13 Risk Factors for the Development of Osteopenia and Rickets in Infants

In utero
Severe maternal nutritional osteomalacia (i.e., vitamin D +/− calcium deficiency)
Maternal hypoparathyroidism and hyperparathyroidism
Prolonged maternal magnesium or phosphate treatment
Birth weight < 1 kg
Postnatal
Prolonged organ dysfunction: intestine, kidney, liver, pancreas
Nutritional
Preterm infants
Prolonged low calcium and/or low phosphate total parenteral nutrition
Soy formula or unfortified human milk for small preterm infants
Chronic loop diuretic therapy given to preterm infants
Term infants
Vitamin D deficiency
Calcium deficiency
Macrobiotic diet
Toxin contamination: aluminum
Inherited defects
Renal tubular disorder
Disorders of vitamin D or parathyroid hormone metabolism
Hypo- or hyperphosphatasia

misdiagnosed as the primary cause of osteopenia, fracture, and rickets in preterm infants, and treated with more vitamin D supplement without improving the mineral and general nutritional support.

Chronic diuretic therapy, commonly used in infants with bronchopulmonary dysplasia may aggravate the calcium deficiency. Contamination of nutrients with toxins such as aluminum is an added risk factor (91). The extent, however, to which each specific risk factor responsible for the development of osteopenia, fractures, and rickets is difficult to define in critically ill infants receiving multiple therapies and suboptimal nutritional support (2). Isolated nutritional deficiency of copper and ascorbic acid has been reported in preterm infants with clinical and radiographic manifestations similar to rickets.

In infants born at term, insufficient endogenous production or exogenous supply of vitamin D is important in the cause of rickets and osteopenia. In one report, almost all children with vitamin D deficiency have ethnocultural risk factors and 80% of the mothers are also vitamin D deficient (152). However, calcium deficiency also is important in older infants and young children (153). Clinical risk factors thus include prolonged exclusive human-milk feeding, limited sunshine exposure, macrobiotic diet, and prolonged total parenteral nutrition with concomitant gastrointestinal or hepatic dysfunction.

Acquired and heritable forms of rickets that develop despite adequate availability of vitamin D usually are associated with renal tubular disorders and metabolic defects in vitamin D and PTH metabolism. Both hypo- and hyperphosphatasia are autosomal-recessive disorders associated with disturbed bone resorption and formation. These causes of rickets are rare, but their skeletal manifestations may present during infancy.

Diagnosis

A history of significant nutritional defect in the mother, either from self-selected dietary restriction or cultural habits, e.g., extensive covering of the body with lack of sunlight exposure, or family history of metabolic disorders and disturbed bone mineral metabolism, should raise the awareness of the potential for nutritional and skeletal problems in both the mother and the infant.

Infants with congenital rickets may be asymptomatic at birth leading to a delay in diagnosis unless investigations are performed as part of the work up for disturbances in maternal mineral metabolism. Most postnatal cases of rickets and osteopenia are diagnosed incidentally during the radiographic investigation of skeletal complications such as fractures, or nonskeletal problems such as respiratory illness. Radiographic features such as generalized bone demineralization and widening, cupping, and fraying of distal metaphyses confirm the presence of osteopenia and rickets. Traditionally, the assessment of osteopenia and rickets is based on radiographic changes (153–156). The introduction of dual energy X-ray absorptiometry (DXA) allows a more accurate quantification of the degree of bone mineralization (157–160), although its role in the diagnosis of bone disorders remains to be defined.

Classic clinical features of rickets such as severe skeletal deformities, including kyphoscoliosis and bowing of the legs, may not be present if the diagnosis is made early in infancy, before significant growth and weight bearing have occurred. This is particularly true for the preterm infant whose skeletal problem typically is diagnosed between two and six months postnatally (161). With the current practice of early discharge of preterm infants from Neonatal Units, it is possible that some nutritional rickets could be diagnosed after hospital discharge, and if there are associated fractures, it may be misdiagnosed as child abuse, as is the case with fractures from other medical illnesses. Clinical hypotonia is probably due to a decrease in intracellular phosphate pool of skeletal muscle.

Serial biochemical changes (92,162,163) commonly include persistently low serum inorganic phosphate, elevated serum alkaline phosphatase activity more than five times the normal adult upper limit, and other bone turnover markers in serum and urine also can be elevated. Serum Ca is usually normal except in late severe nutritional vitamin D deficiency rickets. Vitamin D deficiency as indicated by low or undetectable serum 25 OHD is possible; however, it is more likely in preterm infants to be secondary to mineral deficiency. There may be elevated serum $1,25(OH)_2D$ and IPTH. The elevated PTH and $1,25(OH)_2D$ still may be relatively insufficient to maintain Ca and P homeostasis if the Ca and P intake remain low. Urine changes may reflect increased serum IPTH with increased urine P excretion and Ca conservation. However, in chronic P deficiency, urine findings may reflect changes of P deficiency-related PTH resistance, in which case, urine P would be minimal while there is calciuria. Measurement of specific trace mineral status may be useful if deficiency is suspected (164,165). Additional investigations are needed if inherited renal tubular disorders or disorders of vitamin D and PTH metabolism are suspected.

Treatment and Prevention

Rickets and fractures from nutritional deficiencies respond well to adequate nutrient intake. The best treatment for nutritional osteopenia, fractures, and rickets is prevention. For preterm infants, the use of high Ca- and high P-parenteral nutrition, until establishment of enteral feeding with human milk containing commercial fortifier, or formulas designed specifically for preterm infants is appropriate (2,166,167). Human milk alone is inadequate in a number of nutrients including protein, sodium, Ca, P, and possibly other nutrients for the needs of the

very small preterm infant. All human-milk–fed small preterm infants, particularly those with birth weights less than 1500 g should receive commercial fortifier containing multiple nutrients in human milk during their hospital stay and probably posthospital discharge.

The use of Ca and P supplementation alone is inappropriate for the treatment or prevention of osteopenia with or without fractures or rickets, because bone growth requires protein and multiple other nutrients for matrix formation and mineralization. In addition, further large increases in Ca and P intake beyond the current recommended intake is probably not advisable, because of the risks of bezoar and even intestinal obstruction with excessive oral intake, and hyperphosphatemia with intravenous intake. In preterm infants, most fractures have significant callus formation at diagnosis and only require splinting support; short-term analgesia is needed if the fracture is recent and without callus formation.

For healthy term infants human milk or standard infant formulas should provide adequate amounts of Ca and P. Other appropriate nutrients also should be introduced during latter half of infancy to maintain adequate nutritional status for all infants.

In preterm infants receiving infant formulas with lower Ca and P content (compared to the preterm infant formulas currently available in the United States), normal vitamin D status as indicated by serum 25 OHD concentrations has been reported in infants who received daily supplement of 400 to 2000 IU vitamin D. However, enterally fed preterm infants given adequate volumes of high Ca-, high P-, and vitamin D-fortified preterm infant formula, or human milk fortified with commercial human-milk fortifiers, a daily total intake of 400 IU vitamin D should be adequate and additional vitamin D supplementation may be excessive.

Prophylactic vitamin D supplementation has been recommended for all breastfed term infants (168) because adequate endogenous production of vitamin D cannot be assured with infants living in varied geographic regions, and in families with varied ethnocultural practices. A daily intake of 200 to 400 IU vitamin D should be sufficient to maintain normal vitamin D status in term infants.

For infants who require parenteral nutrition as the major source of nutritional support, 25 to 40 IU vitamin D/dL of parenteral nutrition solution with a maximum total daily intake of 400 IU vitamin D is sufficient to maintain vitamin D status regardless of the gestational age of the infant (169).

For infants with established nutritional vitamin D deficiency, a daily supplementation of 400 IU vitamin D (138) in addition to adequate overall nutritional support is appropriate. Provision of pharmacologic doses of vitamin D to the infants is associated with hypercalcemia, nephrocalcinosis, and hypertension.

Specific therapies are required for inherited renal tubular disorders and for disorders of vitamin D and PTH metabolism, and usually include one or more of the following: Ca, phosphate, and $1,25(OH)_2D$.

Monitoring and Follow-up

The goal is for the affected infants to grow normally without residual defect. Regular clinical assessment and growth measurements are essential. On follow-up during infancy (138,161,162), there was no major residual physical deformity with the use of 400 IU vitamin D for the treatment of rickets for infants born at term or preterm. Skeletal maturation as assessed by ossification centers of the wrists for preterm infants, is similar to term infants at one year of age (161). However, long-term linear growth in the extremely low-birth-weight infants may remain delayed suggesting that bone mineral status in the smallest preterm infants still may be suboptimal (170) despite the relatively uncommon occurrence of radiographic rickets and fractures on follow-up. Current data on DXA measured TB BMC data in the small preterm infant are difficult to interpret because few studies have used the validated techniques (157–160) and inconsistencies in the techniques among different studies (171–173).

Biochemical monitoring of nutritional rickets includes measurement of serum Ca, P, and alkaline phosphatase and avoiding hypercalciuria (< 0.15 mmol and 6 mg/kg/day especially in human-milk–fed preterm infants) at q one- to two-week intervals until stable then at one- to two-month intervals. Bone turnover markers, IPTH, vitamin D metabolites, and any other abnormal biochemical parameters should be monitored at one- to two-month intervals. All biochemical monitoring should be continued until standard radiographs show completion of healing and remodeling of skeletal defects. Radiographs of the wrists and the fracture site/s should be taken at two- to four-month intervals. Standardization of the DXA measurement in infants with serial measurements at two- to three-month intervals should provide an added means to better understand bone mineral status in the developing skeleton (174–177). Screening for other affected family members and molecular studies may be warranted in heritable conditions. Other specific monitoring would depend on the underlying cause of the skeletal defect.

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23

Rickets and Osteoporosis

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RICKETS

Classification and Nutritional Rickets

Rickets is a syndrome caused by a defective mineralization of cartilage in the growth plate (1). Osteomalacia is caused by defective mineralization in bone, and can occur in both children and adults (Table 1) (2). Rickets can be secondary to different etiologies. Among them, nutritional, genetic, drug induced, and prematurity are the most common. John Pettifor (3) classified rickets in ''calciopenic'' (secondary to alterations of vitamin D metabolism or dietary calcium deficiency), ''phosphopenic'' (caused by dietary phosphate deficiency, impaired intestinal absorption of phosphate, increased renal phosphate loss, etc.), and ''inhibition of mineralization'' (e.g., hereditary hypophosphatasia, aluminum and fluoride toxicity, and first-generation bisphosphonates).

Nutritional rickets, once thought defeated, is reappearing (4), and it remains a major health problem in many developing and developed countries. Rickets affects dark-skinned infants receiving human milk without supplemental vitamin D (5). Infants feed exclusively with mother's milk can have nutritional rickets because of the low content of vitamin D in breast milk. Vitamin D content of breast milk varies from 4 to 100 IU/L (6). Reserves of vitamin D in the neonate are dependent on mother's vitamin D status. Very low or no sun exposure causes rickets in infants, particularly in those with dark skin, because vitamin D is produced by the skin after exposure to ultra violet (UV) light. Concentration of vitamin D is lower in breast milk of African-American mothers (7), but it has been shown that vitamin D levels in breast-fed infants are more influenced by sun exposure than by vitamin D concentration in breast milk (8). Thus, the insufficient exposure of the skin to sunlight in people living in latitudes away from the equator may explain the increased prevalence of rickets among northern native populations (9), which prompted the Canadian Pediatric Society to issue their specific guidelines for increased vitamin D intake (10). These recommendations differ from those of the Section on Breast feeding and Committee on Nutrition of the American Academy of Pediatrics that recommend 200 IU of vitamin D per day throughout childhood and adolescence, including those infants fed breast milk (11).

Congenital rickets can be a consequence of maternal hypovitaminosis D (12). During pregnancy, there is normally an increase of calcitriol circulating levels (13); therefore, vitamin D intake should be increased, usually doubled, during the second half of pregnancy. Rickets can be part of the prematurity syndrome (Fig. 1) (Vol. 2; Chap. 22). Chronic anticonvulsant therapy (particularly with combination medications, i.e., Phenobarbital and Phenytoin) may cause rickets regardless of an appropriate vitamin D intake (14). The main mechanism is related to generation of inactive metabolites by induction of hepatic cytochrome P-450 hydroxylation (15). 25-Hydroxy vitamin D levels were reported to be low in children on chronic anticonvulsant therapy (16) and in those with rickets the response to 25-hydroxycholecalciferol was prompt and more effective than with vitamin D_2 (17). The number of fractures was found to be associated with the use of anticonvulsants in patients with cerebral palsy (CP) in an institution (18). A different study did not find a relationship between serum 25 (OH) vitamin D levels and use of anticonvulsants in patients with CP (19). Furthermore, calcitriol levels in plasma are reportedly normal in patients taking medication for seizures (20,21). It is not clear what dose of vitamin D is required to prevent rickets in children taking medications for seizures, or even if any supplementation is needed at all (21). Possibly 800 to 1000 IU/day is sufficient to keep 25(OH) vitamin D_3 levels above 30 ng/mL.

Table 1 Causes of Metabolic Bone Diseases in Pediatric Patients

Primary
- Osteogenesis imperfecta
- Syndromes resembling osteogenesis imperfecta
- Idiopathic juvenile osteoporosis
- Rickets
  - Nutritional
  - Prematurity
  - Genetic
  - Neoplastic
  - Hypophosphatemic
- Hypophosphatasia
- Hyperphosphatasia
- Parathyroid disorders
  - Hyperparathyroidism
  - Hypoparathyroidism
  - Pseudohypoparathyroidism
  - Pseudo-pseudohypoparathyroidism
- Primary hypomagnesemia
- Ehrles–Danlos syndrome
- Marfan syndrome
- Fibrous dysplasia of bone
- Osteopetrosis
- Other sclerosing bone diseases
- Disorders of the calcium-sensing receptor

Secondary
- Endocrine causes
  - Type I diabetes mellitus
  - Cushing syndrome
  - Anorexia nervosa
  - Turner syndrome
  - Hypogonadism
  - Hyperprolactinemia
- Gastroenterologic causes
  - Malabsorption
  - Chronic liver disease
  - Total parenteral nutrition
  - Cystic fibrosis
- Inborn errors of metabolism
  - Galactosemia
  - Lysinuric protein intolerance
  - Glycogen storage diseases
  - Gaucher disease
  - Phenylketonuria
- Neurological causes
  - Cerebral palsy
  - Spina bifida
  - Muscular dystrophy
- Renal causes
  - Renal osteodystrophy
  - Fanconi syndrome
  - Renal tubular acidosis
- Drugs/toxics
  - Corticosteroids
  - Anticonvulsants
  - Aluminum-containing antacids
  - Rifampicin
  - Cadmium, lead
  - Heparin
  - Methotrexate, cyclosporine
  - Medroxyprogesterone acetate, GnRH agonists
- Other causes
  - Prolonged immobilization
  - Transplant
  - Malignancy
  - Tumor-induced osteomalacia
  - Epidermal nevus syndrome
  - Neurofibromatosis, neurinoma, paraganglioma1

Source: From Ref. 1.

Sunlight, Vitamin D, and Calcium for Bone health

There is no recommended daily allowance (RDA) for vitamin D (22). Recommendations for vitamin D intake are actually referred to as ''adequate intake'' (AI) (23), because there is no strong data to support an RDA (22). Vitamin D intake should be as much as needed to keep 25(OH) vitamin D_3 levels above 30 ng/mL. Most humans depend on sun exposure to fulfill their requirements for Vitamin D. Several factors influence the amount of vitamin D produced in the skin, including the latitude of the country, and factors as the season of the year, atmospheric pollution, sunscreen use, skin pigmentation, and age (Fig. 2).

The jejunum is the primary absorption site for vitamin D. Absorption rate ranges from 55% to 99% after an oral dose (24). The two main sources of vitamin D for humans are vitamin D_3 (cholecalciferol), produced by the skin after UV radiation (290–320 nm), and vitamin D_2, (ergosterol), provided by vegetal sources. Both have identical biological actions, and both are concentrated in the liver, where they undergo hydroxylation in position 25 by a microsomal and mitochondrial enzyme, generating 25 (OH) vitamin D_3 (calcidiol) (25). The nutritional vitamin D status of a patient is reflected by the serum levels of 25(OH) vitamin D_3. From there, 25 (OH) vitamin D_3 is hydroxylated again in the kidneys. 1,25 $(OH)_2$ vitamin D_3 (calcitriol) is yielded by hydroxylation in position 1 in the mitochondria. Calcitriol is at least 10 times more potent than 25 (OH) vitamin D_3. Calcitriol is considered to be the most active form of the vitamin, but at least 30 other metabolites are generated by the kidney (26). The biological significance of these metabolites is not clear. Particularly, 24,25 $(OH)_2$ vitamin D_3 probably has a biological role, perhaps as a way to regulate the production of 1,25 $(OH)_2$ vitamin D_3. The physiopathology of rickets is not completely understood, as the specific role of each of the many vitamin D metabolites is not clear. Interestingly, calcitriol levels may be normal in patients with rickets, suggesting that it is not the only active form of the vitamin (27,28). It has also been suggested that calcidiol might be an agonist for calcitriol receptor (29).

For calcium, the recommended intake is listed as an AI, which is a recommended average intake level based on observed or experimentally determined levels (Table 2) (30). There is overwhelming evidence that calcium requirements vary in relation to multiple factors, including protein intake and dietary source (31). Numerous nutrition policy statements recommend the consumption of calcium largely from dairy products, although there is scant evidence that increasing milk intake or other dairy products promote bone mineralization (32). Milk supplementation at school in certain populations showed positive effects on periosteal and endosteal apposition of cortical bone (33). It should be kept in mind that there is an increased consumption of carbonated beverages and sodas and other sweetened drinks with or without

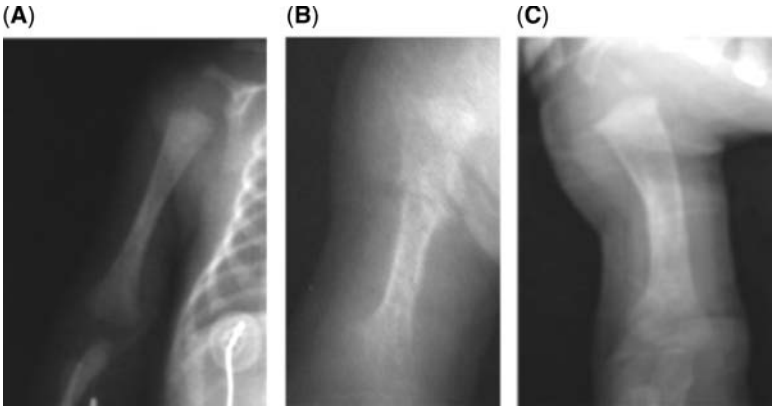

Figure 1 Evolution of rickets of prematurity: (**A**) at birth, no signs of rickets, (**B**) unequivocal signs of rickets at four months of age, (**C**) recovery after one month of vitamin D treatment.

nutritional benefits; these drinks have displaced milk intake (34). However, there are other modifiable factors that influence bone health, primarily physical activity (35), although there may be a synergistic effect on bone density of calcium intake and vigorous exercise (36).

Clinical Features and Treatment

Clinical features of patients with rickets are summarized in Table 3, and typical radiographic findings of florid rickets characterized by widening, cupping, and frying of the metaphyses with uncalcified and

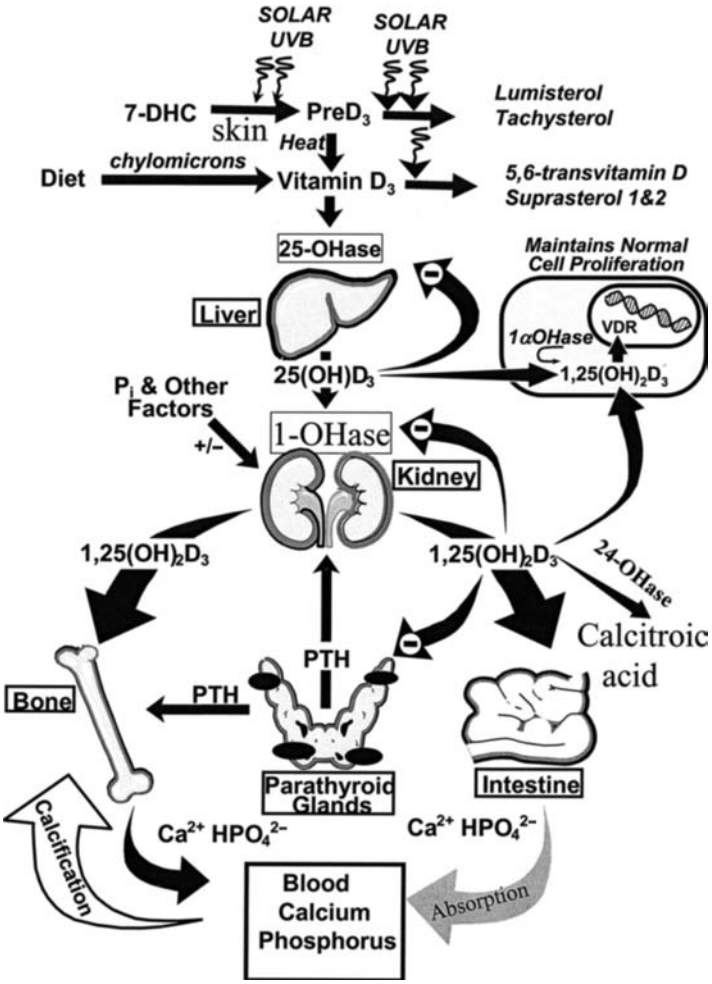

Figure 2 Schematic diagram of vitamin D metabolism and regulation for calcium homeostasis and cellular growth. *Abbreviations:* VDR, vitamin D receptor; PTH, parathyroid hormone; DHC. *Source:* From Ref. 24.

Table 2 Adequate Intake for Calcium for Children and Adults in the United States

Age	Male/female	Pregnancy/lact
0–6 mo	210	N/A
7–12 mo	270	N/A
1–3 yr	500	N/A
4–8 yr	800	N/A
9–13 yr	1300	N/A
14–18 yr	1300	1300
19–50 yr	1000	1000
51+ yr	1200	N/A

Abbreviation: N/A, not applicable.
Source: From Ref. 30.

poorly mineralized epyphysis and generalized osteopenia are depicted in Figure 3. The biochemical abnormalities of children with nutritional rickets as well as those with other types of the disease are shown in Table 4 .

There are several different treatment modalities for rickets. It has been estimated that a young adult exposed to sunlight in the whole body, at a dose causing minimal erythema, generates an amount of vitamin D equivalent to 10,000 IU of vitamin D_3 (37). Rickets can be treated orally with 5000 to 15,000 IU/day of vitamin D for four weeks (38). In cases when compliance cannot be assured, 100,000 to 500,000 IU may be given PO or IM every six months, although 600,000 IU in a single dose may be sufficient (Stoss therapy) (39). It should be kept in mind that preparations of ergocalciferol in propylene glycol (usual vitamin D preparations) should not be used for the treatment of rickets with high dosages because propylene intoxication may ensue. Ergocalciferol capsules containing 50,000 IU may be used by soaking them in water to soften them, and given with blended food to infants and children who cannot swallow the intact capsule. The vitamin D oil preparation for IM use may also be given orally. Calcium intake must be optimized at the same time. Stoss treatment rapidly induces vitamin D and Ca homeostasis, avoiding the risk of hypocalcemia and tetany that may result from the effects of small dosages of vitamin D. The latter may produce precipitation of calcium and phosphate into the osteoid and a sudden increase of extracellular fluid phosphate with a transient drop in calcium levels. Calcium, phosphorus, and parathyroid hormone (PTH) should normalize in one to three weeks. Radiological lesions and clinical symptoms improve rapidly with treatment. Alkaline phosphatase (ALP) may remain elevated for several months after radiological resolution of rickets. A second treatment course may be required in patients with intestinal malabsorption or liver disease who develop rickets.

Table 3 Clinical and Radiological Features of Rickets

Infants: apnea, seizures, tetany
Hypotonia
Delayed motor milestones
Enlargement of wrists and knees
Progressive bowing of long bones
Rachitic rosary
Harrison's sulcus
"Violin case" deformity of the chest
Late closure of anterior fontanelle
Parietal and frontal bossing
Craniotabes
Craniosynostosis
Delayed teeth eruption
Enamel hypoplasia
Low bone mineral density
Proximal myopathy with *N* deep tendon reflexes
Infections (impaired fagocytosis and neutrophil motility)
X rays
Widening of ephyphisial plates
Cupping
Deformities in shaft of long bones
X rays of the costochondral junction are not useful in the diagnosis of rickets
Healing: broadened bands of increased density

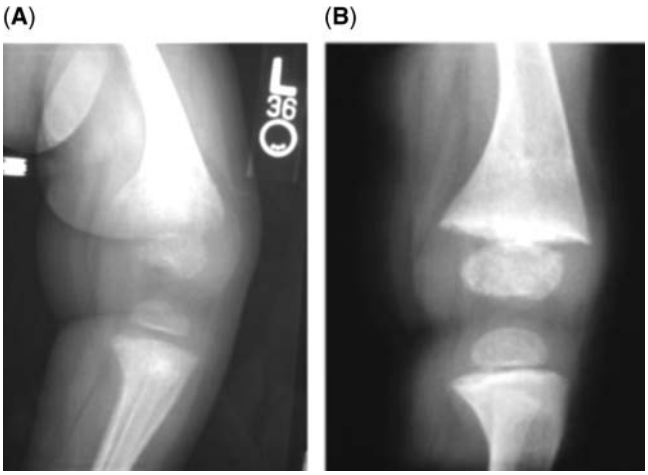

Figure 3 Knee radiograph showing the typical signs of rickets before treatment (**A**) and after one month of treatment (**B**).

VITAMIN D PSEUDODEFICIENCY

Prader et al. (40) described this form of vitamin D–resistant rickets for first time in 1961. Vitamin D pseudodeficiency (PDDR) is a genetic type of rickets caused by a defect in the conversion of calcidiol [25 (OH) vitamin D_3] to calcitriol by the 1α-hydroxylase in the kidney (41,42). It follows that patients have low levels of 1,25 $(OH)_2$ vitamin D in serum, clinical signs of rickets, and they respond to treatment with calcitriol. An injection of PTH extract will fail to increase the serum levels of 1,25 $(OH)_2$ vitamin D in these patients (43). PDDR is inherited in an autosomal-recessive fashion (44). The genetic defect causing PDDR has been mapped to chromosome 12q13.3 (45). Affected patients usually have no clinical manifestations at birth, but during the first two years of

Table 4 Biochemical Characteristics and Treatment of Different Types of Rickets

	Nutritional	PDDR	HVDRR	HHR
Ca	L or N	L	L	N
P	L or N	H, N, or L	L	L
Alkaline phosphatase	H	H	H	H
25 vitamin D	L	H	N	N
1,25 vitamin D	L, N, or H[a]	L	H	N
PTH	H	H	H	N
BMD	L	L	L	N or H
Treatment	Ca, vitamin D, and/or P	Calcitriol	High-dose Ca	P and calcitriol

[a]1,25 $(OH)_2$ vitamin D_3 levels are elevated in hypophosphatemia secondary to low phosphate intake due to 1α-hydroxylase stimulation.
Abbreviations: PDDR, vitamin D pseudodeficiency; HVDRR, hereditary 1,25-dihydroxyvitamin D_3–resistant rickets, HHR, hypophosphatemic rickets; BMD, bone mineral density, PTH, parathyroid hormone.

life, proximal muscle weakness, poor gross motor development, irritability, and growth retardation become evident (46). Physical and radiological signs are not different from those of nutritional rickets (Fig. 4). Serum calcium, phosphate, and 1,25 $(OH)_2$ vitamin D_3 levels are low, and serum ALP activity and PTH are above normal, while 25 (OH) vitamin D_3 levels are usually within normal limits (47–49). Levels of 1,25 $(OH)_2$ vitamin D_3 may be inadequately low for the levels of calcium, phosphorus, and PTH (50). This disease shows some phenotypic variation, which is not explained by variations in the 1a-hydroxylase activity, because it was found to be completely abolished in these patients (51). Calcitriol treatment (52) rapidly restores biochemical parameters to normality, and dramatically improves signs and symptoms of rickets.

Another potential defect that may lead to severe genetic-type rickets was described by Casella et al. in two siblings aged two and seven years who developed the disease despite adequate vitamin D intake. They presented lower levels of serum calcium and phosphate and elevated ALP and low levels of 25 (OH) vitamin D_3 concentrations. Pharmacologic doses of ergocalciferol were required for healing and maintenance of the biochemical variables at normal levels. They had normal vitamin D absorption and they were considered to have a genetic defect of the 25 hydroxylation step in the vitamin D activation (53).

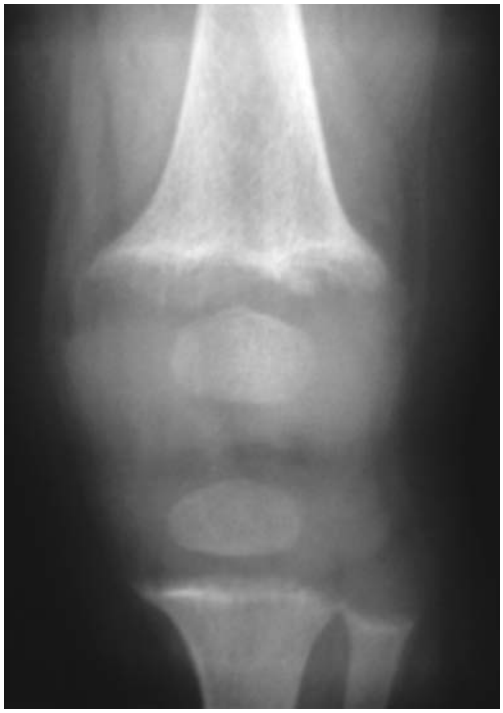

Figure 4 Knee X ray in a case of vitamin D pseudodeficiency.

HEREDITARY 1,25-DIHYDROXYVITAMIN D–RESISTANT RICKETS

Hereditary 1,25-dihydroxyvitamin D–resistant rickets (HVDRR) is also known as vitamin D–dependent rickets type II. It was first described in the literature in 1978 (54,55). The defect in target cells of these patients is heterogeneous and commonly appears to be a mutation in the gene encoding the vitamin D receptor (VDR) (56), causing an end-organ resistance to the vitamin (57). The VDR is a member of the thyroid-retinoid group of receptors (58), and it is present in several tissues, including fibroblasts. The VDR gene comprises eight coding exons and at least three noncoding exons in 75 kbs of DNA (59). The highly conserved DNA-binding domain (DBD) of the receptor is encoded by exons 2 and 3, and the ligand-binding domain (LBD) is encoded by exons 6 to 9 (60). Inheritance is autosomal recessive. The clinical picture is of rickets with severe hypocalcemia and alopecia, and serum levels of 1,25 $(OH)_2$ D_3 are elevated. HVDRR can be lethal in the perinatal period (61,62). Two different phenotypes have been described. In the ''receptor-negative'' (or ''ligand-binding–negative'') phenotype, 3H-1,25$(OH)_2D_3$ binding is negative in cultured fibroblasts (63), and mutations are localized in the LBD region of the gene. In the ''receptor-positive'' (or ''ligand-binding–positive'') phenotype, mutations are localized in the DBD region of the gene.

3H-1,25$(OH)_2D_3$ binding is normal in cells, but VDR binding to DNA is defective (64,65). At least 10 different mutations causing this phenotype are known (46). In mice with HVDRR, maternal intestinal Ca absorption, dependent on VDR, can be replaced by passive Ca absorption entrained by a higher Ca intake to ensure normal fetal mineralization (66).

There is a variant of this disease that occurs with typical clinical and biochemical features of HVDRR but without alopecia (61,67). Different mutations in the LBD of the VDR have been described to be associated with this variant (67,68). Although the presence of alopecia has been regarded as a sign of severity (69), it has also been suggested that its absence is not a predictive sign of a lesser resistance and of responsiveness to vitamin D treatment (61). It appears that patients without alopecia respond better to treatment with vitamin D metabolites (69).

Some patients respond to high doses of 1 (OH) vitamin D or to 1,25 $(OH)_2$ vitamin D_3. Patients with mutations in LBD are more likely to respond to high-vitamin D treatment than those with mutations in the DBD region of the receptor (70,71). Very large doses of IV calcium (400–1400 $mg/m^2/day$) are necessary to treat this condition in patients that do not respond to vitamin D (72). Progression to a very high calcium-intake diet (3.5–9 $g/m^2/day$) can be achieved in some cases, although it implies the risk for hypercalciuria and nephrolithiasis and cardiac arrhythmias (73).

HYPOPHOSPHATEMIC SYNDROMES

Hypophosphatemic Rickets

Albright et al. (74) were the first to publish the concept of hormonal resistance when they described rickets resistant to vitamin D therapy in 1937. This was probably a case of X-linked hypophosphatemic rickets (XLHR). Several different conditions may lead to hypophosphatemia in children (Table 5). XLHR is a dominant disorder, and the most commonly used example of an X-linked disease. As such, there is no male-to-male transmission. Patients with familial hypophosphatemic rickets are prone to have gum abscesses (75), and normal or high bone mineral density (BMD) (Fig. 5). Results of serum phosphate have to be interpreted under the light of calcium and PTH results. High PTH in the presence of low phosphate suggests a primary parathyroid problem, whereas hypophosphatemia with normal PTH suggests a primary phosphate-wasting condition.

Table 5 Hypophosphatemic Syndromes in Pediatrics

Familial
X-linked hypophosphatemia
Autosomal-dominant hypophosphatemic rickets
Hereditary hypophosphatemic rickets with hypercalciuria
Nutritional rickets
PDDR
HVDRR
Hyperparathyroidism
Tumor-induced osteomalacia
Epidermal nevus syndrome
McCune Albright syndrome
Neurofibromatosis, neurinoma, paraganglioma
Ifosfamide treatment

Abbreviations: PDDR, vitamin D pseudodeficiency; HVDRR, hereditary 1,25-dihydroxyvitamin D_3–resistant rickets.

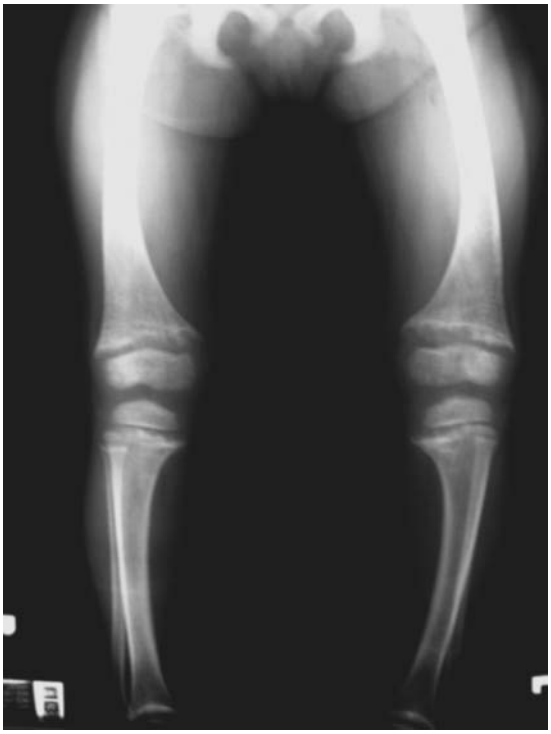

Figure 5 Lower limb X ray of a patient with hypophosphatemic rickets.

Mutations in the PHEX gene are the cause of XLHR. PHEX codifies for a protease—an enzyme that catalyzes the hydrolysis of a protein. But such protein has not yet been identified. Because this protein causes phosphate wasting in patients with XLHR (that have a mutation preventing degradation of the protein), it has been named ''phosphatonin.'' It has also been shown that somatic and germline mosaicism for a mutation of the PHEX gene can lead to genetic transmission of XLHR that mimics an autosomal-dominant trait (76).

Levels of 1,25$(OH)_2$ vitamin D are normal in these patients, which is actually an abnormal response to hypophosphatemia, when levels of 1,25$(OH)_2$ vitamin D should increase. This suggests a defect in 25-hydroxyvitamin D-1α-hydroxylase in kidney. Furthermore, levels of 24,25$(OH)_2$ vitamin D are increased in mice with XLHR (called the ''Hyp mouse''), whereas they are not changed in mice receiving hypophosphatemic diets. Therefore, patients with XLHR are producing more 24,25 $(OH)_2$ vitamin D at expense of 1,25$(OH)_2$ vitamin D. A possible explanation for this is that Hyp mice need lower serum calcium

concentration to initiate increased synthesis of $1,25(OH)_2$ vitamin D (77).

Fibroblast growth factor-23 (FGF-23) has been implicated in the renal phosphate wasting in tumor-induced osteomalacia (TIO) (78) and autosomal-dominant hypophosphatemic rickets (ADHR) (79). Mutations in the gene coding for the main renal sodium-phosphate cotransporter (NPT2a) have been reported in some patients with familial renal calcium stones and hypophosphatemia due to a decrease in renal phosphate reabsorption (80). These patients have hypercalciuria and elevated levels of 1,25-dihydroxyvitamin D_3.

ADHR is a genetic condition first described by Bianchine et al. in 1971 (81). This condition is different from XLHR in that male-to-male transmission is possible. Patients present with hypophosphatemia, normal calcium levels, and inappropriately normal $1,25(OH)_2$ vitamin D serum levels. Age of onset is variable, as well as penetrance. The mutation for ADHR has actually been mapped to locus 12p13 (82), and later a mutation was identified in the gene that codifies for a member of the fibroblast growth factor family, FGF-23 (79). Administration of recombinant FGF-23 decreased serum phosphate in mice within 12 hours, and injection of cells overexpressing FGF-23 to mice caused osteomalacia (83). ADHR mutations may protect FGF-23 from proteolysis (84). It has been suggested that FGF-23 may be a substrate of PHEX, but there is no conclusive evidence so far. Furthermore, there is evidence against cleavage of intact FGF-23 (25–251), as well as of N-terminal [FGF-23 (25–179)] and C-terminal [FGF-23 (180–251)] fragments by the endopeptidase PHEX (85). No functional FGF-23 mutation was detected in a small group of patients with hypophosphatemic rickets (86). Interestingly, patients with fibrous dysplasia of bone have increased levels of FGF-23 when compared with normal age-matched controls. These levels are significantly higher in fibrous dysplasia patients with renal phosphate wasting compared with those without it, and correlates with disease burden (87). There is an unanswered question about the role of NPT2a in mediating (directly or indirectly) the actions of FGF-23 (88).

Hereditary hypophosphatemic rickets with hypercalciuria (HHRH) is an autosomal-recessive form of hypophosphatemia due to isolated renal phosphate wasting that can be distinguished from other forms of hypophosphatemia by increased serum levels of 1,25-dihydroxyvitamin D resulting in hypercalciuria and increased intestinal calcium absorption. It was first described in a large consanguineous Bedouin kindred. This condition was mapped in the end of the long arm of chromosome 9 (89). The candidate region contained a sodium-phosphate cotransporter gene, SLC34A3, which encodes for the renal sodium-phosphate cotransporter NaP(i)-IIc, which has been shown to be expressed in proximal tubulus cells. Loss of function of the SLC34A3 protein presumably results in a primary renal tubular defect and is compatible with the HHRH phenotype. The phosphaturic factor FGF-23, which is increased in XLHR and carries activating mutations in ADHR, is at normal or low-normal serum levels in the patients with HHRH, further supporting a primary renal defect (89). A different group found that disease mapped to a 1.6 Mbp region on chromosome 9q34, which contains the SLC34A3 gene. This mutation is predicted to truncate the NaP(i)-IIc protein in the first membrane-spanning domain and thus likely results in a complete loss of function of this protein in individuals homozygous for c.228delC. These findings suggest that NaP(i)-IIc has a key role in the regulation of phosphate homeostasis (90).

Laboratory includes calcium, phosphorus, magnesium, ALP, creatinine, PTH, 25(OH) vitamin D, and $1,25\ (OH)_2$ vitamin D. Serum phosphate levels must always be measured in the fasting state. It should also be kept in mind that the serum phosphate levels are higher in infants and a bid lower in children, both being above the usual adult normal levels reported by laboratories. Calcium and phosphorus/creatinine ratio in urine, and cAMP in serum and urine if available, can help in the diagnosis, as well as calculation of tubular resorption of phosphate (normal value is 85–95% for children). Other phosphate indices can be calculated:

$$\text{Phosphate and creatinine clearance ratio (PCCR)} = \frac{(\text{Urine phosphate} \times \text{Plasma cretinine})}{(\text{Plasma phosphateurine} = \text{Creatinine})}$$

$$\text{Phosphate excretion index (PEI)} = \text{PCCR} + 0.07 - (0.17 \times \text{Plasma phosphate})$$

Normal value: -0.20 to +0.04 for children

Genetic testing may be done, although it is not available commercially. Diagnosis is based on clinical, radiological, and biochemical features.

Treatment of hypophosphatemic rickets is based on phosphorus replacement and calcitriol. In the past, pharmacologic dosages of vitamin D and dehydrotachysterol were used (91). Phosphate salts are rapidly excreted in the urine after intake, so it must be administered five times a day, including a dose in the middle of the night. This treatment will trigger PTH secretion, which is controlled with concomitant administration of calcitriol. Efficacy of the treatment is reflected by growth (Fig. 6) and decrease in the ALP levels. Children with hypophosphatemia do not grow well and present short stature, with improvement following an appropriate treatment (92). The combination of phosphate intake and calcitriol being the most effective form (93,94). Efficacy of the calcitriol treatment is followed with measurements of PTH levels and its toxicity by measuring the calcium/creatinine ratio in the urine. Doses should be frequently adjusted based on these results. Surgical treatment to correct the bone deformities should be delayed until after growth is over, otherwise deformities may recur. Hemiepiphysiodesis has been proposed as a surgical technique option in these patients (95).

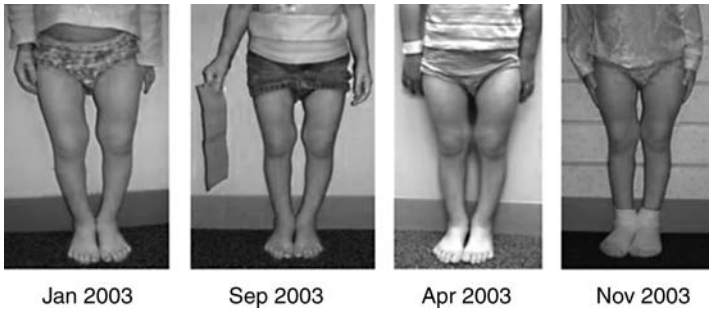

Figure 6 Progression of lower limb deformity under proper treatment for hypophosphatemic rickets.

Tumor-Induced Hypophosphatemia

Tumor-induced osteomalacia (TIO) was first described by McCrance in 1947 (96). TIO is a paraneoplastic syndrome with hypophosphatemia secondary to decreased renal phosphate reabsorption, normal or low serum 1,25-dihydroxyvitamin D concentration, osteomalacia, and myopathy (97). It has been related to different tumors, mostly of mesenchymal origin [epidermal nevus (98), linear sebaceous nevus (99), etc.,] but has also been described in patients with prostatic cancer (100), and in T-cell lymphoblastic lymphoma (101). It was first discovered that certain adenylate cyclase-stimulating proteins from tumors associated with humoral hypercalcemia of malignancy inhibit phosphate transport in PTH-responsive renal cell lines (102). Transplantation of a tumor-derived cell line from a patient with oncogenic rickets produced hypophosphatemia in mice (103). It was later recognized that FGF-23 causes renal phosphate wasting in TIO (83). The transgenic mice expressing human FGF-23 present hypophosphatemia due to increased renal phosphate wasting, inappropriately low serum 1,25-dihydroxyvitamin D level, and rachitic bone, and reduced expression of sodium phosphate cotransporter type IIa in renal proximal tubules (104). Treatment with phosphates has been attempted in the past, but this often caused hyperparathyroidism (105). Treatment is extirpation of the tumor, whenever possible, which leads to normalization of phosphorus serum levels and remineralization of bone (106). After tumor resection, $1,25(OH)_2$ vitamin D_3 normalizes within 24 hours, while renal tubular phosphorus reabsorption and serum phosphorus reach normal levels in two to three days; serum Ca declines slightly, and serum intact PTH, osteocalcin, and urinary pyridinium cross-link excretion increase dramatically, whereas urinary cAMP excretion declines immediately and then begins to increase, concomitant with the increase in serum intact PTH (107).

Phosphate Homeostasis

Most extracellular phosphate is located in bone mineral, whereas the intracellular phosphate is in nucleic acids and phospholipids. Thus, phosphate has two important roles namely bone health and phosphorylation of proteins and lipids. Phosphate and ionic calcium in extracellular fluids are at or exceed the solubility product constant and require elaborate controls to ensure the maintenance of phosphate levels in blood while allowing the deposition of calcium-phosphate into the bone without precipitating in other tissues. Dietary phosphate is readily absorbed and its movement in and out of the bone mineral is regulated by PTH and 1,25-hydroxyvitamin D. In the kidney, phosphate transports against electrochemical gradients from the urine into the proximal tubular epithelial cells through the activity of a sodium-phosphate cotransporter, NPT2a (108). This crucial step is regulated by PTH, and within minutes after the administration of this hormone NPT2a leaves the plasma membrane on endocytic vesicles, which ferry it to lysosomes, and over longer periods the synthesis of NPT2a decreases (109). Consequently, phosphate tubular reabsorption and blood levels fall. Most studies pertaining to the important role of NPT2a in phosphate homeostasis have been carried out in experimental models. In 2002, Prie et al. provided evidence of the role of this cotransporter in humans (80). They sequenced most of the NPT2a in 20 patients with hypophosphatemia due to renal phosphate wasting, who had nephrolithiasis and low bone mass. Furthermore, this work suggested that heterogeneous mutations of NPT2a might also play an important role in the actions of FGF-23 and thereby in the consortium of hypophosphatemic rickets and TIO described above.

HYPOPHOSPHATASIA

Hypophosphatasia is a rare inborn error of metabolism, characterized by rickets or osteomalacia secondary to low activity of the tissue nonspecific isoenzyme of alkaline phosphatase (TNSALP) (110–112). Biochemically, it is characterized by reduced activity of the TNSALP, and increased levels of TNSALP substrates, namely pyridoxal-5′-phosphate (PLP), inorganic pyrophosphate (PPi), and phosphoethanolamine (PEA) in serum and urine (113,114). There is no animal model for hypophosphatasia (115). The estimated incidence of the disease is of one per 100,000 births (115). Clinical presentation is extremely

variable, ranging from death in utero (116) to pathologic fractures first presenting only in adulthood. Severe forms of the disease are inherited in an autosomal-recessive fashion (117). The pattern of transmission of mild forms is uncertain (118). It is possible to perform prenatal genetic diagnosis of hypophosphatasia looking for mutations in the TNSALP gene (119). The mutation has been mapped to chromosome 1p36.1–34 in the case of infantile hypophosphatasia (120). Compound heterozygosity in the TNSALP gene can be responsible for childhood and adult hypophosphatasia (118).

Six clinical forms of hypophosphatasia have been distinguished, although form assignment may be challenging in some cases. This classification is based on the age when skeletal lesions are discovered: perinatal (lethal), infantile, childhood, and adult. Two particular forms include odontohypophosphatasia (only biochemical and dental manifestations are present, with no clinical changes in long bones), and pseudohypophosphatasia. Pseudohypophosphatasia is clinically indistinguishable from infantile hypophosphatasia, but serum ALP activity is normal (121). It has been suggested that in the case of pseudohypophosphatasia there might be a mutant TNSALP that still has activity in vitro, but not in vivo (115). In these patients, PEA, PPi, and PLP are elevated in serum and urine, in spite of normal or elevated ALP activity levels (122). In perinatal hypophosphatasia, affected subjects have lethal short limb dwarfism with very soft calvaria, polyhydramnios, blue sclerae, and spurs in the mid-portion of the forearms and lower legs (116). There is considerable variability in the skeletal manifestations. Well-known radiographic features include generalized decrease in the size of ossified bones and lack of ossification in some bones. Other clinical signs that may be observed include marked variability in the amount of bone ossification, and unusually dense, round, flattened, butterfly shaped, and sagittally clefted vertebral bodies. Different bones are affected in different degrees, and bones affected are different among patients. Variability is also found in femoral shape, and osteochondral projections (Bowdler spurs) of the mid-shaft of the fibula and ulna can be present. Affected newborns may survive briefly, but prognosis is very poor. Cause of death is usually severe respiratory compromise, accompanied by fever of unknown origin, anemia, irritability, bradycardia, seizures, and intracranial hemorrhage (110).

Laboratory work should include calcium, phosphorus, magnesium, ALP, creatinine, PTH, 25(OH) vitamin D, and $1,25(OH)_2$ vitamin D. PLP, PPi, and PEA in serum and urine will make the diagnosis. Measurements of ALP in amniotic fluid yields results too variable to be of value in the prenatal diagnosis of this entity (123). ALP in cultured amniotic cells may be quantified but interpretation of the results is difficult. Monoclonal antibodies against TNSALP may serve to detect a deficiency in chorionic villous tissue (124). There is no known effective treatment for hypophosphatasia. The effects of bone-marrow transplant in hypophosphatasia are transient, and bone lesions may recur six months after the transplant (125). Nonsteroidal anti-inflammatory drugs have been used in patients with childhood hypophosphatasia with some clinical improvement (126), although more experience is warranted before this therapy can be recommended.

VITAMIN D TOXICITY

Vitamin D half-life ranges from 20 days to several months, due to its high liposolubility. Biological half-life of 25(OH) vitamin D is 15 days (127), and about 15 hours for $1,25\ (OH)_2$ vitamin D_3. Interestingly, levels of 1,25 (OH) vitamin D_3 are not elevated in cases of overdose with vitamin D_3 or 25 (OH) vitamin D_3 (128). Administration of $1,25(OH)_2\ D_3$ to mice upregulates the VDR, but increases in endogenous $1,25(OH)_2\ D_3$ do not (129). This may contribute to exogenous calcitriol toxicity. There are variations in the hypercalcemic response to vitamin D overdose in different individuals, probably related to differences in absorption, metabolism, concentration of free vitamin D in serum, and capacity of storage sites (fat tissue) (130).

Symptoms of vitamin D overdose are the consequence of hypercalcemia, and include hypotonia, lethargy, confusion, drowsiness, apathy, abdominal pain, anorexia, constipation and vomiting. The presence of polyuria and nocturia is also very prominent. Patients can even be in a coma. Hypertension may be present (131). EKG changes are typical, with shorter Q-T intervals. Depression and psychosis may also be present. Nephrogenic diabetes insipidus may develop secondary to interference with vasopressin by high calcium concentrations. In that case, polyuria will be present, and dehydration can worsen the hypercalcemia. This is worsened by increased renal excretion of sodium and potassium, with reduced ability to concentrate the urine. Hypercalciuria precedes hypercalcemia, and can lead to nephrocalcinosis (128). Hypercalcemic crisis is a life-threatening emergency. Ectopic calcifications may be present. Patients with granulomatous diseases are ''hypersensitive'' to the hypercalcemic effects of exogenous vitamin D. Diagnosis of vitamin D toxicity is done through a thorough history of present illness and dosage of total and ionized calcium, phosphate, PTH, and vitamin D metabolites in serum. Calcium levels will be increased, PTH levels will be decreased and phosphate levels will be normal or elevated. Additional blood work includes albumin, total protein, BUN, and creatinine levels and calcium, phosphate, and creatinine in urine. The bone density of patients with vitamin D intoxication is also increased, and radiographs of long bones may show the increased calcification lines in the methaphysis that occurred during these episodes even after recovery (132).

The first step for treatment of hypercalcemia is hydration. Furosemide and glucocorticoids (hydrocortisone, prednisone) and pamidronate have been successfully used to decrease calcemia in these patients (133,134).

OSTEOPOROSIS

Causes and Assessment

Growing bones are particularly sensitive to injury; it follows that there are many conditions that cause osteoporosis in children, including primary bone conditions and diseases (Table 1). There is no consensus about a definition for ''osteoporosis'' in pediatrics. Ideally, the term osteoporosis should relate to bone fragility and not just areal bone mineral density (BMD) measured by dual-energy X-ray absorptiometry (DEXA). DEXA is the method most commonly used to assess bone density in pediatrics. The technique has several drawbacks and technical issues that make it sometimes difficult to diagnose abnormal bone density with certainty in the pediatric population (135). For example, children with mild OI, who may not suffer fractures, usually have BMDs (as measured by DEXA) that are several standard deviations below the mean for age (136). Different factors such as weight, height, and bone age influence the bone density results (137–141). Ideally, the absolute BMD should be interpreted considering all those factors. Moreover, bone density should always be measured in at least two different regions (i.e., lumbar spine, femoral neck, distal femur, whole body). For example, patients with CP spend more time in the sitting position than standing. This elicits constant mechanical stimulation to their spines, whereas their hips do not support any weight except when they are placed in standing frames. Measuring BMD for only the lumbar spine may give a falsely normal or near-normal result. In addition, BMD should not be used as the sole outcome of clinical research studies in children. Fracture occurrence and clinical changes (level of energy, pain, etc.) should be primary outcomes.

Lately, some publications have reported results of ''volumetric'' vertebral bone density (vBMD), which is calculated based on the two-dimensional bone density reported by DEXA (142). The formula for vBMD is: vBMD = bone mineral content (BMC)/(projection area)$^{1.5}$. The drawback of this approach is that it assumes a perfectly cubic vertebra. Therefore, it should not be used in the case of patients with vertebral fractures, in whom the result of vBMD will be artificially higher. Other methods to correct BMD by height have not received general acceptance (140). For the purposes of this chapter, we will define osteoporosis in pediatrics as BMD less than two standard deviations below the mean for age. Reference values for different devices have been published (143–149), although accurate reference data for infants are still warranted. The use of ''T-scores'' in pediatrics is not appropriate (150), and there are no epidemiologic studies of the relationship between fractures and BMD in pediatrics, which would allow defining ''osteopenia'' and ''osteoporosis'' as definite entities.

There are several other methods to measure ''bone density'' [e.g., quantitative computed tomography (QCT), ultrasound, and peripheral quantitative computed tomography (pQCT)], but their use has not been generalized to pediatrics. Particularly, cost and radiation exposure with QCT is very high, and is not justified in pediatrics. pQCT is currently used in research, and it may yield very useful information about bone biomechanics. The lack of general agreement on different variables that are decided by the operator is a problem and highlights the need for standards in metabolic bone diseases in children.

Although laboratory workup will vary depending on the suspected cause of osteoporosis, certain basic phosphocalcic biochemical tests should always be performed when evaluating a pediatric patient for osteoporosis. This includes serum calcium, phosphorus, magnesium, ALP, creatinine, PTH, 25 vitamin D, 1,25 vitamin D, and urinary calcium, phosphorus, and creatinine. Vitamin D levels in serum have seasonal variations (higher in summer, lower in winter), as well as regional variations (lower as one goes away from the Equator). Phosphate measurements are highly influenced by intake, and should always be measured with the patient fasting. The new biointact iPTH renders lower values than the ''classic'' iPTH. For intact PTH equivalence, the result should be multiplied by 2. In specific situations, other tests may be requested, including cAMP, biochemical markers of bone formation (osteocalcin, procollagen type I C-terminal propeptide, and procollagen type I N-terminal propeptide), and biochemical markers of bone resorption (serum: tartrate-resistant acid phosphatase, type I collagen telopeptide, urine: collagen cross-links—pyridinoline, deoxypyridinoline, NTx-hydroxyproline, and galactosyl-hydroxylysine). Interpretation of these markers in the pediatric population may be difficult (151), and these are used mostly as markers to follow-up treatments, not for diagnosis.

Acquisition of Bone Mass

Inadequate acquisition of bone mass during childhood and adolescence is considered as a key risk factor for osteoporosis in adult age (152,153). The peak of bone mass accrual is attained around puberty (144,154). Adequate calcium intake during growth is paramount, because it may influence accrual of peak bone density and therefore may be very important in preventing subsequent postmenopausal and senile osteoporosis. Skeletal calcium retention appears to be directly affected by calcium intake during adolescence, but it has been noted that preadolescence years are probably even more important for bone mass acquisition. A daily calcium intake of up to 1600 mg may be required during puberty (155),

although in European countries the daily intake recommended is significantly lower. There is no evidence that the components of soda beverages (e.g., sugar, caffeine, and phosphate) are linked to decrease in bone mass in teenagers (156). Particularly, phosphate intake does not appear to affect calcium absorption (157). The problem with consuming soda beverages seems to be related to milk intake displacement (158). It may be necessary to prescribe calcium supplements to children and adolescents who consume low amounts of dairy products. Different calcium salts (carbonate, lactate, sulfate, and citrate) appear to have similar absorption rates when administered as supplements (159,160), and gastric acid is not necessary for absorption of even poorly soluble preparations of calcium, as long as they are taken with meals (161). Therefore, antacids are not contraindicated in children receiving treatment for osteoporosis. On the other hand, it should be noted that calcium content in calcium-fortified products does not guarantee its bioavailability (162).

Other factors may influence bone mass acquisition, but their relative influence is not totally clear. For some, bone mass acquisition is a purely mechanical phenomenon (163). Mechanical factors dominate control of the biologic mechanisms that control changes in postnatal bone and mass, and nonmechanical agents could help or hinder the influence of the mechanical factors but could not replace them. This line of thought suggests that the view that nonmechanical agents (hormones, calcium, vitamin D, cytokines, gender, genetics, etc.) dominate control of osteoblast and osteoclast (164). For others, genetics counts for about 85% of the bone mass under normal circumstances (165,166). Family and twin studies have found a strong genetic component to the determination of BMD. From this particular point of view, BMD is a complex trait whose expression is confounded by environmental influences and polygenic inheritance. The number, locations, and effects of the individual genes contributing to natural variation in this trait are all unknown.

Smoking has been found to have a deleterious effect on bone mass (167,168). The effects of cigarette smoking on bone are not clear. Tobacco use is not associated with a change in prevalence of osteogenic connective tissue progenitor cells in bone marrow, or their intrinsic biologic capacity to undergo early osteoblastic differentiation (169).

Probably all hormonal changes have influence on bone. The beneficial effects of sex steroids on bone have largely been recognized. It has even been shown that several common aromatase polymorphisms are associated with cortical bone size although not with trabecular volumetric BMD (170). On this regards, treatment with GnRH agonists may predispose to osteoporosis (171). Recently, it has been shown that patients with hypothyroidism have elevated plasma levels of osteoprotegerin, an inhibitor of bone resorption (172). These levels normalize after normalization of thyroid function.

Clinical Features and Treatment

The clinical features of osteoporosis are classically associated with the findings present in women in later life, particularly after menopause. It has been called the silent epidemic, and it is even more silent in children who may have decreased bone quality with osteopenia and/or osteoporosis. These alterations usually manifest as bone fractures that occur with relatively minor trauma, not as spinal deformity and kyphosis classically occurring in elderly persons. Osteopenia and consequent fractures are prevalent in premature infants, as discussed in Vol. 2; Chap. 22 of this book. In older children, osteoporosis is usually discovered fortuitously by radiological examination done for a variety of reasons, or after a fracture. Fractures of the extremities are more common in the lower limbs occurring after trauma, and often the presence of underlying osteoporosis is not considered. Vertebral fractures in children and young adults in the absence of severe trauma are almost invariably due to osteoporosis. These may produce pain, kyphosis, and loss of height. The latter may not be easily appreciated in a growing child as in an adult whose height has been unchanged prior to the fracture.

Fractures due to osteoporosis are a principal cause of disability and death among the elderly, but less than one-third of patients who have had fragility fractures are appropriately evaluated for osteoporosis despite their high risk of future fractures (173). The underdiagnosis of alterations in bone health among children who have had a fracture may even be more prevalent than that reported for high-risk adult populations. A history of a fragility fracture increases the risk of future fractures, and there is an association of traumatic fractures and osteoporosis (174). Thus, a high index of suspicion should be exercised to consider bone health alterations in children who have had fractures.

In the treatment of osteoporosis, the ability to monitor the response to the therapy is of paramount importance. BMD and other biochemical markers of bone remodeling do not provide an optimal approach to the monitoring response and/or predicting the fracture risk improvement or lack of it. In children, prevention of the disease should be the first goal. At present, there are two important approaches to the prevention of osteoporosis: increasing bone mass acquisition at skeletal maturity and reducing bone loss (175). Assuring an appropriate calcium intake throughout life is essential for bone health (176). Calcium intake and supplementation plays an important role on bone mass in adolescent girls and it significantly increases bone accretion during the pubertal growth spurt (177,178).

Bisphosphonates have been used to treat secondary osteoporosis in the pediatric population

(179–183). It is believed that one of the mechanisms of action of bisphosphonates is through inhibition of the mevalonate pathway in the osteoclasts enhancing apoptosis and reducing bone resorption, but effects of these drugs on osteoblasts have also been proven (184,185). Pamidronate is the most widely used bisphosphonate in the pediatric population. Protocols for administration of the drug vary from center to center. Doses range from 4.5 (186) to 9 mg/kg/yr. Most institutions administer pamidronate every two months to children up to two years of age, every three months to children two to three years of age, and every four months for older children. Administration should be very slow (in about 3–4 hours) and dilution should be at least 1:10 in normal saline solution or dextrose solution. Ringer lactate solutions should not be used.

The dose of 9 mg/kg/yr was chosen based in the doses used for adults with Paget's disease of bone. It has been shown that this dose of pamidronate cause retention of calcified cartilage within secondary spongiosa in children with osteogenesis imperfecta (187), and higher doses have caused osteopetrosis in a patient with no clear diagnosis (188). This strongly suggests a dose-related effect of pamidronate. The need to test the efficacy of lower doses of pamidronate in the pediatric population has been recognized in the past (189–191). Treatment with low doses of pamidronate (4.12 mg/kg/yr) is generally safe and increases bone density significantly in both lumbar spine and femoral neck in nonambulatory children (186), although the efficacy of this treatment regime to prevent fractures is yet to be determined. Other IV bisphosphonates are available in North America and/or Europe, including neridronate (192), zoledronic acid, and olpadronate (193). The benefits and side effects of these drugs in children are currently being tested in different research studies. Although the use of these medications, particularly intravenous pamidronate, has been extended to children through off-label use, bisphosphonates have a prolonged half-life in bone and their safety and efficacy have not been well established in children. Although the medical literature is uniformly positive, and patients and parents are encouraged by the initial decrease in fractures after initiation of treatment, there is a risk that the prolonged administration of high doses of these medications may excessively increase bone density, with bones potentially becoming more brittle rather than stronger.

Treatment of osteoporosis in children should always be multidisciplinary. All different aspects of the condition should be taken into account, including orthopedic surgery, physical therapy, occupational therapy, nutrition, psychology, and social services.

IDIOPATHIC JUVENILE OSTEOPOROSIS

Idiopathic juvenile osteoporosis (IJO) was first described in 1965 by Dent and Friedman (194), as a syndrome with osteoporosis starting prior to puberty, back and appendicular pain, gait abnormalities, which characteristically resolves spontaneously after puberty (also see Vol. 2; Chap. 24 of this volume). Presentation may be with long bone fractures, back pain (195), or gait problems (196). ''*Neo-osseous porosis*'' (metaphyseal osteopenia) has been described as a radiological lesion pathognomonic of IJO (197), but it has also been observed in polyglandular autoimmune syndrome (198). The mean age of onset is seven years (199), with a range of one to 13 years, highlighting the fact that IJO may start at a very early age. Other causes of osteoporosis must be ruled out, particularly those related to malignancy (200). The etiology of IJO is obscure and the diagnosis is based both on the exclusion of other diseases and on the clinical evolution of the patients (201). Hystomorphometric analysis of bone biopsies of patients with IJO showed that the condition is characterized by a decreased cancellous bone volume associated with a very low bone formation rate on cancellous surfaces (202). The disturbance of bone remodeling in IJO is limited to cancellous bone, but there may also be a modeling defect affecting the internal cortex. The process causing IJO appears to mainly affect bone surfaces that are in contact with the bone-marrow cavity (203). Several treatments have been attempted in the past for IJO, including calcitonin (204), sodium fluoride (205), calcitriol (206), and bisphosphonates (207). Because spontaneous recovery is the rule, it remains impossible to assess the efficacy of these treatments. On the other hand, vertebral fractures may not recover completely without treatment, and may be an indication to start bisphosphonate therapy in these patients.

SECONDARY OSTEOPOROSIS IN PEDIATRICS

Numerous pathological circumstances put growing bones at risk of osteoporosis (Table 1). Mechanical stimulation is paramount for bone strengthening at any age (208), and immobilization is a common cause of osteoporosis (209,210). Immobilization due to paralysis from injury to the central nervous system or peripheral nerves, prolonged therapeutic bed rest, and application of cast to treat fractures are common causes of disuse osteoporosis. In normal individuals, bone mass appears to be closely related to muscle mass (211). Children who require prolonged total parenteral nutrition in early life are at risk of abnormalities in growth and nutritional status in later childhood, requiring long-term dietary, growth, and nutritional monitoring, including BMD (212–214). The metabolic bone disease of patients receiving total parenteral nutrition was extensively reviewed in a previous edition of this book (215).

Children with Turner syndrome, growth hormone deficiency (GHD) and short bowel syndrome have decreased BMC compared with control subjects; however, this differences disappear when adjusted

for differences in weight or height (216,217). Although it appears that growth hormone (GH) treatment increases bone density Z-scores related to age (218), it is not clear what the effect of growth itself on these changes is. Neely et al. reported that volumetric BMD in adolescents with Turner syndrome receiving GH therapy was not abnormal (219) and Lanes et al. found similar results in prepubertal girls with this syndrome (220). Hogler et al. found no differences in any of the DEXA Z-scores between the GH-treated and GH-untreated groups of patients (221) Furthermore, there may be an increased risk of scoliosis (222). An increase of fracture rate during GH therapy has been reported in children with osteogenesis imperfecta (223,224). In a controlled study, comparing seven children with mild osteogenesis imperfecta with seven receiving no treatment, fracture rate was not different between the groups (225). This suggests that although bone density may increase, fracture risk is not reduced.

Immobilization

The molecular mechanism of mechanical stress-induced bone formation remains unclear. The ''mechanostat'' theory of bone enunciated by Frost et al. (226,227) analyzes the skeleton from a biomechanical perspective. From this point of view, the skeleton has been described by J. Ferretti as ''a biomechanically-regulated structure that can be systemically disturbed (in the cybernetic sense), the strength of which depends on the intrinsic stiffness (material properties) and the spatial distribution (architectural properties) of the mineralized tissue'' (228). The biomechanical feedback system involved (bone ''mechanostat'') would not control bone mass to optimize bone strength; it would rather control bone material quality and architecture (through a modulation of bone modeling and remodeling), in order to optimize bone stiffness. The natural stimuli for the bone mechanostat would be the customary strains of bone tissue (sensed by osteocytes) that are induced by gravitational forces and, more importantly, the contractions of regional muscles (228). Mechanical stimulation is paramount for bone strengthening (229), and immobilization is a well-known cause of osteoporosis (209,210). Reduction of mechanical stress on bone inhibits osteoblast-mediated bone formation and accelerates osteoclast-mediated bone resorption, and leads to the so-called ''disuse osteoporosis.'' Fractures may be a consequence of decreased bone density, although their occurrence in fact depends on multiple factors.

Neuromuscular diseases

CP is the most common physical disability of childhood, with an incidence of two to three per 1000 live births (208). It has been estimated that over 100,000 children have some degree of neurological impairment attributable to CP in the United States (230). Children with severe CP are unable to increase bone mass, and even lose bone mass progressively (19,231). There is no correlation between BMD and the age when the child first walked, fracture history, use of anticonvulsant medication, or serum vitamin-D levels after adjustment for walking and nutritional status in children with CP (19). This means that the main cause of osteoporosis in these children is lack of activity. Extra mechanical stimulation of their bones is warranted, provided that nutritional factors are controlled. Weight bearing for short periods of time is not enough to keep bone mass stable, as suggested by occurrence of severe osteoporosis in patients with CP despite being placed in the vertical position for one to two hours per day (19).

Severity of the condition varies widely. In fact, most children with CP are ambulatory and are not cognitively impaired. The most profoundly involved subgroup has spastic quadriplegia, comprising roughly 20% of all children with CP (232,233). This subgroup characteristically has severe physical and cognitive impairments, and a high prevalence of poor growth and/or malnutrition. These patients are non-ambulatory, and often have multiple other problems including seizure disorder, swallowing difficulties, and gastric reflux requiring feeding tubes, and osteoporosis that results in fractures. Low doses of IV pamidronate (4.5 mg/kg/yr) appear to be effective and generally safe to increase bone density in children with severe CP (234).

Decreased BMD is also present in patients with advanced Duchenne's muscular dystrophy. Low bone density may be present even in ambulatory patients (235,236), and is often worsened by corticosteroid therapy (237). As a consequence, the incidence of vertebral fractures is increased in this population, particularly after initiation of corticosteroid therapy (238). Vertebral fractures appear after about three years under steroid treatment, and after about eight years under treatment, 75% of the patients may have vertebral fractures (238).

Type I Diabetes Mellitus

Even though pediatric patients with type I diabetes of recent onset may not be at particularly risk of low BMD and growth problems (239), poor metabolic control may expose adolescents with long-standing type I diabetes to the risk of developing osteoporosis as adults (240). Adult patients with IDDM may have reduced BMD (241). Studies with pQCT showed that there is a decrease of trabecular, total, and cortical bone density in children and adolescents with type I diabetes (242). Trabecular bone density appears to be inversely correlated with the duration of the disease and the concentration of glycosylated hemoglobin (HbA1), whereas total BMD correlates inversely with serum levels of HbA1 (242). A different study using QCT, however, found that cortical bone density is slightly but significantly lower in diabetic children than in controls, but there is no difference

between patients and controls regarding trabecular bone density (243). The decrease in cortical bone density did not correlate with age, sex, duration of diabetes, or glycosylated hemoglobin levels in the diabetic group (243). There are no current recommendations for prevention or treatment of osteoporosis in pediatric patients with type I diabetes. Still, optimizing calcium and vitamin D intake is warranted and of course maintaining the best possible control of the disease.

Renal Causes

Chronic metabolic acidosis may increase alkali (calcium) mobilization from the bone and thus promote the development of osteoporosis (244). Children with idiopathic nephrotic syndrome are at risk of low bone mass, probably related to high doses of steroids (245). Decreased lumbar spine BMD in kidney transplant patients is related to height and repeated transplants, but interestingly, it does not appear to be influenced by weight, preexisting renal disease, gender, or rejection events (246). The factors that affect BMD and the long-term effects of transplantation on BMD in children are still unknown (247).

Renal tubular acidosis (RTA) is the result of a group of disorders with pure tubular damage without concomitant glomerular abnormalities. RTA can be hereditary or secondary to different conditions such as obstructive uropathy, sickle-cell disease (SCD), renal transplant, autoimmune diseases, or drugs. The disorder is characterized by the presence of hyperchloremic metabolic acidosis with a urinary pH greater than 5.5 in the presence of systemic acidemia, and absence of an easily identifiable cause of the acidemia (248). Defects in potassium homeostasis may be associated. The bone disease found in RTA is more likely related to the acidosis and hypercalciuria prevalent in this disorder, than to abnormalities in vitamin D metabolism resulting from systemic acidosis (249).

Fanconi syndrome is a disorder of proximal renal tubular transport characterized by wasting of phosphate, amino acids, glucose, bicarbonate, and uric acid. Dent's disease is a familial form (X-linked) of Fanconi (250), although family history may be negative in some cases of Dent's disease (251). Clinical manifestations include severe hypophosphatemic rickets, failure to thrive, and metabolic acidosis. Osteomalacia associated with adult acquired Fanconi syndrome is thought to result from hypophosphatemia and relative 1,25-dihydroxyvitamin D deficiency. A potential drug-induced Fanconi syndrome has been noticed in children treated for relapsed Wilms' tumors with ifosfamide, a derivative of cyclophosphamide, presenting with radiological changes compatible with rickets (252,253). It has been suggested that continuous nasogastric infusion of phosphorus and bicarbonate may be a useful alternative therapy in patients with renal Fanconi syndrome who are resistant to conventional bolus therapy (254). Regardless of the underlying cause, osteomalacia associated with adult-acquired Fanconi syndrome appears to respond well to calcium, phosphate, and vitamin D replacement. These patients do not appear to necessarily require 1,25-dihydroxyvitamin D replacement (255). Treatment of RTA includes avoidance of precipitating factors when possible, treatment of any underlying disease, cessation of any drug possibly causing the syndrome, correction of electrolyte imbalance, particularly hypokalemia and hyperkalemia, and the use of alkali (248).

Endocrinological Causes

Osteoporosis is a common aspect of different hypogonadal states (256). The syndrome of ''athletic amenorrhea'' causes bone demineralization in girls participating in heavy training. These results in the ''female athlete triad'': disordered eating, amenorrhea, and osteoporosis (257) with increased risk of skeletal fragility, fractures, and vertebral fractures (258). Menstrual abnormalities in the female athlete result from hypothalamic suppression of the spontaneous pulsatile secretion of gonadotropin-releasing hormone (258). Patients with anorexia nervosa are also at risk of osteoporosis. The occurrence of low bone mass in this particular group is so common that it has been suggested that an eating disorder should be suspected in underweight young individuals (primarily girls) presenting with low-impact fractures (259). The bone loss experienced by patients with anorexia nervosa is the product of multiple factors. The associated estrogen deficiency in anorexia nervosa may not be the major contributor for developing osteoporosis (260) because bone density abnormalities are present before any alteration in estrogen levels can be detected.

Turner syndrome is caused by a 45,XO monosomy, or a variety of mosaic patterns, such as XX/XO. Clinically, these girls have wide, webbed neck, small mandible, anomalous auricles, and increased carrying angle of the arms, cardiovascular defects, renal abnormalities, and perceptive hearing impairment. Typically, they do not develop pubertal changes (Vol. 2; Chap. 12). Both areal (261) and volumetric (262) BMD are reduced in these patients. Interestingly, this is seen not only in adults with Turner syndrome but also in affected girls (263). Some authors found that areal BMD appears to be normal after being corrected for height or bone age (217,219,264,265), but trabecular lumbar spine volumetric BMD appears to be reduced when measured with QCT (266). More importantly, fracture risk appears to be increased in this group (264,267). It has been suggested that estrogen replacement may optimize bone mass acquisition in girls with Turner syndrome (256,268,269) and that optimizing bone mass in patients with Turner syndrome may require earlier induction of puberty than currently recommended (221). Estrogens act directly on bone cells

through high-affinity estrogen receptors (270). Estrogen deficiency accounts for the bone loss in menopause and even in prolonged functional amenorrhea that occurs in athletes leads to bone loss in young women (271).

Prader–Willi syndrome is characterized by characteristic facies, obesity, hypogonadism, and short stature, infantile hypotonia, developmental delay, mental retardation, and behavioral disorders. Affected patients have elevated biochemical markers of bone resorption and formation compared with controls, which suggests a high bone turnover, and low BMD (272).

GHDs in adults presents with a low bone mass, despite prior GH substitution (273,274). Osteopenia is present in both patients with isolated GHD and multiple pituitary deficiencies. The pathogenesis of the reduced BMC and density in childhood-onset GHD is likely due to deficient buildup of the bone mass, rather than premature bone loss. Adults who develop GHD may present decreased bone turnover (273). The degree of GHD seems to be correlated with the severity of bone mass and turnover impairment (274) and age of the patient (275).

The BMC is affected in GHD adults, while the calculated true bone density was normal (276). The prevalence of lifetime low-energy fractures was not increased in patients with isolated GHD, but it substantially exceeded the expected prevalence in patients with multiple pituitary hormone deficiencies.

Malabsorption

Bone mineralization and remodeling can be affected in patients with celiac disease, inflammatory bowel diseases, gastrectomy, cholestatic liver diseases, liver transplantation, or hepatitis C (277). Although children with celiac disease are at risk of lower BMD, it has been suggested that a strict gluten-free diet may improve their bone mineralization (278,279). Osteoporosis is a frequent complication in children with cystic fibrosis (CF) regardless of their age (280,281), but it is more common in children with poor nutritional status. Despite lower bone density, these children may not be at greater risk of fracture (282). Patients with end-stage liver disease are prone to develop osteoporosis (283). Prevalence of osteoporosis in this group ranges from 10% to 56%, depending mostly on the nature of liver disease (284). The so-called ''hepatic osteodystrophy'' has been defined as a syndrome in children with cholestatic liver disease (285). Patients who undergo liver transplant are at higher risk of bone fractures secondary to osteoporosis. The association of cholestatic liver disease and osteoporosis is well recognized, but the underlying etiology is unknown (286). Immunosuppression protocols that use lower doses of prednisone administration over shorter time intervals may help prevent bone loss after orthotopic liver transplant (283). Greater disease severity is associated with a higher risk of osteoporosis (287).

Levels of calcidiol and calcitriol were found to be lower in patients with CF than in controls. Low calcidiol levels are related to malabsorption, but it is not clear why these patients have low levels of calcitriol. Older patients with CF may be at increased risk of development of osteoporosis because of progressively diminishing sunlight exposure (288). Osteoporosis in patients with CF probably is related to nutritional factors (289) and respiratory complications [e.g., decreased forced expiratory volume in one second (290)] and is thus not related to a primary defect in bone mineral metabolism. This has a significant impact in therapeutic decisions. Prevention of osteoporosis with adequate vitamin D supplementation (enough to reach a serum vitamin D level of at least 30 ng/mL) is warranted in children with CF and other gastrointestinal diseases.

Inborn Errors of Metabolism

Inborn errors of metabolism include a large list of defects in the metabolism of carbohydrates (disorders of carbohydrate transport, of gluconeogenesis and glycogen storage, of glycerol metabolism, and of galactose and fructose metabolism), amino acid transport and peptide metabolism (aminoacidopathies, organic acidurias, disorders of ammonia detoxification), vitamin (cobalamin and folate), mineral (copper, iron, zinc, etc.), and fatty acids, as well as errors in mitochondrial energy metabolism, problems with biosynthesis and breakdown of complex molecules (including lysosomal storage diseases, peroxisomal disorders, and several other), and neurotransmitter defects.

Lysosomal storage disorders are caused by genetic defects of lysosomal enzymes that lead to accumulation of different substrates in the lysosome, which impairs cellular function. There are more than 40 different diseases caused by this mechanism. There is a misconception that all lysosomal storage diseases cause skeletal changes (291). In fact, the vast majority of them affect predominantly the central nervous system. The radiological manifestations of lysosomal storage diseases have been termed ''dysostosis multiplex'' (292,293). Typically, the shafts of bones are widened with thin cortices, and sometimes pathological fractures are present. Limbs are short, epiphyseal centers are poorly developed; and the mid-shafts are enlarged. Metacarpals are broadened distally and taper at their proximal ends. Phalanges have a characteristic bullet shape. Small femoral heads coxa valga and poorly developed pelvis are often seen. Lower ribs are broad and the lateral part of the clavicle is hypoplastic. Vertebrae are hypoplastic. Other common characteristics are pectus carinatum, short stature and kyphosis, hypoplasia of the odontoid, vertebral fractures, macrocephaly, and hydrocephalus.

Gaucher's disease is a multisystemic lipidosis characterized by bone-marrow infiltration leading to hematological problems and organomagaly

(hepatosplenomegaly). Bone involvement includes osteonecrosis and bone thinning (294). Abnormal cells containing undegraded glycosphingolipids, particularly glucosylceramide, are present in the reticuloendothelial system (295). There are three clinical forms of the disease. Type 1, the most common, is the adult, non-neuronopathic form, common among Ashkenazi Jews (296). Types 2 and 3 are infantile and juvenile, respectively. All forms are autosomal recessive, and severity and age of onset vary widely. The mutation has been mapped to chromosome 1 (q21–q31), where a gene encoding for acid b-glucosidase is located (297). It has been suggested that there may be more types of Gaucher disease (298,299). Fractures are a consequence of infiltration of the medullary space by Gaucher cells, erosion of bone, osteonecrosis in the area of the fracture, and disuse osteoporosis in this group (300). Bone lesions on patients with Gaucher's disease may also be secondary to high concentrations of interleukin-6 in serum (301).

Lysinuric protein intolerance (LPI) first appears in the infant as failure to thrive, vomiting, and diarrhea after weaning from mother's milk. Delayed signs are failure to thrive, hepatomegaly, muscular weakness, and osteoporosis, associated with aversion to animal protein and, in some, mental retardation (302). It is inherited in an autosomal-recessive fashion. Skeletal manifestations may include severe osteoporosis leading to fractures after minor trauma, abnormal thickening of cortex of the metacarpals and thin cortices of the long bones, endplate impression of vertebrae, rickets-like metaphyses, or early destruction of cartilage, and delayed bone age (303). Transport of cationic amino acids at the basolateral membrane of intestinal and renal epithelia is impaired, which leads to renal hyperdiaminoaciduria (especially lysinuria), and to impaired formation of urea with hyperammonemia after protein ingestion (304). LPI is distinguished from other hyperdibasicaminoacidurias by the presence of the transport defect of diaminoacids into the hepatocytes (305). The transport defect in intestinal and renal membranes can actually be found in skin fibroblast (306). Treatment with citrulline (307) and lysine (308) supplements have been used for LPI. It appears that they correct the deficiency of the urea cycle intermediates, thus protecting patients from hyperammonemia and its consequences.

Phenylketonuria may cause low bone density in affected children, despite an adequate diet as per current recommendations (309). The risk of late complications of dietary therapy, such as osteoporosis may be increased because of long-lasting dietary restriction in these patients (310). It is unclear whether deficits in bone mineralization in this population are a consequence of the disease itself or its treatment (311).

Malignancy and Steroid-Induced Osteoporosis

Pediatric patients with cancer often have impaired development of BMD (Fig. 7) (312,313). Osteoporosis

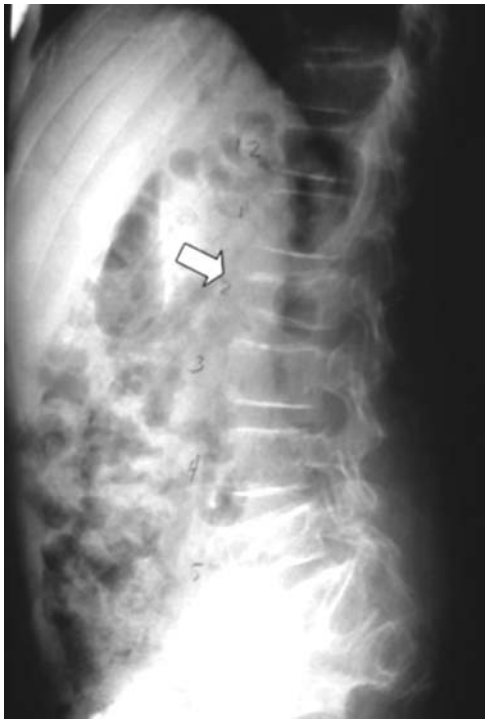

Figure 7 Vertebral fractures in a patient with lymphoma.

at the level of the femoral neck can be evident as early as one year after initiation of chemotherapy, independently of the type of tumor. This decrease in bone density appears to be independent of the well-known effects of chemotherapy on growth (313–315). On the other hand, a study found that the whole-body bone mass of a group of survivors of childhood Hodgkin disease or non-Hodgkin lymphoma was only slightly reduced and the size-adjusted bone mass was normal 11 years after diagnosis (316).

The precise cellular and molecular basis of the effect of glucocorticoids on bone has begun to be elucidated in recent years, despite being known for a long time (317). Glucocorticoid-induced bone disease is characterized by in situ death of isolated segments of bone (osteonecrosis) (318), and a decrease in bone formation (319). Ischemic necrosis is actually caused not only by necrosis but also by apoptosis in pigs (320). Glucocorticoids promote osteoclast survival (321), and prednisolone in particular stimulates apoptosis in osteoblasts and osteocytes in mice and humans (322,323). Inhaled corticosteroids are widely used in the long-term management of asthma in pediatric patients. An increased risk of fracture was found in association with use of inhaled corticosteroids, although it is not clear if this is the result of the underlying illness, rather than being directly attributable to inhaled corticosteroid therapy (324). A recent case-controlled study involving more than 20,000 children receiving systemic glucocorticoids found an

increase in risk of fracture (325). Patients with conditions that require prolonged corticosteroid treatment should be evaluated for osteoporosis. Prescription of bisphosphonates concomitant with the initiation of steroid therapy is an option to be considered in those cases (326).

Patients with sickle cell anemia (SCD) may have low bone density (327). The cause of decreased bone density in these patients is multifactorial. In fact, there are no solid studies that had addressed this issue. Serum calcium, phosphate, and ALP concentrations are normal in children with SCD (328).

Prematurity

Eighty percent of bone mineralization in the fetus occurs during the third trimester in normal circumstances (329). Infants with osteopenia of prematurity (OOP) may have decreased BMC as a result of inadequate calcium and phosphorus intake after birth (Vol. 2; Chap. 22). The intrauterine accretion of calcium and phosphorus is much higher in the fetus compared to extrauterine life. As an example, a fetus who weighs 1 kg and is at 27 weeks of gestational age incorporates daily approximately 110 mg/kg of Ca and 76 mg/kg of P into the body from placental transfer (330). This accretion rate of the ''reference fetus'' corresponds to intake of 12.2 mL/kg/day of 10% Ca gluconate and 3.6 mEq/kg/day of potassium phosphate in parenteral nutrition solutions. However, this comparison may not be valid. The ''reference fetus'' usually develops in a normal metabolic environment and has sustained weight gain and growth. In contrast, very low birth weight infants, especially the so-called ''micropremies,'' are usually very sick, are growing in an unstable metabolic environment, and usually do not achieve sustained weight gain for at least several weeks postnatally. The proper approach to OOP is prevention. An adequate amount and ratio of calcium and phosphorus intake is required, together with an adequate caloric intake. The proportion between Ca and P in parenteral nutrition of premature babies should be 1.7:1 (330,331). Particularly, intake of vitamin D should be monitored. It has been shown that increased parenteral intakes of calcium and phosphorus result in greater retention of these minerals during parenteral nutrition therapy and in greater BMC after therapy (332). Daily physical therapy may be a useful adjunct, as it has been found to be associated with a significant increase in BMD and BMC (333).

Juvenile Rheumatoid Arthritis

Patients with chronic juvenile rheumatoid arthritis (JRA) are often affected with osteoporosis Bone loss results from multifactorial processes which lead to bone resorption through the activation of osteoclasts (334). Steroid intake is obviously involved in the development of osteoporosis in JRA, but the role of other factors affecting bone mineralization remains unclear. Early onset disease is also an important factor in the development of osteoporosis in JRA (335). Furthermore, the role of calcium and vitamin D supplementation for the prevention and treatment of osteoporosis associated with pediatric rheumatic diseases remains to be established (336).

Methotrexate is commonly used for management of moderate-to-severe polyarthritis (337), although in certain cases other ''disease-modifying antirheumatic drugs'' such as sulfasalazine and cyclosporine are being used in combination with methotrexate. These drugs allow avoiding steroid treatment in many children with severe JRA, therefore reducing the risk for osteoporosis and growth failure (337). It appears that most young adults with JRA attain a normal BMD if the disease goes into remission, while the risk for osteopenia and osteoporosis is increased in young adults with active disease (338). A stress fracture should be suspected if a patient with rheumatic disease experiences sudden and unexplained localized pain, particularly in the forefoot, above the ankle, below the knee, or in the pelvis (339).

Drugs and Toxics

Low molecular weight (LMW) heparins have potential significant advantages over unfractionated heparin and oral anticoagulants for both the prevention and the treatment of thromboembolic events in children (340), but chronic therapy with heparin may cause osteoporosis. The advantages of LMW heparins over unfractionated heparin include higher bioavailability, a longer half-life (allowing once- or twice-daily subcutaneous dosing), and predictable anticoagulant response, and a lower risk of heparin-induced thrombocytopenia and osteoporosis (341). Long-term exposure to treatment and prophylaxis of venous thromboembolism cause a modest but progressive decrease in BMD. This effect is more evident in patients receiving LMW heparins than in those taking acenocoumarol (342).

Methotrexate osteopathy can occur in patients treated with low doses of the drug (343). Severe lower extremity pain and osteoporosis involving the lower extremities, as well as thick dense provisional zones of calcification and growth arrest lines resembling scurvy are characteristics (344). These effects may lead to fractures (345). Patients with ALL may not have long-term effects on their bones after remission despite high doses of steroids (dexamethasone) and methotrexate without cranial irradiation (346). Lower BMD appears to be related to low physical activity levels in these patients (347), but it is probably multifactorial, including previous chemotherapy, limited exercise capacity, and relative physical inactivity (348).

Treatment of Secondary Osteoporosis

The first step in the treatment of secondary osteoporosis is of course prevention. To preserve bone health, all

disease-related risk factors such as malnutrition, immobilization, sex steroid deficiency or GHD, and inflammation need to be considered and treated appropriately. For example, children with CP may benefit from standing programs, although the benefits of this therapy are yet to be demonstrated (349). An adequate calcium and vitamin D intake is paramount. Fortification of milk with vitamin D in the United States is 400 IU/L. In many occasions prevention is not enough, or even possible. Once the diagnosis is established, and besides treating the primary cause in children with secondary bone disease, the use of bisphosphonates may be indicated (350). Please see Vol. 2; Chap. 24 for a detailed description of bisphosphonate treatments of children with brittle bone syndromes and above for those with primary osteoporosis. It should not be assumed that drugs used to treat osteoporosis in adults are appropriate for children. Randomized control studies are needed to determine the dose as well as the safety and efficacy of bisphosphonates in children (351).

Bisphosphonates, particularly IV pamidronate, have been used for the treatment of different types of secondary osteoporosis. Oral bisphosphonates are potentially beneficial for pediatric osteoporosis, but reliable studies in children with osteogenesis imperfecta showed that besides increase in bone density there are no other treatment-related benefits of these drugs relative to placebo. Particularly, bone pain, bone pain frequency, and pediatric disability scores are no different in treated children compared to those who receive placebo (352). Furthermore, patients taking oral bisphosphonates have the theoretical risk of gastric discomfort or even severe burning of the esophagus if the drug is not taken properly.

There is only limited published information about the use of bisphosphonates in children receiving pharmacologic doses of glucocorticoids. A significant increase in lumbar spine BMD in all subjects after one year of treatment has been noted in children with rheumatologic diseases, low BMD, and receiving glucocorticoid treatment for at least six months, or with a history of bone fragility fractures (353,354), but there is no information about the efficacy of these treatments to prevent fractures. In the only double-blind study of IV pamidronate in children published so far, Henderson et al. showed promising results in the treatment of children with CP (182) using a dose of pamidronate of 9 mg/kg/yr. Lower doses of pamidronate has been administered to children with CP with promising results (234). Osteopetrosis developed in a case of a child with normal bone density who received very high doses of pamidronate (188), suggesting a dose-related effect of the drug. Lower doses of pamidronate will possibly prevent development of osteopetrosis and delayed fracture healing. More research is warranted on these aspects.

Other therapeutic options, such as PTH (355), PTHrP (356), and statins (357) may have a role in the future, but their efficacy and safety have not been assessed in children.

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24

Syndromes with Brittle Bones, Hyperostotic Bone Diseases, and Fibrous Dysplasia of Bone

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OSTEOGENESIS IMPERFECTA (BRITTLE BONES DISEASE)

Introduction

Osteogenesis imperfecta (OI) (1) is a group of heterogeneous disorders with the common feature of congenital bone fragility, caused by mutations in the genes that codify for type I procollagen (*COL1A1* and *COL1A2*)(Table 1). These two genes are located in chromosomes 7 and 17. A comprehensive listing of the mutations within type I collagen genes resulting in OI (2) is maintained in the internet (3). In the vast majority of cases, OI is inherited in a dominant fashion or is caused by a new mutation, but a case of a family in which OI was recessive has been published (4). The prevalence of OI is estimated to be 1 in 20,000 to 50,000 infants (5), but the incidence is probably higher, because being a heterogeneous condition misdiagnosis is frequent. The prevalence of OI appears to be similar throughout the world and in all races (6–9).

Ivar Benløs, eldest son of the Danish legendary king Regnar Lodbrog reportedly had legs as soft as cartilage (he lacked bones), so that he was unable to walk and had to be carried about on a shield (10). Two centuries later, Ivar's skeleton was exhumed and burnt by William the Conqueror; so there is no way to prove that he actually had OI. The earliest example of OI is the skeleton of an Egyptian mummy dating from about 1000 B.C., which is preserved in the British Museum although some argue that OI is not the diagnosis.

Clinical Characteristics

Apart from brittle bones (Fig. 1), clinical characteristics of OI are variable, and there may be a different degree of severity in different members of the same family (11,12). Wormian bones (detached portions of the primary ossification centers in adjacent membranous bones) are present in skull in about 60% of the cases of OI (13), but they can be present in several other genetic conditions including progeria, cleidocranial dysplasia, Menkes syndrome, cutis laxa, Cheney syndrome, and pyknodysostosis (14). Osteopenia due to the basic bone defect is often worsened by immobilization secondary to fractures or surgery and decreased physical activity. Midshaft fractures are the most common in individuals with OI, but it is of note that metaphyseal fractures (often considered as being pathognomonic of nonaccidental injury), can be present in children with OI (15). Other clinical features that may be present are joint hyperlaxity, muscle weakness, chronic unremitting bone pain, and skull deformities (e.g., posterior flattening) due to bone fragility in infants with severe OI. Deformities of upper limbs in the case of severely affected patients may create a compromise of the function and mobility (16). Fractures may still occur after puberty (17), and bone fragility persists throughout life. Individuals with mild forms of the disease may have normal stature, no deformities, and no fractures at all, and the condition be diagnosed when an X ray is obtained for other reasons. People with OI have a high tolerance for pain. Old fractures can be discovered in infants only after X rays are taken for other reasons, and they can occur without any signs of pain. Infants with mild OI can suffer unexplained fractures in the first months of life, with the fracture rate decreasing dramatically thereafter. Exercise tolerance and muscle strength are significantly reduced in patients with OI even in the mild forms (18).

OI can affect several organs and systems. Basilar invagination is an uncommon but potentially fatal occurrence in OI. The prevalence of this complication in patients with OI is not known (19), and its treatment is difficult. Basilar invagination tends to progress despite fusion surgery in 80% of the cases (19). Chiropractic manipulation may cause paraplegia in patients with OI (20). Hypoaccusis may be present in about 50% of the individuals with mild forms of OI after the third decade of life (21). Stapedotomy successfully improved the hearing of patients with osteogenesis type I (22). There is no doubt that hearing screening in children with OI is warranted (23). Vertigo is common in patients with OI (24). The incidence of

Table 1 Syndromes with Congenital Brittle Bones

OI (in order of severity)
Type I
Type IV (moderate OI with short stature)
Type III (severe OI)
Type II (extremely severe OI)
Congenital brittle bones with dense areas in bones
SROI
Congenital brittle bones with rhizomelia
Congenital brittle bones with redundant callus
Osteoporosis-pseudoglioma syndrome
Congenital brittle bones with microcephaly and cataracts
Congenital brittle bones with optic atrophy, retinopathy, and severe psychomotor retardation
Congenital brittle bones with craniosynostosis and ocular proptosis
Congenital brittle bones with congenital joint contractures
Congenital brittle bones with mineralization defect

Abbreviations: OI, osteogenesis imperfecta; SROI, syndromes resembling OI.
Source: from Ref.(1).

congenital malformations of the heart in children with OI is probably similar to that of the normal population (13,25). Hypercalciuria may be present in about 36% of patients with OI (26,27), but it does not appear to affect renal function (27). There is a common misconception regarding malignant hyperthermia in subjects with OI (28). Some patients with the condition have a hypermetabolic state, with excessive diaphoresis, increased oxygen consumption, and elevated thyroxine levels (29). Patients with OI may develop hyperthermia during surgery, but it is rarely malignant. Patients with OI should nevertheless be considered as high risk for anesthesia (30) because they are prone to fracture, may have neck and jaw deformities making intubations difficult, and sometimes severe thoracic deformities and kyphoscoliosis may cause restrictive problems (31). Dentinogenesis severe and valvular heart disease must also be taken into account when evaluating the anesthetic risk of these patients.

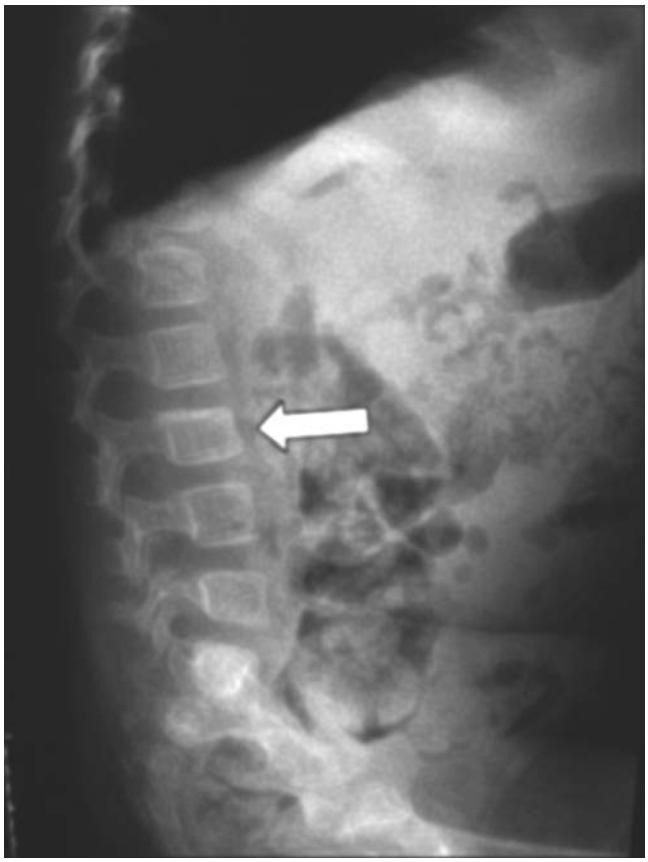

Figure 1 Vertebral fracture in a patient with "type I" osteogenesis imperfecta.

Respiratory complications secondary to kyphoscoliosis are common in individuals with severe OI (32). People with OI have a tendency to bruise easily, probably related to an increase in capillary fragility caused by the underlying collagen defect. Decreased platelet retention and reduced factor VIII R:Ag have also been described in patients with OI (33). Joint hyperlaxity is common in OI (34), and may lead to dislocation of hips and radial heads, sprains, and flat feet. Constipation and hernias are also common in people with OI (35). Dentinogensis imperfecta (DI) is caused by an abnormal dentin, while enamel is normal (36,37). The prevalence of DI in the OI population is of about 28% (38). The presence of DI is not related to severity. For unknown reasons, the permanent dentition is usually less affected than the primary dentition. Subjects with DI do not have an increased susceptibility to cavities and do not always have more dental pain. A more common finding is class III malocclusion [the cusp of the posterior mandibular teeth interdigitate a tooth or more ahead of their opposing maxillary counterparts (39)], with a prevalence of 60% to 80% (40,41).

Life expectancy in subjects with nonlethal OI appears to be the same as the normal population (42), except in cases of severe OI with respiratory or neurological complications (43). Some common misconceptions about OI's clinical presentation are shown in Table 2.

Mode of inheritance in OI is in almost all cases dominant or a new dominant mutation, regardless of the clinical form of OI. A recessive pattern of inheritance has been demonstrated in some families from South Africa (44). The possibility of a germ cell mosaicism (45) has been proposed as an explanation in cases of families with healthy parents that have more than one child with OI (46,47). These cases were previously thought to have been transmitted in a

Table 2 Some Misconceptions about OI

Patients with OI have a tendency to malignant hyperthermia under anesthesia
There are four types of OI
Life expectancy in OI is reduced. Patients rarely reach adult age
All patients with OI have blue sclerae
Infants with mild OI do not present with fractures
Fractures in children with OI take longer to heal, therefore immobilization should be prolonged after a fracture

Abbreviations: OI, osteogenesis imperfecta.

recessive fashion. It has been estimated that in at least 6% of the cases of lethal OI, one of the parents is carrier of a germ cell line mosaicism (48). Interestingly, a person with 40% to 75% burden of osteoblasts heterozygous for a COL1A1 mutation may have normal skeletal growth, density, and histology, and no clinical signs of OI (49).

Classification

Clinical severity of OI ranges from very mild cases with no deformity, normal stature, and no fractures, to forms that are lethal in the perinatal period. An old classification proposed in 1906 by Looser divided OI into two forms (50): ''congenita'' (Vrolik) and ''tarda'' (Lobstein). In OI congenita multiple fractures may happen in utero, whereas in OI tarda, fractures occur at the time of birth or later. OI tarda has also been subdivided in ''gravis'' and ''levis'' (50). This classification is no longer current as it is an oversimplification of the complex clinical picture of OI.

In 1979 a classification was proposed, reflecting the spectrum of clinical presentation of OI (8,51). The classification has received general acceptance despite the fact that there is no consistency in the literature about the characteristics of the different types of OI, and members of the same family (that should have the same OI type) may differ dramatically in severity and clinical presentation (52). Furthermore, this classification does not allow for prognosis. Four types of OI are defined, but it is of note that the general description that follows has not found consensus among experts, who are currently debating the characteristics that define each type. The main drawbacks of this classification are the overlap among different types and the impossibility to do prognosis based on the type. The numeric classification of OI should be used with caution, and severity must always be referred in each individual case. There have been reports in the literature of at least 12 different forms of OI, including two syndromes named ''type V OI.'' There is intense debate about including certain syndromes (e.g., Cole–Carpenter and osteoporosis-pseudoglioma) as forms of OI or not, as described below.

There have also been attempts to classify OI according to radiological characteristics (53), and to severity (17,54). The latter approach appears to be practical in terms of therapeutic decisions, and further development is warranted.

Type I Osteogenesis Imperfecta

Patients have normal stature, and a slightly low stature does not preclude the diagnosis of type I OI. It is important to note that ''type I OI'' is not equal to ''mild OI'' (Fig. 1). While individuals with type I OI may have few or no fractures happening mostly during the first years of life or at birth (55), they may have numerous fractures throughout their lives. They may have triangular face. They are fully ambulatory, and typically do not have bowing of the long bones. Vertebral fractures may be present. For some experts all the subjects with type I have blue sclera, for others most have blue sclera, but it can be white, or blue color may fade as the individual grows older. In all cases this condition is transmitted as an autosomal-dominant trait. Bone density can be very low, with no relation with clinical severity, and despite the absence of fractures. This underscores the relative lack of significance of bone density measurements assessing severity in patients with OI. Bone density is usually normal during the first months of life, progressively failing to increase with age. Diagnosis can be an incidental finding after a fracture (56). DI can be present even in very mild cases. Cardiovascular problems, particularly aortic valvular disease (55) and early hypoacusia (57,58) can be present in these subjects. Individuals with type I OI may continue to sustain fractures into adulthood, and the majority reports some functional impairment (often significant) due to musculoskeletal issues (59).

The most common mutation causing type I OI causes a reduction in the production of otherwise normal type I collagen. Frameshift mutations within the terminal exon of either procollagen gene can lead to synthesis of a full-sized procollagen chain that is rapidly degraded intracellularly after it fails to incorporate into the collagen molecule (60). A process called ''nonsense-mediated RNA decay'' causes production of a severely affected RNA, promoting its intracellular destruction (61–63) causing a clinical picture of mild OI with less, but normal, collagen. Other less common mechanisms for underproduction of collagen include mutations that lead to retention of an intron within the mature transcript (64), mutations within the 3 ft untranslated region affecting polyadenylation, or synthesis of a procollagen chain that is unable to incorporate within the triple helical molecule. The result is production of less than 50% of normal collagen. Often pediatricians encounter patients, particularly teenagers, with several fractures after relatively minor trauma, with bone density in the lower limit of the reference chart, and no bone deformity. In many cases, a skin biopsy will reveal less than normal collagen but more than 50%. It is not clear if these patients actually have a bone disease; studies on this regard are warranted.

Type IV Osteogenesis Imperfecta

This is the least clearly defined type of OI. These individuals have short stature, bowing of some or all long bones, and vertebral fractures, but differential diagnosis with types III and I is not always easy. Scoliosis and joint laxity may also be present. Those with this type of OI are generally able to ambulate, but they may require aids for walking. Type IV OI has been subdivided into two forms (''a'' and ''b'') based on the presence of DI (65). It has been suggested that

all subjects with type IV OI have white sclera, but this is not always the case. Furthermore, in clinical practice, scleral hue has very little significance for the diagnosis and classification of OI unless is very dark blue, as blue sclera may be present in normal children and in other genetic diseases, and has no relation to clinical severity. Precise diagnosis of this type of OI is often difficult, as the clinical characteristics are not clear in the literature, and different centers make the diagnosis based on different criteria.

Type III Osteogenesis Imperfecta

An enlarged head and underdevelopment of the facial bones give the impression of a ''triangular face'' in these individuals. They are frequently nonambulatory, although some are able to walk with aids. Chest deformities (usually pectus carinatum), marked short stature, and severe deformities of the long bones, vertebral fractures, and scoliosis are typical (Fig. 2). Long bones may be severely bowed, and suffer frequent fractures (Fig. 3). A particular structural alteration of the metaphyses and epiphyses and altered structure of the growth plates lead to ''popcorn appearance.'' Long-term survival may be compromised by respiratory complications in very severe cases.

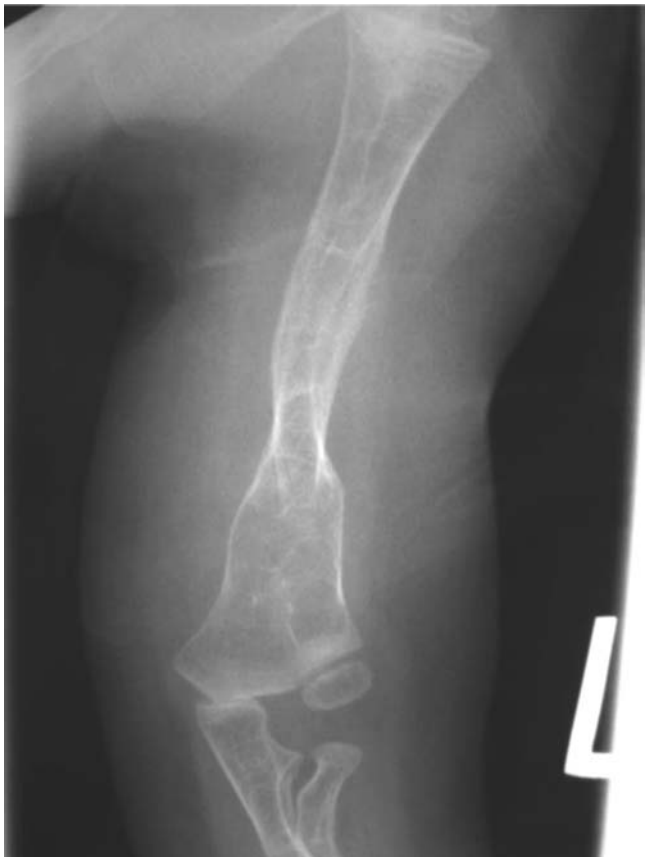

Figure 2 Severe osteogenesis imperfecta.

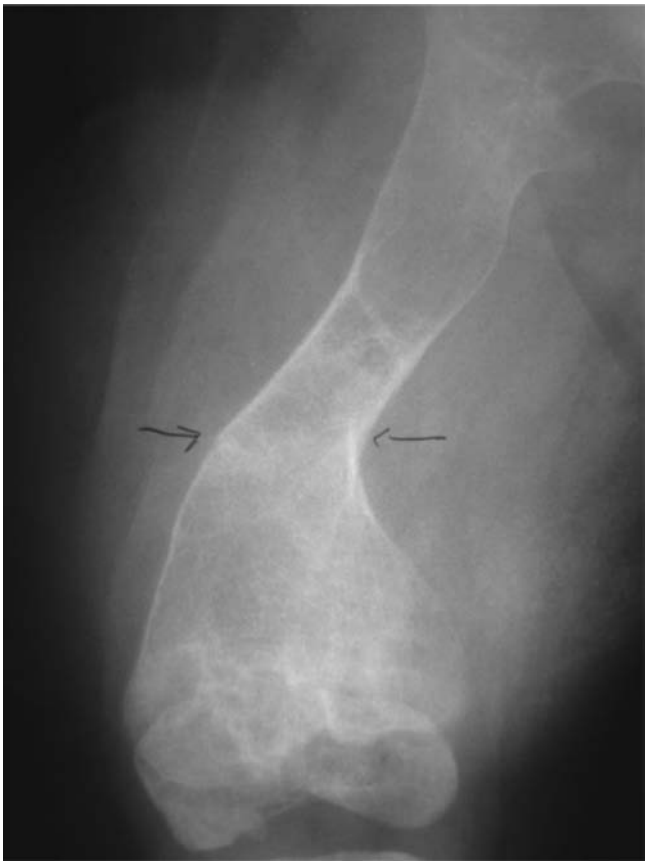

Figure 3 Fracture in a patient with severe osteogenesis imperfecta.

Type II Osteogenesis Imperfecta

Most newborns with type II OI do not survive the perinatal period. Causes of death include extreme fragility of the ribs, malformations or hemorrhages of the central nervous system (66), and pulmonary hypoplasia (67). Newborns present with multiple intrauterine fractures, which may include skull, long bones, and vertebrae; ribs are beaded, and long bones are severely deformed (Fig. 4) (68). Usually, it is not possible to make prenatal differential diagnosis between very severe and lethal OI. There has been a description of an extremely severe baby that was dismembered during delivery (69). Inheritance is autosomal-dominant new mutations in the vast majority of cases (48,70,71). It has been suggested that there may be different clinical forms of lethal OI (72). It is of note that despite severity, a few patients have survived for several years.

Congenital Brittle Bones with Dense Areas in Bones

This form of OI, with documented procollagen gene mutation, has been described in only one infant (73). The girl died shortly after birth and presented with an OI phenotype that differed from the usual type II OI.

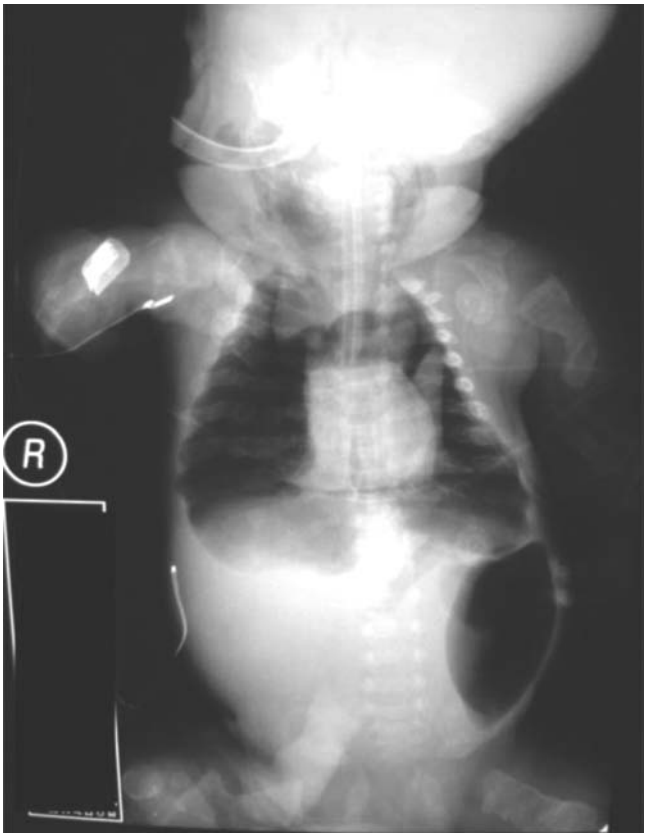

Figure 4 X ray of a newborn with lethal osteogenesis imperfecta. Note the pneumoperitoneum and pneumothorax. The patient died from complications secondary to pulmonary hypoplasia.

Increased bone density was noted in regions of the skeleton, and had dysmorphic facial features, including low set ears, loss of mandibular angle, soft skull, and large anterior and posterior fontanelles. Other features included bilateral upper and lower limb contractures with multiple fractures in the long bones and ribs. X rays also showed dense metaphysis of the long bones. The patient died after a few hours of birth and histopathological studies identified little lamellar bone formation, decreased number of osteoclasts, abnormally thickened bony trabeculae with retained cartilage in long bones, and diminished marrow spaces similar to those seen in dense bone diseases such as osteopetrosis and pyknodysostosis. There was also extramedullary hematopoiesis in the liver. The child was heterozygous for a COL1A14321GàT transversion in exon 52 that changed a conserved aspartic acid to tyrosine (*D1441Y*). Abnormal proa1(I) chains were slow to assemble into dimers and trimers, and abnormal molecules were retained intracellularly for an extended period (73). Because the procollagen I gene mutation was documented, this form of congenital brittle bones should be considered as a form of OI.

Diagnosis and Treatment

There is an interactive network between the cells that make the extracellular bone matrix, with collagen playing an essential role (74) and noncollagenous proteins leading to proper mineralization of bone. X-ray diffraction has shown small collagen fibers with less well-defined lateral growth and more fiber disorganization in tissue obtained from OI subjects (75). Mutations that interrupt the helix decrease the thermal stability of procollagen molecules and render the molecule more susceptible to proteolytic attack by tissue proteases (76). Tissue proteases probably selectively affect mutant molecules (77) allowing relatively normal collagen fibers to accumulate in cases of mild OI. It is of note that other matrix proteins can modify the size and organization of otherwise normal type I collagen fibrils; thus, affecting the mechanical properties of the collagen fibers (78,79), despite lack of mutations in the procollagen genes. Mutations in certain noncollagenous proteins [e.g., decorin (80), fibromodulin (81), and microfibrillin (82)] can affect the organization or structure of type I collagen fibers, indicating that physical interaction among them play an important role in this process. Moreover, mutations that distort triple helix conformation and slow folding rate may favor overglycosylation, which may contribute to bone fragility (83).

The diagnosis of OI may be confirmed by determination of mutations in COLIA1 and COLIA2 genes. This test is now available commercially and may be indicated when a child presents multiple unexplained fractures and there are not enough clinical elements to diagnose or rule out OI, particularly in cases of suspected nonaccidental injury. Currently, several laboratories in the United States and Europe offer molecular diagnostic services, either based on DNA sequencing from peripheral blood or cultured fibroblasts, or on collagen products from cultured cells. However, the cost of the test is between $2000.00 and $4000.00 and the results can be negative in patients with OI for reasons discussed above.

Treatment of OI is a multidisciplinary effort. Medical management has to go together with orthopedic surgery, physical therapy, occupational therapy, nutrition, psychology, social services, and audiology. Other specialists may also need to be involved (pneumonologist, neurologist, gastroenterologist, etc).

Physical and occupational therapy must focus on ''independence,'' as walking is not always a choice, and in that case the use of a wheelchair will be more beneficial for the patient than false expectations of walking. Still, advances in treatment and orthopedic surgery have made it possible for children with severe OI to walk with aids in some cases (Fig. 5) (84). Development of new telescopic intramedullary rods has improved the morbidity of rodding surgeries dramatically. The current tendency is to minimize the number of osteotomies and early weight bearing after surgery. Cochlear implantation in patients with OI is technically possible,

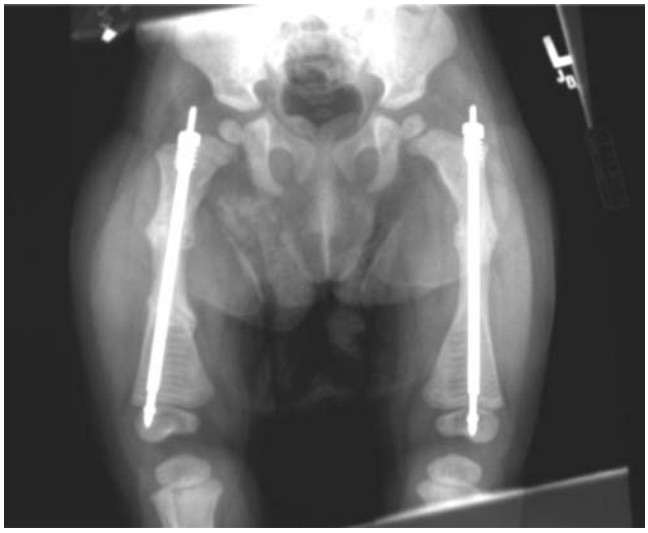

Figure 5 The Fassier–Duval intramedullary rod.

and results appear to be similar to implant outcomes for other patients with sensorineural hearing loss (85). In the section ''Treatment of low bone density in pediatrics'' the medical treatment of OI is discussed.

SYNDROMES RESEMBLING OSTEOGENESIS IMPERFECTA

There is a group of disorders with congenital bone fragility that in many cases have been classified as ''OI'' (Table 1). Mutations in the procollagen genes could not be demonstrated in these syndromes, and in some cases a mutation was identified. In many of these syndromes, inheritance is autosomal recessive (which is exceptional in OI). I have proposed to group these syndromes as ''syndromes resembling OI'' (1). This classification is important for the development of a possible gene therapy. Treatment of low bone density in these patients is similar to that of OI, with the possible exception of lack of response in patients with congenital brittle bones with mineralization defect.

Congenital Brittle Bones with Rhizomelia

This particular form of syndromes resembling osteogenesis imperfecta (SROI) with short humerus and recessive inheritance was only described in a First Nations community of Quebec. It was published as ''type VII OI'' (86), but mutations in the procollagen genes could not be found. Typically, affected individuals have short humerus and femora. Severity in terms of fractures and disability is moderate to severe. Fractures may be present at birth. This condition courses with lower limb deformities early in life, coxa vara, and low bone mineral density. Under the microscope the bone in this SROI is not different to that of mild OI. Linkage studies have mapped the genetic defect to the short arm of chromosome 3 (87), where there are no genes that codify for type I procollagen.

Congenital Brittle Bones with Redundant Callus Formation

Patients with this SROI develop hyperplastic calluses in long bones after a fracture or intramedullary rodding surgery (55). Reports of this complication in patients with apparent OI are numerous in the literature (50,88–94). It was suggested that this SROI could be called ''type V OI'' (95), but again, mutations in the type I procollagen genes could not be found in these patients. Initial presentation often resembles OI with bone fragility and deformity, but these patients develop hard, painful, and warm swellings over long bones that initially may suggest inflammation or osteosarcoma. In X rays a redundant callus can be observed around some fractures (Fig. 6). The size and shape of the callus may remain stable for many years after a rapid growth period (88). Large calluses may also be present in flat bones (96). Osteosarcoma may occur in patients with OI and should be always taken into consideration (97). Histomorphometry studies show that

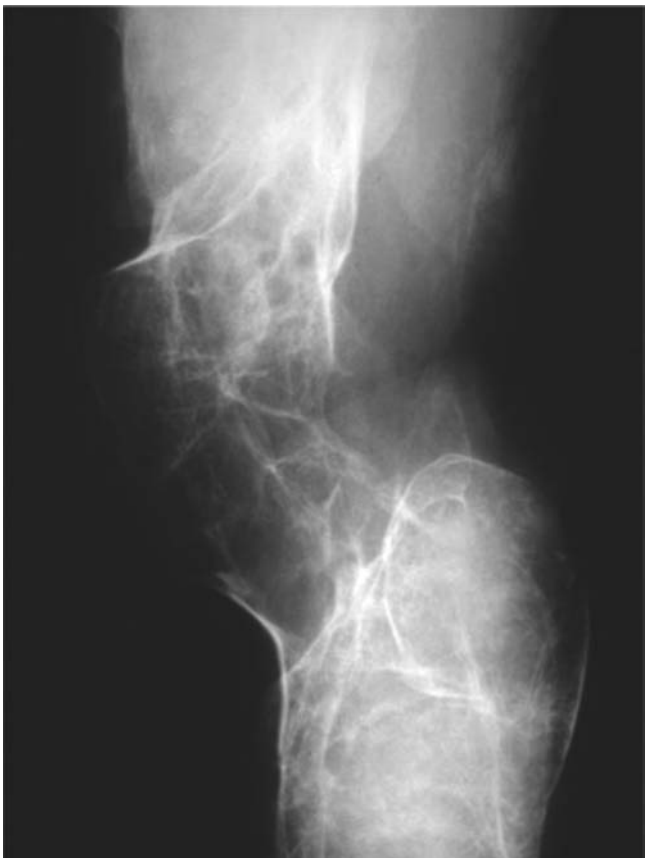

Figure 6 Knee of an adult with congenital brittle bones with redundant callus formation.

the bone lamella are arranged in a mesh-like fashion, as opposed to the typical parallel arrangement in patients with OI (95). Patients with this SROI have white sclera and normal teeth. Mutations in the procollagen genes could not be identified. Inheritance appears to be autosomal dominant. Many patients with congenital brittle bones with redundant callus formation are not able to pronate and supinate the forearms. This is caused by calcification of the interosseous membrane between radius and ulna.

Osteoporosis-Pseudoglioma Syndrome

This SROI was first described in three families in 1972 (98–100), and a different report described a South African family of Indian origin with the same clinical presentation (101). This SROI is inherited in an autosomal-recessive fashion. Bone fragility is mild to moderate. Blindness is due to hyperplasia of the vitreous, corneal opacity, and secondary glaucoma. Failed regression of the primary vitreal vasculature during fetal growth may be the cause of the ocular pathology (102). The genetic defect has been identified and mapped to chromosome region 11q12–13 (103). Specifically, the defect is in the LDL receptor-related protein 5 (LRP5) gene encoding for the low-density lipoprotein receptor-related protein 5 (102). Treatment with IV pamidronate has shown promising results in these patients (104).

The LDL receptor-related protein 5 gene is involved in osteoporosis-pseudoglioma syndrome, the high bone mass phenotype, endosteal hyperostosis, van Buchem disease, autosomal-dominant osteosclerosis, and osteopetrosis type I (105).

Other Ocular Forms of Syndromes Resembling Osteogenesis Imperfecta

At least two other forms of SROI with ocular involvement have been described in the literature. One variant includes optic atrophy, retinopathy, and severe psychomotor retardation (106), and another microcephaly and cataracts (107).

Congenital Brittle Bones with Craniosynostosis and Ocular Proptosis (Cole–Carpenter Syndrome)

Two boys (108) and a girl (109) have been described in the literature as having this particular form of SROI. In the case of the boys, diagnosis was made after several months of life, as they were apparently normal at birth. They developed craniosynostosis, hydrocephalus, ocular proptosis, and facial dysmorphism, multiple metaphyseal fractures, associated with generalized low bone density. Hypercalciuria was present in one of the patients. Neurological development is normal in this form of SROI (1). By adult age, both boys where nonambulatory, with very short stature, severe osteopenia and bone deformity and normal intellectual and neurological development (1). No specific mutation has been identified yet as responsible for this syndrome.

Congenital Brittle Bones with Joint Contractures (Bruck Syndrome)

Patients with Bruck syndrome have congenital brittle bones with congenital joint contractures. Infants are born with brittle bones, leading to multiple fractures, and joint contractures and pterygia (arthrogryposis multiplex congenita) (110,111). Wormian bones are present, and inheritance appears to be recessive (112,113). The syndrome was first described by Bruck et al. in 1897 in an adult patient (114). No mutations in the COL1A1 and COL1A2 genes could be found in three patients with Bruck syndrome that underwent procollagen mutation testing (111). Furthermore, the basic defect was mapped to locus 17p12 (18 cM interval), where a bone telopeptidyl hydroxylase is located (115). The mutation leads to underhydroxylated lysine residues within the telopeptides of collagen type I, and therefore to aberrant cross-linking in bone, but not in cartilage or ligaments. Recently, two mutations in the lysyl hydroxylase 2 gene (*PLOD2*, 3q23–q24) have been identified in congenital brittle bones with congenital joint contractures, showing genetic heterogeneity (116). The original mutation found in OI with congenital contractures leads to underhydroxylated lysine residues within the telopeptides of collagen type I, with aberrant cross-linking in bone, but not in cartilage or ligaments.

Congenital Brittle Bones with Mineralization Defect

This rare form of SROI is undistinguishable from moderate-to-severe OI on a clinical basis. It has been published as ''Type VI OI'' (117). Diagnosis is only possible by bone biopsy, in which a mineralization defect affecting the bone matrix and sparing growth cartilage is evident. Teeth are normal in these patients, and they do not have wormian bones. There are no radiological signs of growth plate involvement, despite the mineralization defect evident in the bone biopsy. The pattern of inheritance is not clear, but gonadal mosaicism or a somatic recessive trait is suggested by the case of two siblings from healthy consanguineous parents (117). Collagen structure appears to be normal in these patients, and no mutations of COL1A1 and COL1A2 genes have been found. This form of SROI shares several characteristics with fibrogenesis imperfecta ossium (118,119). There may be a mild form of this condition, which is very rare (three patients in a series of 128 bone biopsies performed for bone fragility) (120).

Treatment

Treatment of SROI is based on the same principles as treatment for OI. It is also a multidisciplinary effort, including medical management, orthopedic surgery, physical therapy, occupational therapy, nutrition,

psychology, social services, and audiology. As is the case with OI, other specialists may be involved (pneumonologist, neurologist, gastroenterologist, etc). High doses of anti-inflammatory drugs have been used after a fracture to prevent development of excessive callus after a fracture in patients with SROI with redundant callus formation, but more studies are warranted in this regard.

IDIOPATHIC JUVENILE OSTEOPOROSIS

Idiopathic juvenile osteoporosis (IJO) is a syndrome with onset of osteopenia prior to puberty, back and appendicular pain, gait abnormalities, and that resolves spontaneously after puberty. Initial symptoms may be related to long bone fractures, gait problems (121), or back pain (122). This condition was first described by Dent and Friedman in 1965 (123). The mean age of onset is seven years of age (124), with a range of one to 13 years. This highlights the fact that IJO may start at a very early age. It is important to rule out other causes of osteoporosis, particularly those related to malignancy (125). Metaphyseal osteopenia (neo-osseous porosis) has been described as a radiological lesion pathognomonic of IJO by Dent in 1977 (126), but this sign may also be present in polyglandular autoimmune syndrome (127). The etiology is not known. Diagnosis is based both on the exclusion of other diseases and on the clinical evolution of the patients (128). Bone biopsies of patients with IJO showed decreased cancellous bone volume and a very low bone formation rate on cancellous surfaces (129). The typical disturbance of bone remodeling in IJO is limited to cancellous bone, although there may also be a modeling defect affecting the internal cortex (130). IJO appears to mainly affect bone surfaces that are in contact with the bone marrow cavity (130).

Several treatments have been attempted in the past for IJO, including calcitonin (131), sodium fluoride (132), calcitriol (133), and bisphosphonates (134). Because spontaneous recovery occurs it remains impossible to assess the efficacy of these treatments. On the other hand, vertebral fractures may not recover completely without treatment.

OSTEOPETROSIS AND OTHER SCLEROSING BONE DISEASES

Types

The list of sclerosing bone diseases is very long including more than 40 different conditions. Causes can be metabolic (including fluorosis, heavy metal poisoning, hypophosphatemic osteomalacia and rickets, adynamic renal osteodystrophy, and milk-alkali syndrome) or dysplastic (osteopetrosis, melorheostosis, endosteal hyperostosis, Caffey disease, osteopathia striata, van Buchem disease, autosomal-dominant osteosclerosis, pyknodysostosis, and tubular stenosis, among others), and there is a big group that can only be classified as ''others,'' including the high bone mass phenotype, ionizing radiation, diffuse idiopathic skeletal hyperostosis, sarcoidosis, some bone metastasis, and Erdheim–Chester disease.

Osteopetrosis, also known as Marble Bone Disease and Schöenberg disease (Figs. 7 and 8), is a syndrome caused by the inability to reabsorb bone and calcified cartilage. It was first described in 1904, by H. Albers-Schöenberg (135), and it may be secondary to osteoclast malfunction or reduced number of osteoclasts. The defining sign is histological: presence of calcified cartilage, remnant of the primary spongiosa. The reported incidence of osteopetrosis ranges between 1:100,000 and 1:500,000 (136).

Clinical Forms

There are several clinical forms of osteopetrosis: an autosomal-dominant adult type (Albers–Schonberg disease) (137), an infantile malignant autosomal-recessive form (138), a mild autosomal-recessive form with slow progression (139), an autosomal-recessive form with renal tubular acidosis and cerebral calcifications, and a transient form presenting in infancy and gradually improving during the first year of life. A different form is associated with a neuronal storage disease and is lethal. It was described in two brothers who presented with osteopetrosis and accumulation of neuronal ceroid lipofuscin (140). Another form described in the literature has immunodeficiency, lymphoedema, and anhidrotic ectodermal dysplasia (141). This form is inherited in an X-linked fashion. The coincidence of osteopetrosis and rickets has been termed ''osteopetrorickets'' (142). Rickets is a common and paradoxical feature of infantile malignant osteopetrosis, resulting from the inability of osteoclasts to maintain a normal calcium–phosphorus balance in the extracellular fluid (143).

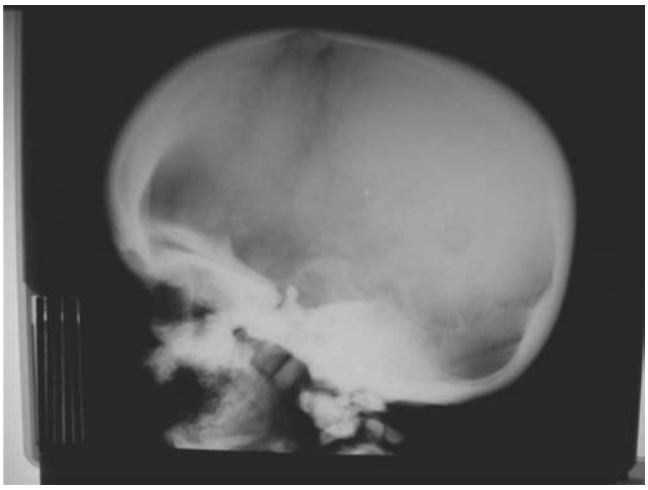

Figure 7 Skull of a patient with osteopetrosis.

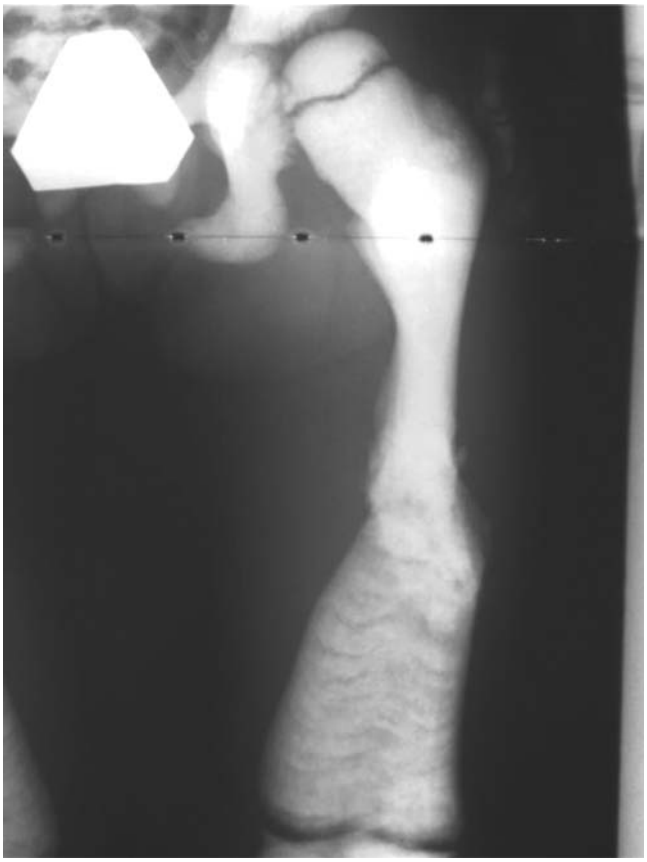

Figure 8 Femur of a patient with osteopetrosis.

Creatine kinase isoenzymes BB-CK is normally predominantly found in brain and is not normally detected in the blood, but it can be measured in patients with osteopetrosis (144). BB-CK is not present in blood of patients with other sclerosing disorders. BB-CK in serum appears to distinguish the osteopetroses among the sclerosing bone disorders (144).

Infantile Malingnant Osteopetrosis

Infantile malignant autosomal-recessive osteopetrosis is a genetically heterogeneous disease characterized by osteoclast malfunction. As a result, patients present with generalized osteosclerosis and obliteration of cranial foramina and bone marrow spaces. The classical clinical features are pathological fractures because the bones are too rigid, progressive visual impairment, and decreased blood cells secondary to bone marrow failure. Hepatosplenomegaly may be present. Ocular involvement starts at about two months of age and is the first sign in about 50% of patients (138). Infections after tooth extraction and fracture of sclerotic bones after mild trauma are serious, hard to treat complications (145). It has been suggested that a combination of elevated serum CK-BB fraction, decreased biochemical markers of bone resorption and soluble receptor activator of nuclear factor kappa-B ligand (sRANKL) with normal–high osteoprotegerin (OPG) concentration (resulting in an elevated OPG to sRANKL ratio) may help in early diagnosis of this condition, particularly in familial cases (146). The probability of survival until the age of six years is about 30% for this type of osteopetrosis (138). Bad prognosis signs are early hematological problems (before three months of age) associated with early visual impairment. It has been suggested that abnormal function of circulating monocytes and granulocytes may contribute to impaired host resistance to infection in these patients (147).

Infantile malignant autosomal-recessive osteopetrosis has been associated with mutations in two different genes: the T-cell immune-regulator-1 gene (in about 50% of the patients), and the chloride channel 7 gene (in about 10% of cases). In one case, osteopetrosis was caused by a mutation in the gray-lethal gene (148). Mutations in the LRP5 gene have been shown to be involved in osteopetrosis type I. Linkage at 11q12–13 has been found in both asymptomatic adult autosomal-dominant osteopetrosis and severe infantile autosomal-recessive osteopetrosis (149,150). A spontaneous 8 bp deletion in exon 2 of the Rank gene causes lethal autosomal-recessive osteopetrosis in mice (151).

Carbonic Anhydrase Type II Deficiency

Carbonic anhydrase type II deficiency is a cause of mild osteopetrosis. It is usually benign in nature and compatible with long-term survival (152). This form of osteopetrosis is associated with metabolic disorders of bone, kidney, and brain. Renal acidification mechanisms appear to be impaired in both the proximal and the distal tubule (153) leading to tubular acidosis (154,155). It is usually diagnosed late in infancy or early in childhood, and clinical features may include short stature, history of pathological fractures, weakness, cranial nerve compression, dental malocclusion, and psychomotor retardation (152). Since first described in 1983, several different mutations within the CA-II gene have been identified (156,157). One mutation associated with this syndrome entails a His107Tyr substitution, which is a destabilizing folding mutation (158).

Treatment

There is no proven medical therapy for osteopetrosis. Bone marrow transplant (BMT) should be considered in severe cases (143,159). Bone marrow transplantation is not a promising approach to correct the renal manifestations of CA-II deficiency (160), and does not guarantee improvement of the condition despite successful engraftment in osteoclast-deficient autosomal-recessive osteopetrosis (161,162). Attempts have been made to treat osteopetrosis patients with high doses of calcitriol (163) and recombinant human interferon gamma (164). Patients with osteopetrosis may develop hypercalcemia after BMT. This complication may be managed with pamidronate infusions (136).

Other Hyperostotic Conditions

Progressive Diaphyseal Dysplasia

Progressive diaphyseal dysplasia (or Camurati–Engelmann disease) is characterized by gradual development of hyperostosis on both the periosteal and the endosteal surfaces of long bones (Fig. 9). The epiphyses are characteristically spared, but it may progress to involve metaphyses. Symptoms include leg pain, limping, a broad-based and waddling gait, muscle wasting, and decreased subcutaneous fat in the extremities, with different degree of severity. Presentation is typically during childhood. Laboratory tests may show elevated serum alkaline phosphatase activity, mild anemia and leucopenia, and elevated erythrocyte sedimentation rate. Bone density values may be elevated. Pamidronate treatment has been showed to actually increase bone pain in these patients (165). Low doses of prednisone may be beneficial if pain interferes with activities of daily living.

Ribbing's Disease

Ribbing's disease is a mild form of Camurati–Engelmann disease (166). It was first described in 1949 in four siblings (167). The disease is confined to the diaphyses of long bones, particularly tibias and the femurs. Engelmann disease is bilateral and symmetric, Ribbing's disease can be unilateral and asymmetric. In Engelmann disease there may also be involvement of the skull. Patients with Engelmann disease may have gait and neurological abnormalities and anemia with extramedullary hematopoiesis. Pain is frequently associated with the disease. This is probably caused by bone marrow edema (168). Patients with Ribbing's disease may also have osteosclerosis (169). Differential diagnosis include stress fracture, chronic infection, systemic metabolic or endocrine disorders (170), and bone-forming tumors (171). As could be expected, pamidronate treatment is not effective to treat Ribbing's disease (168).

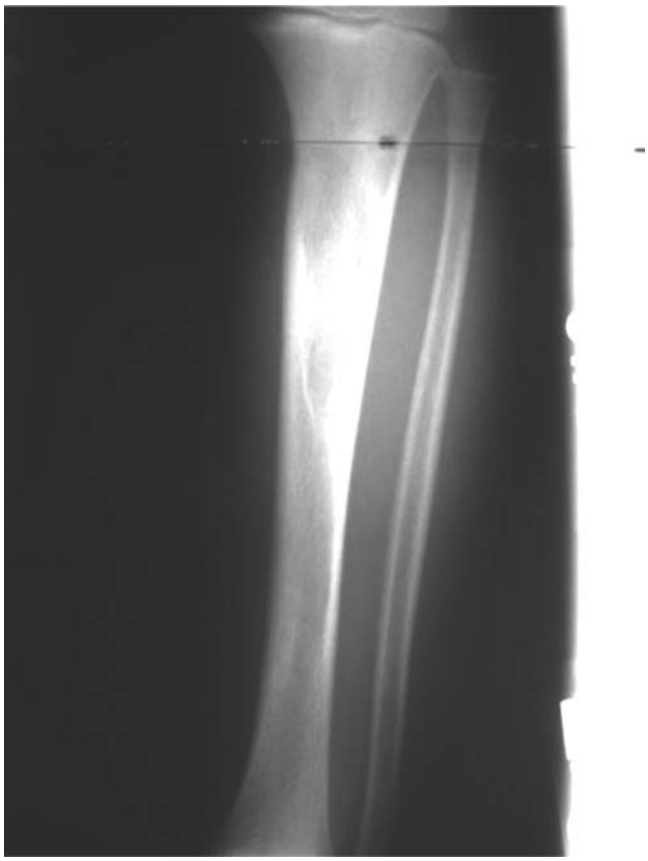

Figure 9 Ribbing's disease.

Caffey Disease—Infantile Cortical Hyperostosis

In Caffey disease (infantile cortical hyperostosis) patients present with spontaneous episodes of subperiosteal new bone formation in one or more bones. Usually, it starts within the first five months of life. This disease is rarely diagnosed after five months of age and usually resolves after two years of age (172). It may be present at birth. Clinically, patients present with inflammatory processes, with fever and hot, tender swelling of involved bones, including mandibles and ribs (173). The causal mutation appears to be in chromosome 17q21. Mutations in the same locus have been described in patients with OI and Ehlers–Danlos syndrome type I. Affected individuals and obligate carriers were heterozygous for a missense mutation in exon 41 of the gene encoding the alpha1(I) chain of type I collagen (*COL1A1*). Fibroblast cultures from an affected individual showed larger, more variable in shape and size, and less densely packed dermal collagen fibrils than those in control samples. Individuals bearing the mutation had joint hyperlaxity, hyperextensible skin, and inguinal hernias resembling symptoms of a mild form of Ehlers–Danlos syndrome type III, independently of the presence of cortical hyperostosis (174). This is the first time that a mutation in COL1A1 gene is related to a hyperostotic disorder.

van Buchem Disease

Classified as a ''craniotubular hyperostosis,'' together with sclerosteosis (175), van Buchem disease (176) (hyperostosis corticalis generalisata familiaris) is an autosomal-recessive disorder characterized by generalized progressive osteosclerosis more evident in the skull and mandibular bones (177). As a consequence, deafness, visual impairment facial nerve palsy, and pain due to narrowing of the cranial foramina are common. Recently, a mutation of the SOST gene was described in two siblings of German origin with van Buchem disease (178). The SOST gene codifies for sclerostin, an osteocyte-expressed negative regulator of bone formation (179).

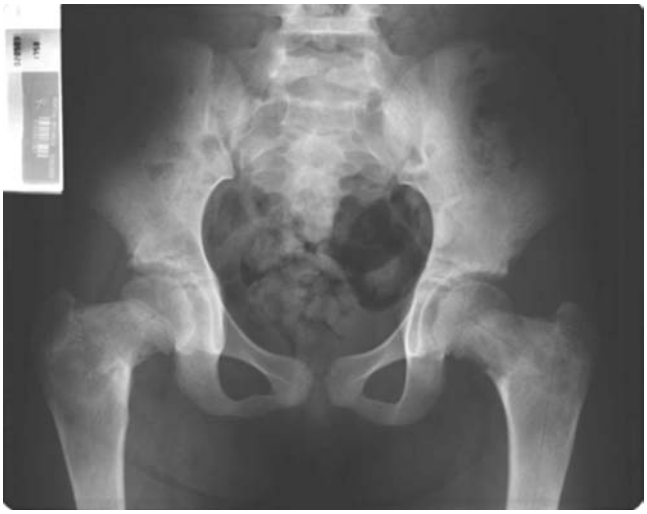

Figure 10 Fibrous dysplasia lesions in an adolescent.

Sclerosteosis is a more severe, sometimes lethal, form of sclerosing bone disease. Elevated intracranial pressure, syndactyly and tall stature are frequently observed, aiding with the differential diagnosis with van Buchem disease. Mutations in the SOST gene were also identified in patients with sclerosteosis (180).

FIBROUS DYSPLASIA OF BONE

Fibrous dysplasia of bone (FD) is an uncommon nonheritable congenital disorder affecting both sexes. It is characterized by expanding fibrous lesions that contain bone-forming mesenchymal cells, and disruption of normal bone architecture. In the majority of cases, only one bone is involved (Fig. 10) (181–183), but the disease may be polyostotic and even panostotic (184). The triad of polyostotic fibrous dysplasia, abnormal skin pigmentation and precocious puberty (or various other endocrinopathies) is identified as McCune–Albright syndrome (181). It has been estimated that precocious puberty affects about 20% of girls and a smaller percentage of boys with polyostotic fibrous dysplasia (185). In children, FD can be associated with hypophosphatemic rickets (186).

G Protein Alteration

This metabolic disorder is caused by a sporadic postzygotic-activating mutation in the GNAS1 gene that codes for the G(s) alpha protein in the cAMP-signaling cascade, resulting in increased production of cAMP, and thus the effects of hormones normally utilizing cAMP as a second messenger occur without actual stimulation by the hormones (187).

The Gs protein is part of the PTH receptor. Because of the activating Gs protein mutation, the cells ''think'' that the PTH is stimulating its receptor, and respond in consequence.

The distribution of the mutation follows a mosaic pattern, and it can be present in different tissues, in particular kidney and bone (188,189). In bone cells (190,191), the mutation leads to increased production of c-fos protein and interleukin-6 (192,193). The mutation is also found in affected skin tissue and endocrine tumors present in patients with McCune–Albright syndrome (FD associated with café-au-lait spots and gonadotropin-independent precocious puberty) (187,188).

Clinical Findings

Spinal lesions and scoliosis are frequent in patients with polyostotic fibrous dysplasia. Since there is a strong correlation between a spinal lesion and scoliosis, these patients should be screened clinically for scoliosis (194). The natural evolution of FD is highly variable (185). Lesions may remain stable for decades, but may progress relentlessly, leading to multiple fractures and severe bone deformities (Fig. 11). The disease progress appears to be more rapid in the growing skeleton (195), in line with the high turnover rate characteristic of children and adolescents.

Radiological signs of FD consist mainly of lytic and cystic lesions, with reduction of cortical thickness, and sometimes widening of the diaphysis. Radioisotopic bone scans usually disclose increased uptake in affected areas. Indices of mineral metabolism and bone turnover have been reported to be within the normal range in some cases. However, hypophosphatemia may be present in some patients and biochemical markers of bone turnover may be altered (particularly alkaline phosphatase), especially if lesions are widespread (186).

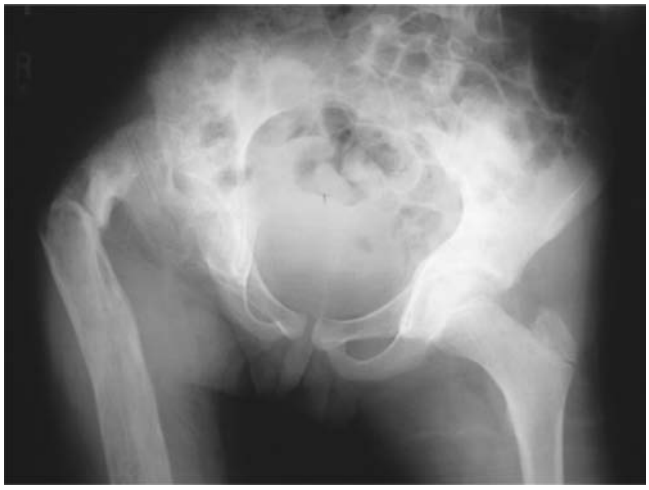

Figure 11 ''Shepper's hook'' deformity in a case of fibrous dysplasia of bone.

Histological examination of the bone lesions shows spindle-shaped cells, which have been identified as incompletely differentiated osteoblasts (196,197). They produce a matrix of randomly distributed collagen fibers and islands of woven bone (196). There is also evidence for increased bone resorptive activity, as the number of osteoclasts within the lesions is slightly higher than normal (193,196,197). In addition, the number of nuclei per osteoclast appears to be increased (193). Presumably, osteoclasts are part of the mechanism responsible for the spread of the lesions through normal adjacent bone (185). An interesting method to measure the disease burden using bone scintigraphy with ^{99m}Tc-labelled bisphosphonate was recently published (198).

Diagnosis and Treatment

Laboratory tests to be requested include a complete phospho-calcic profile (fasting serum calcium, phosphorus, magnesium, alkaline phosphatase, creatinine, PTH, 25(OH) vitamin D, 1,25(OH) 2 vitamin D, and studies of calcium and phosphorus excretion in urine). A radiological skeletal survey, including lateral views of the spine and a scintigraphic bone scan should be ordered. In selected cases, head CT scan may be necessary to rule out compromise of the base of skull.

Treatment of FD has been largely confined to orthopedic surgery, consisting of preventive measures (curettage, bone grafting, and internal fixation of long bones), and management of fractures (199). Curettage and bone graft does not prevent the recurrence of the bone lesions (195). Medical treatment with calcitonin and mithramycin in individual cases has shown little effect (200,201). However, an open-label study of treatment with the bisphosphonate compound pamidronate has yielded promising results in adults (202,203). The positive response in treatment of Paget disease [an entity that has similarities with FD (204)], has led to the use of bisphosphonates in patients with the disease. Several studies have been published showing results of the use of pamidronate treatment in children with FD. In one, nine children were treated once per year initially, and then every six months, without radiological signs of healing (205). In the other, five children were treated every six months for two years (206) but there was no description of the radiological changes of the lesions in this report. Both publications reported clinical and biochemical improvement. On the other hand, a report published in 2004 (207) also showed that levels of serum alkaline phosphatase and urinary collagen type I N-telopeptide (elevated at baseline), decreased continuously during the first three years of therapy, but there was no radiographic evidence of filling of lytic lesions or thickening of the bone cortex surrounding the lesions in any of the 18 patients studied. Analysis of histomorphometry results in dysplastic bone tissue of patients receiving pamidronate was similar to those of patients without medical therapy. Therefore, pamidronate therapy appears to be safe and may have a positive effect on pain in children and adolescents with polyostotic FD. Pamidronate may slow down the progression of the lesions; however, there is no clear evidence that pamidronate has a ''curative'' effect on dysplastic lesions (207).

TREATMENT OF LOW BONE DENSITY IN PEDIATRICS

Prevention of metabolic bone diseases is often possible, and should be the main goal of any pediatrician or pediatric specialist. This is particularly true in the case of nutritional rickets and many secondary bone diseases. For example, children with cerebral palsy may benefit from standing programs, although the benefits of this therapy are yet to be demonstrated (208). A good vitamin D intake is paramount. Usual fortification of milk with vitamin D in the United States is 400 IU per liter. Supplements may be required if milk intake is low.

Several medical therapies failed to increase bone density and decrease the incidence of fractures in patients with OI (209), including magnesium (210,211), anabolic steroids (212,213), vitamin C (214,215), sodium fluoride (17,216,217), and calcitonin (218,219). BMT has been attempted in children with OI. Very few patients have received this type of treatment. One of them suffered sepsis, pulmonary insufficiency, and a bifrontal hygroma after BMT. There have been no publications on the subject since 1999.

Other important therapeutic aspects that need to be considered include psychology, dentistry, social services, audiology, nutrition, physical therapy, and occupational therapy.

Bisphophonates

Bisphosphonates have been used to treat osteopenia of primary and secondary origin (220). These drugs have a chemical structure based on pyrophosphate (221). Effects on both osteoblasts (222,223) and osteoclasts (224–226) have been documented. It is not completely clear what is the mechanism of action of bisphosphonates on bone resorption. It may depend on the induction of osteoclasts apoptosis, but there is also evidence that the antiabsorptive action of bisphosphonates does not require osteoclast apoptosis. Furthermore, it is not known why they have a clear effect on pain that is not explained by cessation of microfractures, as it is very rapidly evident after each infusion.

Several noncontrolled studies using bisphosphonates in children have been published for treatment of OI (134,227–236) and secondary osteoporosis (237–241), all showing promising results in terms of increased bone density. Different protocols use

pamidronate at different doses, ranging from 4.5 mg/kg/yr (242, 245) to 9 mg/kg/yr. There may be a dose-related side effect of retention of calcified cartilage with pamidronate (242). A child with normal bone density that received very high doses of pamidronate developed osteopetrosis (243). The dose can also be calculated per body surface or at a fixed dose, depending on the protocol used (235,244).

Pamidronate treatment of children with osteoporosis secondary to severe cerebral palsy has been successfully tried in the past (240). The dose used was 9 mg/kg/yr, based in the doses used for adults. It has been shown that pamidronate doses of about 9 mg/kg/yr cause retention of calcified cartilage within secondary spongiosa in children with OI (226), whereas higher doses have caused osteopetrosis in a patient with no diagnosis (243). This suggests a dose-related effect of pamidronate. The need to test the efficacy of lower doses of pamidronate in the pediatric population has been recognized in the past (233,240,244,245). Treatment with low doses of pamidronate (4.12 mg/kg/yr) is generally safe and increases bone density significantly in both lumbar spine and femoral neck in nonambulatory children (242). Other bisphosphonates are under evaluation for the treatment of patients with OI. Neridronate, has shown promising result in the treatment of prepubertal children with OI (246), and zoledronic acid is currently under study in a multicenter, international study.

Oral bisphosphonates [pamidronate, alendronate, risedronate, and olpadronate (247)] can be used in children with OI that are able to swallow pills and have no gastroesophagic reflux. Patients taking oral bisphosphonates have the theoretical risk of gastric discomfort or even severe burning of the esophagus if the drug is not taken properly. Oral alendronate has been used in children with OI in a double-blind study (248). Basically, alendronate, 5 or 10 mg, produced a substantial increase in lumbar spine BMD (increase of 1 BMD *z*-score unit, equivalent to a placebo-subtracted increase of 26% in one year), but radiologically confirmed and investigator-reported long-bone fractures, found no treatment-group differences. Increases in vertebral bone area and metacarpal cortical width were also seen in both treatment groups over the 12 months of double-blind therapy, but there were no significant between group differences in these parameters. There were no other treatment-related benefits of the drug, relative to placebo. Particularly, bone pain, bone pain frequency, and pediatric disability scores were no different in treated children compared to those who received placebo. It is of note that oral bioavailability of alendronate and risedronate in children is very low (249,250).

Oral olpadronate has also been used in a double-blind study treating children with OI of three to 28 years of age (251). This study, as the alendronate study, found no differences between olpadronate and placebo on functional outcome, and anthropometrics. There were also no differences in vertebral height. Interestingly, markers of bone turnover were no significantly different in the treated group. An explanation for this is not offered in the paper.

Changes in bone density with IV pamidornate treatment are dramatic in the pediatric population. Increase in infants can be as high as 200% per year (230,252). The deviation of bone mineral density from normal, as indicated by the *z*-score, improves in several points. Other effects include increase of the cortical width of the metacarpals, and increased vertebral height. The mean incidence of radiologically confirmed fractures decreases as well, and fracture healing does not appear to be impaired in these patients compared to OI patients not receiving treatment (253,254). It is of note that in infants with severe OI and preexisting respiratory compromise, the first pamidronate infusion cycle may be associated with an acute deterioration of respiratory function (255) and should be done in a hospital setting. There is a report of two women who became pregnant while under pamidronate treatment. One baby had hypocalcemia at birth and the other had bilateral talipes equinovarus. Both babies were affected with OI and had good evolution thereafter (256).

A trend to decrease the fracture rate is noted with treatment despite higher risk of injury due to increased mobility, suggesting a direct effect of the therapy. Larger studies are warranted to assess the effect of treatment on fracture incidence. The disappearance of bone pain and decreased fracture incidence may contribute to greater mobility (257), an essential factor for the development of the skeletal system (258).

Pamidronate does not have a detrimental effect on growth. Instead, the height *z*-score increases in patients who have started treatment before three years of age (230). After one year of pamidronate therapy in older children height *z*-scores increase significantly in patients with severe OI and do not change in patients with mild and moderate OI (259). After four years of pamidronate therapy, mean height *z*-scores increased significantly in children with moderate OI, whereas nonsignificant trends to increase are characteristic of patients with mild and severe OI (259). There is no significant change in size of bone biopsies from the iliac crest with pamidronate treatment in children with OI, whereas cortical width increases by about 90% and cancellous bone volume increases by about 45% [due to higher trabecular number, with no change in trabecular thickness (226)]. There is no evidence for a mineralization defect in children with OI treated with pamidronate (226).

Serum levels of ionized calcium drop and serum PTH levels almost double after the first pamidronate infusion (260). At the same time, urinary excretion of the bone resorption marker type I collagen N-telopeptide (related to creatinine—uNTX/uCr) decreases by about 60% to 70% during the first infusion cycle, and PTH levels increase by about 30% in the same

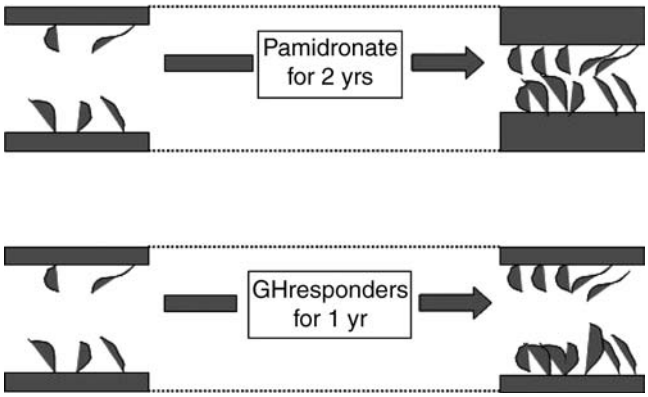

Figure 12 Schematic representation of microscopic changes in bone under bisphosphonates and growth hormone in children with osteogenesis imperfecta. There is an increase in the number of trabecula and the thickness of the cortex with pamidronate treatment, whereas under GH treatment there is no change in cortical width at the level of the iliac crest. *Abbreviation*: GH, growth hormone.

period. Skin rashes have been described in patients receiving pamidronate treatment (261). Pamidronate can also cause anterior uveitis, nonspecific transitory conjunctivitis, episcleritis, and scleritis (262).

Long-term effects and ideal dose scheme of bisphosphonate treatment are not well characterized; therefore, this therapy should be administered only under strict protocols.

Growth Hormone

The use of growth hormone (GH) in children with severe OI is controversial. Children with OI treated with GH can be ''responders'' or ''nonresponders'' in terms of linear growth (263). Treatment determines an increase in cancellous bone volume and trabecular number in ''responders,'' but cortical bone does not change at all, even in those responding in terms of growth. Furthermore, bone resorption is increased in ''nonresponders'' (Fig. 12). PICP appears to be higher at baseline for responders, but response to GH in children with OI cannot be accurately predicted. An increase of fracture rate during GH therapy has been reported (264,265). In a controlled study, comparing seven children with mild OI with seven receiving no treatment, fracture rate was not different between the groups (266). GH should not be used as a first-line therapy in OI (267).

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Abnormal Endocrine Test Results Due to Nonendocrine Conditions

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INTRODUCTION

The endocrinologist is frequently consulted with abnormal test results, which are caused by technical problems or systemic conditions that may be a physiologic response or an adaptive mechanism of the body. It is the clinician's duty to detect these nonendocrine aberrations in order to prevent unnecessary or even harmful diagnostic and/or therapeutic interventions. In this chapter, we will outline some of the possible causes of abnormal endocrine tests not caused by endocrine disease and concentrate on systemic conditions that may cause endocrine alterations.

The problem may be as simple as an error in collecting or handling a sample. The events that precede or surround an abnormal test must be carefully delineated. For example, hyperammonemia following a febrile seizure is most probably due to strenuous muscle activity, and not an inborn error of metabolism (1). The next step for probable errors may be related to the methods utilized in the laboratory. Possible sources of error in this step range from whether or not the ligand was extracted to specificity and sensitivity of the assay, and assay validation (2). Last, but not least, factors that may lead to abnormal test results is nonendocrine conditions such as acute stress or malnutrition (3,4). In addition, the clinician must always try to explain the pathogenetic mechanism that could cause the hormonal alteration. For example, an elevated parathyroid hormone (PTH) in the presence of hypocalcemia represents a secondary compensatory attempt, whereas a high PTH level is more likely to be the primary event when it is associated with hypercalcemia. Therefore, metabolites or hormones that are closely linked such as T4 and thyroid-stimulating hormone (TSH), glucose and insulin, calcium and phosphorus, and sodium and potassium must be measured and interpreted simultaneously.

In addition to the sampling and handling errors there may be variations in laboratory methods and variability among laboratories that need to be considered, although these factors are beyond the scope of this chapter. Here are described the physiologic status or nonendocrine conditions that alter endocrine function and produce abnormal tests that need to be considered by pediatric endocrinologist in assessing a patient.

TIMING AND PHYSIOLOGIC STATUS

Many hormones show significant diurnal and seasonal variations that must be taken into consideration. The serum concentrations of adrenocorticotropic hormone (ACTH) and cortisol peak in early morning hours and decline to nearly undetectable levels in the evening. The establishment of this diurnal rhythm is age dependent and occurs in late infancy. In contrast, the concentration of TSH, 17-hydroxyprogesterone, and testosterone rise to higher levels later in the day. Growth hormone (GH) and gonadotropins are released in pulses and single measurements may not represent their deficiency or excess. The pain and stress of a venipuncture may cause transient elevations in cortisol, prolactin, or catecholamines. A high-carbohydrate diet must be consumed for several days preceding an oral glucose tolerance test for appropriate interpretation of the glucose levels attained. Failure to follow a special diet before measuring urinary vanyllilmandelic acid may cause a false increment leading to an erroneous diagnosis of pheochromocytoma (2). It is common practice that the endocrinologist may have to reassure anxious parents regarding the test results that are out of the normal range on the laboratory report. However, the normal range may differ according to the various stages of growth in children including the gestational age, postnatal age, gender, and pubertal stage of the patient. The female sex hormones must be interpreted with relevance to the time of menstrual cycle. Vitamin D concentrations may change seasonally. Postural change affects the concentration of renin, aldosterone, and catecholamines. Acute physical exercise raises the

serum concentration of catecholamines as well as ACTH, cortisol, vasopressin, and glucagon (2,3).

The interpretation of the thyroid hormone levels obtained by newborn screening may be difficult particularly when the infant is premature or has a low birth weight (Vol. 2; Chap. 16). The majority of the babies born before 30 weeks of gestation have a low total and free T_4 level (5). Premature infants also have lower T_3 and thyroxine-binding globulin (TBG) levels. Most of the babies have a normal TSH (6). The usual course in these infants is normalization of the thyroid hormones in six to 10 weeks (7). The cause of low T_4 levels in low-birth-weight newborns may be due to iodine deficiency in certain geographic regions or to increased urinary excretion of iodine because of renal immaturity (8,9). Other responsible factors may be a decreased rate of thyroglobulin synthesis, partial organification defect due to the immaturity of the thyroid gland, low TBG, or increased peripheral utilization of the thyroid hormones under stress (10–12). The reason for the normal TSH may be hypothalamic or pituitary immaturity (13).

The clinician may have to make a critical decision about whether or not the infant requires thyroid hormone replacement. Treatment is certainly recommended if TSH is elevated (5–7). Infants with normal TSH but low free T_4 or signs and symptoms suggestive of congenital hypothyroidism should also be treated (14,15). It has been reported that very-low-birth-weight babies born at 25 to 26 weeks of gestation and treated with l-thyroxine had higher developmental scores at 24 months of age than the placebo control infants (16). However, no benefit of thyroid replacement has been reported on growth and development of premature babies with low total T_4 (17). A thorough review of the alterations in thyroid function in infancy is made in Vol. 2; Chap. 16.

NONENDOCRINE SYSTEMIC CONDITIONS

The accumulation of knowledge has been accelerating exponentially, which warrants the development of new subspecialties under the disciplines that have already become established. During this inevitable process, the clinician who applies the knowledge to a variety of patients must maintain the unity of pediatrics and continue to see the patient as a whole. While the researchers advance and produce new knowledge, the clinicians establish the bridges between the systems. Furthermore, the clinicians contribute a lot to this evolution by observing the intricate relationship between the details. This is most evident when interpreting an abnormal endocrine test result because it applies to a particular patient.

Endocrine Alterations in Psychiatric Disease

The symptoms of many psychiatric diseases and endocrine abnormalities overlap. It is well known that certain affective disorders and eating disorders originate from hypothalamic dysfunction. As described below, alterations in appetite, sleep, circadian rythms, and reproductive function may be due to depression. Anorexia nervosa may cause variations in hypothalamic–pituitary–adrenal axis as well as changes in gonadotropin secretion. Central neurotransmitters that play role in the pathogenesis of psychiatric diseases also control the synthesis and release of hypothalamic peptides and pituitary hormones. In addition, hypothalamic hormones via their receptors widely dispersed in the central nervous system, control the functional effectiveness of the neurotransmitters. The end result of these complex pathophysiological alterations may result in altered endocrine function that often triggers a consultation with the endocrinologist.

Depression

Hypercortisolism is the most common somatic abnormality in major depression (18). This issue must be considered before diagnosing cortisol excess in a depressive patient. A defect in the hypothalamus or higher cortex may produce excessive corticotropin-releasing hormone (CRH). Therefore, ACTH response to CRH stimulation is blunted in these patients (19). This finding demonstrates that the corticotrophic cells in the pituitary gland are sensitive to the negative feedback of cortisol and the source of hypercortisolism is suprapituitary. In addition, the adrenal response to ACTH is increased in depressive patients leading to bilateral adrenal hyperplasia (19). The astute clinician may appreciate that such findings even those visualized by imaging techniques can be caused by a psychiatric illness, not by a primary alteration of the endocrine system.

Loss of libido and secondary amenorrhea may also be due to depression. The secretion of luteinizing hormone-releasing hormone (LHRH) is under the tonic inhibition of β-endorphin released from the arcuate nucleus (20). Injection of CRH into the arcuate nucleus in animals increases β-endorphin and suppresses reproductive function. Glucocorticoids may also inhibit reproductive function at the hypothalamic, pituitary, and gonadal level (21).

The thyroid functions may also be altered in depression. For example, TSH response to TRH stimulation is blunted and the expected nocturnal rise in TSH may be absent. The peripheral conversion of T_4 to T_3 is also decreased (22). These changes are consistent with euthyroid sick syndrome and may be all mediated by glucocorticoids (23). Frank hypothyroidism may develop due to the treatment of depression with lithium (24).

GH response to hypoglycemia, l-DOPA, clonidine, and GH-releasing hormone is decreased in depression. In contrast, 24-hour GH release as well as insulin-like growth factor-1 (IGF-1) is increased in major depression. This increase is accomplished by suprapituitary mechanisms despite the decrease in

sleep. Hypercortisolism seems to play a major role by inducing resistance to IGF-1, thereby increasing the concentrations of both GH and IGF-1 (24,25).

It has been reported that bone mineral density is decreased in major depression. Depression is a risk factor for osteoporosis and pathologic fractures (26). Although the exact mechanism is not clear, both bone formation and resorption are decreased. Possible contributing factors are inadequate nutrition, decreased activity, hypercortisolism, and hypoestrogenemia associated with menstrual irregularities. On the other hand, serum calcium, vitamin D, and PTH are usually normal. Although these alterations are usually manifested long term, often beyond the scope of pediatric endocrinologists, attention must be given to assure that depressed youth receive the appropriate care to help prevent these alterations in adult life.

Anorexia Nervosa

The neuroendocrine manifestations of anorexia nervosa include hypercortisolism, hypothalamic hypogonadism, alterations in vasopressin secretion, and decreased thyroid functions (27,28). Any one of these may be manifested by an abnormal endocrine test result requiring an appropriate interpretation and management. These endocrine changes may be primary or secondary to weight loss. The plasma- and urinary free cortisol concentrations are frequently above the limits observed in depression and in the range encountered in Cushing's syndrome. However, in contrast to Cushing's syndrome, the diurnal variation is preserved. The pituitary and adrenal responses to CRH stimulation are similar to that present in patients with depression and denotes that hypercortisolism is centrally mediated. The basal plasma cortisol is elevated and the ACTH response to CRH is blunted. Therefore, the source of hypercortisolism seems to be hypothalamic or higher in the cortex (28). The plasma and urinary cortisol levels return to normal in a few weeks after weight gain with treatment and nutritional improvement there is recovery in the central source of hypercortisolism. However, the blunted ACTH response to CRH and adrenal hyperresponsiveness returns to normal slowly, after six months (28).

The alterations in antidiuretic hormone (ADH) due to anorexia nervosa has been well described. During weight loss, ADH response to osmotic stimulus is diminished. The patient may have a low plasma ADH concentration and its elevation in response to increasing sodium concentration may be delayed. Furthermore, the linear relationship between sodium and ADH may be completely demolished. This is similar to the situation described in patients with an anterior pituitary tumor where the osmostat mechanism is damaged, but the neurosecretory cells are preserved. It may take months to normalize the ADH physiology after anorexia nervosa is treated. Similar changes are observed in bulimia; ADH response to osmotic stimulus is decreased, but the linear correlation between sodium and ADH is preserved (29).

Gonadotropin concentrations are decreased and serum estrogen concentration is low and stable in anorexia nervosa. FSH and LH response to gonadotropin-releasing hormone (GnRH) stimulation test is usually normal. These hormonal changes associated with amenorrhea may precede weight loss in anorexia nervosa in 50% of the patients (30). It is important to remember this in the differential diagnosis of secondary amenorrhea and hypogonadotropic hypogonadism before proceeding to more sophisticated tests.

Hypothalamic hypogonadism may also be partially due to hypercortisolism. The increase in CRH may inhibit GnRH and glucocorticoids may suppress reproductive functions at the hypothalamic, pituitary, and gonadal level. Hypercortisolism due to excessive exercise may also impair menstrual physiology during the early stages of anorexia nervosa (21,31).

Hypothalamic amenorrhea, also called functional amenorrhea, is frequently seen in women who are athletic, underweight and/or stressed, and who do not meet criteria for an eating disorder. These patients are usually referred to the endocrinologist because of their amenorrhea that is usually preceded by menstrual irregularity, weight loss, or increased physical activity. The role of energy deficiency in the pathogenesis of these alterations are very important, even when there is no weight loss and when the body weight is normal, because there may be subtle deficits in calorie or micronutrient intake. The central energy-related hormone leptin is the common factor underlying the pathogenesis of this entity now considered a leptin deficiency condition (32). The distinguishing features of hypothalamic amenorrhea from anorexia nervosa as well as the pathophysiology of the disease because it relates to body fat, leptin, and amenorrhea are clearly described elsewhere (33). The patient with anorexia nervosa is typically an adolescent girl who is severely wasted and continues to starve herself with distorted attitudes about food and weight control such as feeling fat despite wasting and exercising excessively to lose weight. The neuroendocrine changes are consistent with starvation and osteopenia and osteoporosis is common. Other clues that may support the diagnosis of anorexia nervosa include hypercarotenemia, dry skin, lanugo-type hair, pedal edema, anemia, leukopenia, thrombocytopenia, low metabolic rate, cold intolerance, hypothermia, dehydration, electrolyte imbalance (hyponatremia and hypokalemia), bradycardia, hypotension, and systolic murmur.

Euthyroid sick syndrome may also occur in anorexia nervosa. The abnormal thyroid tests frequently observed in anorexia nervosa include a low T_3, high reverse T_3 (rT3), normal to low T_4, low-normal free T_4, and normal TSH (34). The TSH response to TRH and iodine uptake on thyroid scan is often diminished. The recovery period for thyroid

Table 1 Endocrine and Nonendocrine Causes of Hyperprolactinemia Other than Prolactinoma

Sleep
Pregnancy
Nursing
Neonatal period
Stress
Exercise
Estrogens
Opiates
Dopamine antagonists
Phenothiazines
Reserpine
Methyldopa
Cimetidine
Metoclopramide
Cholinergic agonists
Serotonergic agonists
Amino acids
VIP
TRH
Hypothyroidism
Hypothalamic lesions
Pituitary stalk section
Renal failure

hormones to become normal, particularly T_3 may be prolonged (35). Furthermore, it may be difficult to interpret the thyroid function tests in anorexia nervosa because thyroid hormones may be abused by these patients to induce weight loss. The etiology of biochemical hyperthyroidism in this setting may be defined by a thyroid scan that will show increased iodine uptake in hyperthyroidism due to pathological causes whereas uptake will be suppressed if exogenous thyroid hormones are taken by the patient.

Although catecholamine deficiency is not a well-recognized endocrine disorder, catecholamine concentrations are decreased in anorexia nervosa, which return to normal with therapy (36). GH resistance in anorexia nervosa is characterized by low IGF-1 and high GH concentrations. Low IGF-1 contributes to osteoporosis (37). Other factors that affect bone health in anorexia nervosa include hypogonadism, hypercortisolism, inadequate nutrition, low intake of calcium and vitamin D, and excessive exercise.

Although it is seldom a clinical problem to find a low serum dehydroepiandrosterone (DHEA) concentration, this is a frequent finding in anorexia nervosa due to the diminished activity of 17-20 lyase (38).

Hyperprolactinemia

A common problem for the clinician is a patient referred for high prolactin levels. A list of endocrine and nonendocrine causes of hyperprolactinemia other than prolactinoma are given in Table 1 (39). The first step to approach a patient with hyperprolactinemia must be to exclude nonendocrine causes such as stress and tactile stimulus. Prolactin is a stress hormone that is acutely elevated in systemic disease such as seizures (40). Many drugs used in the field of psychiatry also raise prolactin levels to the range that would cause galactorrhea. This effect is usually dose dependent and resolves after discontinuation of the medicine. However, in psychiatric patients it may be difficult to stop the medicine that must be given for the control of their illness, to observe the fall in prolactin level confirming that it is a side effect of therapy and not due to prolactinoma. In these cases, a dopamine suppression test may be helpful. The details of the dopamine test are described in Vol. 2; Chap. 33. The prolactin levels after dopamine administration must be reduced by more than 50%. Failure of suppression raises the possibility of prolactinoma, which warrants imaging studies. A TRH stimulation test to assess prolactin status is usually not helpful in this setting. As always, a wise recommendation to the referring physician would be to maintain the ''minimum effective dose'' of the psychotrophic medication to avoid side effects, including hyperprolactinemia. If galactorrhea persists despite these measures, dopamine antagonists may be used for a short time to control the symptoms even though prolactinoma is excluded. It must be remembered that the cause of hyperprolactinemia may be as simple as tactile stimulation of the breasts that may be self-induced or due to abusive behavior. An eight-year-old girl was referred to our clinic for galactorrhea associated with hyperprolactinemia at the level of 50 ng/mL. The patient came with ultrasound of the breasts and MRI of the pituitary gland that were normal. A careful history revealed that she had thelarche and she rubbed her nipples intently. The physical examination revealed galactorrhea with inflammation of the nipples and the surrounding tissue. By avoiding manipulation of the breasts with the help of a local anesthetic, the galactorrhea improved and the prolactin level decreased to 10 ng/mL.

Attention Deficit-Hyperactivity Disorder

While children with attention deficit-hyperactivity disorder (ADHD) may also have hyperprolactinemia due to side effects of therapy with certain drugs that are administered to control their symptoms, i.e., methylphenidate, most children with this condition get referred to the pediatric endocrinologist for growth alterations rather than for increased concentrations of prolactin levels. The multimodal treatment study of patients with ADHD clearly established that the behavioral manifestations of this disorder were improved with medication; however, there was significant deterioration of growth progression among those who took the stimulant therapy for the longest periods (41). The growth suppression persisted as long as the medications were ingested, whereas those with poor compliance or those with treatment interruption regimens had improved growth. Suboptimal nutrition appears to play a significant role in stunting growth due to the anorexic effects of the stimulant medications, although there may also be alterations

in endocrine/growth mechanisms. In a study that aimed to determine the predictors of weight loss in children with ADHD treated with stimulant medication, significant correlations were found between pretreatment weight and change in weight in both methylphenidate-treated patients and dextroamphetamine-treated group. A greater proportion of heavier children experienced a decrease compared with thinner children (41). ADHD therapy was found to be associated with a slight decrease in the change in height SDS during GH treatment in patients with idiopathic GH deficiency but not in those with idiopathic short stature (42). On the other hand, methylphenidate was reported to have no effect on serum GH, GH-binding protein, and IGF-1 in patients with ADHD (43). Similarly, Bereket et al. reported that other than modest reductions in serum T_4 and TSH within normal limits, methylphenidate treatment in ADHD did not significantly affect SDS of height, weight, BMI, IGF-1, and IGF-binding protein (IGFBP)-3 (44). The guidelines of the American Academy of Pediatrics state that ''Recommended stimulants require no serologic, hematologic, or electrocardiogram monitoring'' (45). Growth on stimulant medication has been recently reviewed by Poulton (46). This review demonstrates that the studies in late adolescents and adults who received stimulant medication during childhood showed a height deficit of approximately 1 cm/yr during the first one to three years of treatment. The indication of medical therapy in ADHD must be decided on an individual basis and the minimum effective dose should be utilized. The long-term implications of these drugs on final stature and the causal mechanisms remain to be investigated.

Endocrine Alterations in Hepatogastrointestinal Disease

The liver is the major organ responsible for the metabolism and clearance of hormones. Therefore, liver disease alters the concentrations of hormone in the circulation that may lead to clinical findings. The clinician must consider the possibility of liver dysfunction before assuming that the source of altered hormone levels is an endocrine gland. GH is elevated in liver disease and it has been proposed that the level of GH is related to the severity of the liver failure, whereas serum IGF-1 concentration is decreased again in parallel to the chronicity of the liver disease (47,48).

During acute hepatitis, serum T_4 concentration is increased without clinical hyperthyroidism. The cause of this increase is decreased clearance of T_4 and increased TBG as part of the acute-phase response to inflammation. TBG is also increased because presynthesized TBG leaves the damaged hepatocytes. Both T_4 and TBG return to normal with the recovery of the liver alteration. A positive correlation has been demonstrated between TBG and AST concentrations (49–51). The increase in T_4 is parallel to the elevation in TBG and hyperthyroidism is rarely a problem as demonstrated by diminished ''T_3 resin uptake'' and normal ''free thyroxine index.'' The T_3 concentrations in acute hepatitis are variable. Decreased total and free T_3 are usually accompanied by increased rT3, which returns to normal with recovery of the liver disease. Serum TSH is usually normal; therefore, these levels constitute the best marker of thyroid functions in acute hepatitis. T_3, T_4, and TSH may all be decreased in fulminant hepatitis (52,53).

Chronic liver disease and primary biliary cirrhosis may be associated with autoimmune disease and the incidence of Hashimoto's thyroiditis in this setting may be as high as 18% to 20% (54). The most consistent finding regarding the thyroid hormone alterations in chronic liver disease is decreased serum T_3, which is due to the diminished activity of the hepatic enzyme 5-monodeiodinase. Consequently, the conversion of T_4 to T_3 is reduced providing more substrate for rT_3 synthesis. Although total T_4 is reduced, free T_4 is usually normal. The thyroid hormone concentrations in end-stage liver disease may be variable and difficult to interpret. In general, serum TSH concentration should guide the clinician about the significance of altered levels of T_3 and T_4. TSH will remain normal as long as the patient is euthyroid. However, it is possible that reduced TSH response to a low T_3 level may be due to inadequate nutrition or hypercortisolism frequently observed in patients with cirrhosis (55–57).

It is also important to note that certain medications used in liver disease may change the concentrations of circulating thyroid hormones. These drugs usually cause such changes by inhibiting the monodeiodinase enzymes. There are two types of monodeiodinase; type I is present mostly in the liver and kidney and type II is localized in the pituitary gland. The drugs that inhibit type I deiodinase include propylthiouracil, dexamethasone, and propranolol. Amiodarone, and contrast media used in cholangiography such as iopanoic acid and ipodate block the activity of both type I and type II deiodinase. The net effect of all these medications is reduced peripheral conversion of T_4 to T_3 and diminished clearance leading to increased T_4 and decreased T_3 concentration. Consequently, serum rT_3 and TSH are increased (54,58,59).

Hypothalamic–pituitary–gonadal axis is frequently impaired in liver disease. Males with cirrhosis are feminized due to increased estrogen/androgen ratio. The concentrations of plasma testosterone and DHEA-S are reduced and estradiol is normal or moderately increased. Hemochromatosis affects endocrine function due to the accumulation of iron in endocrine glands (60).

Spironolactone treatment in cirrhosis may contribute to the pathogenesis of gynecomastia by blocking testosterone receptors, reducing testosterone synthesis, and increasing estradiol concentration (61). In severe cirrhosis with encephalopathy, the pulsatile

secretion of GnRH may be impaired because of alterations in central neuromediators such as dopamine and norepinephrine. In addition, the reduction in central dopamine concentration leads to the well-known hyperprolactinemia of cirrhosis further inhibiting GnRH synthesis and secretion (62).

While glucose intolerance and insulin resistance is expected in acute hepatitis, hypoglycemia is a common complication of liver necrosis (63). Hypoglycemia in acute liver failure may be symptomatic, severe, and persistent. Gluconeogenesis and glycogenolysis are decreased to a critical level. In addition, delayed insulin metabolism results in relative hyperinsulinemia, which may also be aggravated by portosystemic shunt that develops with liver necrosis. Glycogen synthesis is also impaired (64–66). On the other hand, glucose intolerance is reported in 54% to 92% of the patients with cirrhosis (67). Overt diabetes may be as high as 40% (68). Although the pathogenesis of diabetes is not clear, both insulin release and sensitivity are reduced. Insulin resistance at the level of beta cells that leads to hyperinsulinemia has been described in liver failure due to hepatitis C (69). Increased concentrations of counterinsulin hormones (GH, glucagon, cortisol, and catecholamines) also contribute to hyperinsulinemia (70).

The associated hyperglucagonemia may increase glucose production and aggravate glucose intolerance (71).

Malnutrition that frequently accompanies cirrhosis may lead to deficiencies of cofactors for certain enzymes that participate in glucose metabolism. The effect of liver disease results in fat malabsorption, and decreased synthesis and transport of lipids. The plasma tryglyceride concentration is initially increased that declines with advanced liver failure (72).

Regarding the bone metabolism, chronic liver disease causes both rickets and osteoporosis. Alterations in GH-binding protein, IGF-1, and vitamin D are responsible for the negative effect on the bones. The serum total calcium concentration is usually low due to hypoalbuminemia. In contrast, the ionized calcium level is normal. Inadequate nutrition and malabsorption may also cause hypocalcemia (52,73).

The PTH concentration is generally normal in cirrhosis. However, it has been reported that C-terminal PTH, which is a proteolytic product of PTH is increased in primary biliary cirrhosis. Its clinical significance is not clear (74). The concentrations of 1,25-dihydroxyvitamin D and 25-hydroxyvitamin D are normal until liver failure is severe, then 25-hydroxyvitamin D level may be reduced. However, low levels of 25-hydroxyvitamin D are more frequently observed in primary biliary cirrhosis. This is due to the reduced activity of 25-hydroxylase. The synthesis of vitamin –D-binding protein is also decreased. Other contributing factors are lack of sun exposure, inadequate nutrition, malabsorption of vitamin D, and impaired enterohepatic circulation of the vitamin. The most common bone problem in advanced liver disease is osteoporosis (21–39%). Prolonged immobilization, malnutrition, and decreased muscle mass are common problems each of which contributes to osteoporosis. It has been demonstrated that conjugated bilirubin inhibits the proliferation of osteoblasts. Therefore, prolonged hyperbilirubinemia in cholestatic liver disease plays an important role in bone loss. Bone resorption is generally preserved in liver disease while bone formation is diminished (73–75).

Endocrine Alterations in Renal Disease

The kidney has endocrine functions because it produces hormones such as 1,25-dihydroxycholecalciferol, renin, and erythropoietin. It also participates in the degradation and clearance of GH, prolactin, insulin, glucagon, PTH, and calcitonin (76,77). Renal failure leads to the accumulation of bioinactive peptides that causes elevation of the serum concentration of the hormones that share the same antigenic properties when measured by radioimmunoassay. In addition, the secretory rate of hormones and the sensitivity of end organs may also be affected (78). The endocrine consequences of dialysis is another issue that must be considered by the clinician who takes care of patients with renal failure (79).

Endocrine dysfunction due to chronic renal failure (CRF) may be caused by three mechanisms: increased hormone concentration, decreased hormone concentration, or reduced tissue response. Serum hormone concentration may be elevated by either increased secretion (PTH and aldosterone) or accumulation of peptides that may lack bioactivity (glucagon, PTH, calcitonin, and prolactin). Hormone concentration may be reduced by either decreased secretion by the kidney (1,25-dihydroxyvitamin-D3, renin, and erythropoietin) or decreased secretion by other endocrine organs (testosterone, estrogen, and progesterone). Hormone resistance is also a feature of CRF as documented for insulin, glucagon, PTH, 1,25-dihydroxyvitamin-D3, and erythropoietin (80).

The basal and stimulated GH concentration is increased in CRF. In addition, a paradoxic rise during oral glucose tolerance test has been reported. The reduced rate of metabolic clearance partially accounts for the increased GH concentration in CRF. Also responsible is the central dysregulation of GH secretion as observed in other chronic diseases such as liver failure and anorexia nervosa (76,77). Serum IGF-1 concentration is usually decreased in CRF, because the kidney is one of the primary sites of IGF-1 synthesis along with the liver. The levels of IGFBP are also altered in CRF. Most importantly, serum IGFBP3 is reduced and serum IGFBP2 is elevated (81,82). Therefore, neither GH stimulation tests nor serum IGF-1 level is helpful in assessing GH pathophysiology in CRF; because somatomedin bioactivity is suppressed due to the inhibitory molecules that accumulate in the serum. This inhibition returns to normal after dialysis. In light of the above

discussion, patients with CRF and growth retardation may be given GH treatment without performing the stimulation tests usually utilized to assess GH in children, but in accordance with the plans for transplantation.

In CRF, the pituitary and thyroid functions are impaired and peripheral conversion of T_4 to T_3 is diminished. Serum T_4 and T_3 levels are usually decreased and rT_3 resin uptake is increased. Serum total rT_3 concentration is usually normal unlike other systemic conditions that cause euthyroid sick syndrome, although the free rT_3 level is increased. The plasma TBG level is usually normal. The TSH response to TRH stimulation and the thyroid response to exogenous TSH are both reduced. Similarly, radioactive iodine uptake by the thyroid gland is decreased at two or six hours after the administration of radioactive iodine. This may be due to the increased free iodine concentration in the serum that dilutes the radiolabeled iodine. Interestingly, iodine uptake at 24 hours may be increased (76,83,84).

The metabolic bone disease of CRF reveals itself with characteristic laboratory tests that seldom cause confusion in interpretation. Secondary hyperparathyroidism in the presence of hypocalcemia and hyperphosphatemia with diminished 1-α hydroxylation, and end-organ resistance to vitamin D are expected features of renal osteodystrophy (76,85). Increased PTH is due to both hypocalcemia and decreased clearance of PTH from the kidneys. The elevated portion of PTH due to the filtration failure is mostly the COOH-terminal that warrants caution when this fraction is measured. Therefore, intact PTH must be measured to prevent confusion between primary hperparathyroidism and secondary hyperparathyroidism due to CRF (86).

The basal plasma cortisol is usually normal although the basal morning ACTH concentration and cortisol response to ACTH stimulation may be slightly increased (76,77). The 17-hydroxycorticosteroids is reduced in urine and elevated in plasma. The setpoint for suppression of the hypothalamic–pituitary–adrenal axis may be elevated leading to false-positive results in overnight and even two-day dexamethasone suppression test (87,88). The cortisol response to insulin-induced hypoglycemia may be blunted, but the response to stress is preserved.

The aldosterone level in CRF may be normal, elevated, or decreased. The clearance rate of aldosterone is normal as long as the liver functions are intact because the liver is responsible for the metabolic degradation of aldosterone (76,77). The secondary hyperaldosteronism of nephrotic syndrome is not only due to hyponatremia, but also exaggerated by reduced 11β-hydroxysteroid dehydrogenase activity (89). CRF may also cause hyporeninemic hypoaldosteronism due to the reduced synthesis and secretion of renin. This situation is characterized by hyperchloremic and hyperkalemic metabolic acidosis and usually occurs in pathologies that affect the renal tubules and interstitium such as diabetes mellitus (80). The plasma catecholamines as well as vasoactive hormones such as angiotensin II, arginine vasopressin, and endothelin are usually increased in patients with CRF (90).

Amenorrhea is common in CRF. The hormonal alterations expected in renal failure include hyperprolactinemia, normal FSH, mildly elevated LH, increased LH/FSH ratio, low estradiol, progesterone, and testosterone. Prolactinemia may contribute to the gonadal failure. Testosterone level in girls may be elevated. FSH and LH may be elevated to menopausal levels if primary gonadal failure occurs due to uremia. The pulsatile gonadotropin release is lost. Gonadotropin response to LHRH test is usually normal although the LH response may be prolonged (91).

Decreased testicular size and gynecomastia are common in males with CRF. Although testosterone level is reduced, serum gonadotropins are modestly elevated. Serum sex hormone-binding globulin and its binding affinity with testosterone are normal. The testosterone response to human chorionic gonadotropin is decreased. The gonadotropin response to LHRH test may be normal, decreased, or increased (76,77).

Delayed puberty is a common problem in CRF. The FSH level is elevated in boys suggesting a damage in germinal epithelium. In contrast, the LH concentration and Leydig cell function seems to be intact (92).

The hyperglycemia frequently observed in CRF is defined as uremic pseudodiabetes that is caused by decreased insulin sensitivity, defective insulin release, and insulin antagonists in the circulation (93). In contrast, insulin requirement of a diabetic patient with CRF may decrease because of decreased food consumption and reduced clearance of insulin (94). Hypoglycemia in CRF may be due to impaired glycogenolysis and substrate limitation for gluconeogenesis. Insulin resistance in CRF does not seem to be related to increased GH or glucagon, because hemodialysis improves insulin sensitivity without changing the GH and glucagon levels presumably by clearing insulin antagonists (95). In addition, the high intracellular calcium concentration in pancreatic islet cells due to the secondary hyperparathyroidism may impair insulin release (96). Urinary glucose measurements are unreliable, because the renal threshold may be altered. The glycosylated hemoglobin levels must be interpreted with caution, because affinity chromatography may underestimate and cation exchange chromatography may overestimate the glycemia (80). Regarding HbA1c, a significant correlation was found between the degree of hemoglobin carbamylation and the mean blood urea concentration in both uremic and control subjects. In this study, carbamylation of hemoglobin was higher in both diabetic and nondiabetic CRF patients in comparison to control subjects without uremia (97).

The lipid metabolism is also affected in CRF. Hypertriglyceridemia with elevated very-low–density lipoprotein is common, whereas high total cholesterol and low-density lipoprotein are rarely observed.

The causes of hypertriglyceridemia include decreased renal clearance and increased synthesis of triglycerides in the liver due to hyperinsulinemia (98). Total cholesterol and triglycerides are increased in nephrotic syndrome (99).

Hypergastrinemia in CRF may be due to decreased renal clearance or achlorhydria associated with atrophic gastritis (100). On the other hand, long-term erythropoietin therapy significantly suppresses plasma gastrin and glucagon concentration (79). The plasma leptin concentration in CRF is higher than expected for body mass index that may contribute to the anorexia of CRF (101).

Endocrine Consequences of Malnutrition and Obesity

Acute and chronic changes in nutritional intake and body composition may lead to adaptive alterations in the endocrine system. We reviewed the endocrine adaptations in different types of malnutrition (Table 2) (128). The clinician must assess and correct the nutritional problem before trying to treat the endocrine test alteration. A classical example is the finding of a low IGF-1 concentration in a short child, which may be associated with malnutrition as well as GH deficiency. Numerous studies have shown that IGF-1 concentration is reduced in nutritional growth retardation. Even suboptimal nutrition without overt malnutrition decreases the synthesis of both IGF-1 and IGFBP-3 (108–118). Therefore, weight and nutritional status of the patient must be carefully assessed before attributing growth failure to endocrine causes. Alterations in thyroid hormones due to malnutrition may mimic central hypothyroidism, but they only reflect euthyroid sick syndrome.

Endocrine and metabolic alterations in obesity must also be kept in mind because the prevalence of obesity is tremendously increasing in the world (Vol. 1; Chap. 1). Pediatric endocrinologists are often asked to evaluate an obese child and to rule out hypothyroidism. This may be easy if appropriate growth records are available as growth proceeds at an appropriate rate whereas weight gain is increased in obesity. In contrast, decreased growth and short stature characterize the hypothyroid child. However, every endocrine

Table 2 Endocrine Alterations in Different Types of Malnutrition

Condition studied	Hormones	Direction of change	References
Acute fasting in adult human	GH pulse frequency	▲	102
	24 hr integrated concentration	▲	
	Maximum pulse amplitude	▲	
Malnutrition in infants	Suppression by glucose	–	103
	GH response to arginine	–	104,105
	GH clearance	N	106
Nutritional dwarfing	Mean overnight GH secretion		109
	Prepubertal	N	
	Pubertal	▼	
	GH response to GHRH		
	Prepubertal	▲	
	Pubertal	N	
Normal short children	Spontaneous GH secretion	Inversely correlated with adiposity	107
Malnutrition in children	IGF-I	▼	108–117
	IGF-II	▼	105
Anorexia nervosa in adolescents	IGF-I		116,117
Fasting in adults (after 72 hr)	IGFBP-3	▼	118
		▼	
Anorexia nervosa in adolescents	IGFBP-1	▼	119
	IGFBP-2		
Acute fasting in adults	TSH	▼	120,121
	TSH response to TRH	▼	
Adolescents with growth failure due to fear of obesity	TSH response to TRH	Delayed but normal amplitude	121
Anorexia nervosa and ND	TSH response to TRH	N or delayed	113,117,122
Acute and chronic malnutrition in rats and children	T_3	▼	121,123
	rT_3	▲	
Malnutrition and anorexia nervosa in children	24 hr urine free cortisol	▲	113,114
	P.M.-free and total plasma cortisol	▲	124,125
	Cortisol suppression after dexamethasone	Inadequate	
Malnutrition in children	Fasting blood glucose	▼	105,126
	Glucose disposal	Delayed	
	Insulin release	Diminished	
Malnutrition in children and adults	Hypothalamopituitary and direct gonadal	Suppression	113,114,125,127

Abbreviations: GH, growth hormone; GHRH, GH-releasing hormone; IGF, insulin-like growth factor; IGFBP, IGF-binding protein; rT3, reverse T_3; TSH, thyroid-stimulating hormone.

Table 3 Endocrine and Metabolic Alterations in Obese Children and Adolescents

Endocrine and metabolic function	Alterations in obese subjects	References
Growth factors	Attenuated basal and stimulated GH release following provocative hypothalamic or pituitary stimuli; normal serum somatomedins and normal or accelerated linear growth	129–131
Thyroid	Normal serum thyroxine, triiodothyronine, free thyroxine, and thyroid-stimulating hormone	132
Adrenal	Normal serum cortisol with increased production and excretion of cortisol and its metabolites, normal circadian rhythm, premature adrenarche, elevated serum adrenal androgens and DHEA, and normal epinephrine and norepinephrine	133,134
Pancreatic	Hyperinsulinemia and insulin resistance Decreased glucagon release	135–139
Reproductive		
Pituitary	Normal or elevated serum FSH and normal LH, advanced bone age, and early onset of puberty	140
Ovarian	Normal serum estradiol, elevated progesterone, decreased SHBG, premature pubarche, hyperandrogenism, hirsutism; increased incidence of polycystic ovarian syndrome	141–143
Testicular	Decreased total serum testosterone due to decreased SHBG, but normal free testosterone, increased serum estrogen, but no clinical feminization; premature pubarche	144–146
Prolactin	Elevated basal serum prolactin but attenuated response to provocative tests	147

Abbreviations: DHEA, dehydroepiandrosterone; GH, growth hormone; LH, luteinizing hormone.

organ is affected in obesity and laboratory tests should be interpreted with caution (Table 3) (148). Although thyroid function tests were reported to be normal in exogenous obesity (132), it has been recently proposed that serum-free thyroxine is inversely and thyrotropin is positively correlated with body mass index suggesting a subclinical hypothyroidism despite having serum hormone concentrations within normal range (149). Of course, one must not conclude that thyroid hormones may be used for the treatment of obesity because thyroid hormones are also appetite stimulants. However, it must be remembered that slight elevation of TSH and slight depression of free thyroxine concentrations (within the normal range) may be associated with obesity although a cause and effect relationship is not clear.

A retrospective study on a large population of obese children revealed that, in addition to elevated cholesterol and insulin levels, 46% had significantly advanced bone age (150). The long-term growth potential and the attainment of ultimate height in obese children may be decreased (Vol. 1; Chaps. 1 and 2).

After the discovery of the hormones synthesized in the adipocytes, the adipose tissue began to be regarded as an endocrine organ (151). In addition to the production of leptin and other adipokines, the adipose tissue can produce cortisol from its inactive precursor, cortisone. Indeed, urinary free cortisol in obesity may be increased above $70\,\mu g/m^2/day$, which is the critical range for hypercortisolism and needs to be differentiated from adrenal hyperfunction. Often an obese child may be referred as possible Cushing's syndrome, if that is the case inadequate growth progression would distinguish them. A low-dose dexamethasone suppression test ($1.25\,mg/m^2$ overnight) should suppress the AM serum cortisol level below $2\,\mu g/dl$ in normal individuals. Failure of suppression warrants further investigation.

More recently, an obese child is being referred for evaluation of hyperinsulinism and prediabetes, particularly when they present acanthosis nigricans. Insulin resistance syndrome, type 2 diabetes mellitus and other related alterations are addressed in Vol. 1; Chap. 11.

CONCLUSION

Clinical experience shows us that things are not always what they look like. The easy assumption is that an abnormal endocrine test is due to an endocrine disease. However, this assumption may often be wrong and the abnormal test may only be the consequence of a physiologic or systemic condition or the side effect of a medicine given for a completely unrelated problem. Failure to recognize this, may lead to unnecessary and even expensive diagnostic and potentially ineffective or harmful therapeutic interventions. In order to prevent these devastating consequences, a systemic and detailed history must be obtained and a careful physical examination must be performed. Treatment of the underlying or coexisting problem will often solve the apparent endocrine abnormality as well. However, endocrine treatment may be needed occasionally such as GH therapy in CRF or thyroxine replacement in hypothyroidism. Although general guidelines help the clinician to make decisions, each patient must be invidualized and the best interest of the particular patient must be weighed against the adverse effects of treatment and the consequences of avoiding therapy. Multidisciplinary approach is often necessary to resolve the endocrine consequences of systemic conditions and medications.

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Autoimmune Endocrinopathies

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INTRODUCTION

This chapter will overview the immune response in health and in autoimmune disorders, classify autoimmune disorders, and examine a variety of autoimmune disturbances. The chapter concludes with a clinical approach to the diagnosis and evaluation of immunoendocrinopathies. The reader is referred to Vol. 1; Chap. 5 for a review of all aspects of autoimmunity of diabetes mellitus (1).

THE ADAPTIVE IMMUNE SYSTEM IN HEALTH

Normal Role of the Immune System

Cells of the immune system, including macrophages, T-lymphocytes (e.g., T-cells), and B-lymphocytes (e.g., B-cells), must recognize one another as well as somatic cells of the body to achieve proper intercellular communication (Fig. 1) (2). This recognition is afforded by polymorphic cell surface molecules encoded by genes within the major histocompatibility complex (MHC), as well as, by various adhesion and other cell–cell recognition molecules (3,4). The human MHC, which is termed the human leukocyte antigen (HLA) complex, is located on the short arm of chromosome 6. By differentiating self from nonself (a process sustained by thymic T-lymphocyte education, B-lymphocyte education, and peripheral T-cell tolerance), the immune system is able to recognize and react to foreign antigens providing protection from microbiological invasion and certain cancers that express "new" antigens.

The immune system must survey or monitor two major spaces of the body: the cytoplasmic space and the extracellular/intravesicular space. Class I MHC molecules (HLA-A, HLA-B, and HLA-C in humans) monitor the cytoplasm of nucleated cells, whereas the extracellular/intravesicular space is surveyed by class II MHC molecules (HLA-DR, HLA-DQ, and HLA-DP in humans). The intravesicular space is the space that exists within cellular vesicles that is formed during pinocytosis and phagocytosis of extracellular materials.

Communication Between Cells via MHC Molecules: Class I MHC Molecules

Class I MHC heavy chains are each encoded by single loci within the HLA complex. At the cell surface, each HLA-encoded chain is coexpressed with β-2-microglobulin as the class I MHC molecule. The molecules present cytoplasmic peptides, both self and nonself, to CD8 T-cells (e.g., lymphocytes). Class I MHC molecules are found on the surfaces of all nucleated cells. If an activated CD8 T-cell recognizes a peptide, usually viral in origin, presented by a class I MHC molecule, that CD8 T-cell will function as a cytolytic-T-lymphocyte [CTL or Tk (killer) cell] and will induce apoptosis in the cell presenting the peptide that was recognized (Fig. 2). Differences in class I and class II MHC molecules between donor and recipient play a major role in foreign-tissue rejection, serving as the classically described transplantation antigens.

Apoptosis is programmed cell death. In apoptosis, the apoptotic cell essentially involutes, breaks into fragments contained within plasma membrane, and undergoes an "intracellular" necrosis where intracellular materials are not released to the extracellular space until the cell has fully autodigested. In response to viral infection, apoptosis does not release viable virions: as the cell autodigests, the intracellular virus is also digested, protecting adjacent cells from exposure to potentially infectious virus. Cytolytic-T-lymphocytes (e.g., Tk cells) represent the "effector" stage of activated CD8-positive T-lymphocytes. T-cells, both CD4 and CD8, predominantly use α/β T-cell receptors (TCRs) to recognize MHC-presented peptides (5,6). γ/δ-bearing T-cells represent about 5% of circulating T-cells and are in high concentration along mucosal surfaces such as in the Peyer patches in the gut. However, the role of such γ/δ T-cells is still poorly understood.

Upon recognizing a "target" cell, the CD8-positive Tk cell juxtaposes its cell membrane against the target cell and inflicts cell membrane damage by the release of perforin (a C9-like protein that forms pores in the target-cell membrane). In the target-cell plasma membrane, holes are produced by perforin, and granzymes

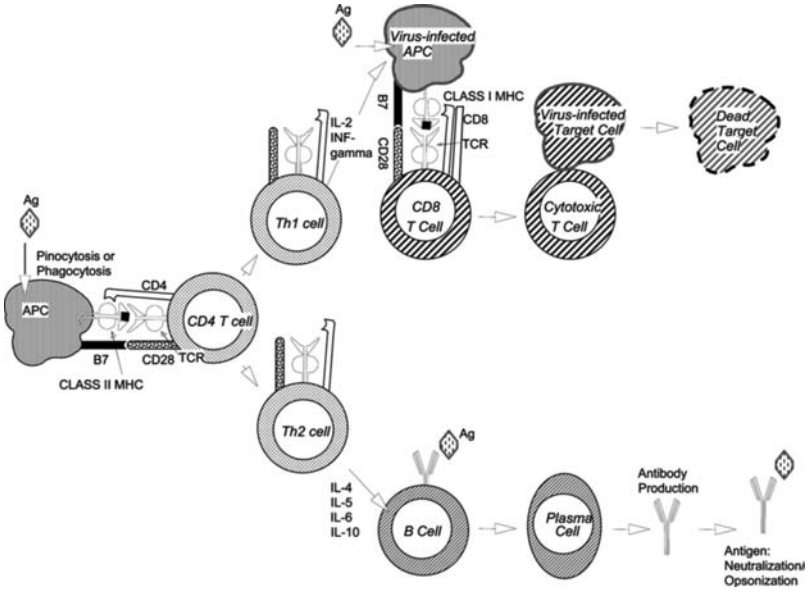

Figure 1 Antigen is taken up by professional APCs such as macrophages (by phagocytosis) or dendritic cells (by pinocytosis). If properly activated, the APC will express B7. Antigen-peptide presentation via class II MHC molecules plus B7 expression when contacted by a responsive T-cell via its T-cell receptor (TCR) and CD4 molecule lead to CD4 T-cell activation. Initial contact of CD4 T-cells with antigen peptide (presented by MHC molecules) plus microenvironmental influences stimulates differentiation of the CD4 T-cell into a Th1 or a Th2 cell. Not shown is the influence of interferon γ from NK cells or CD8 T-cells, and IL-12 from dendritic cells in fostering Th1 responses, whereas IL-4 from CD4T NK1.1+ cells pushes CD4 T-cells toward a Th2 phenotype. The role of Th1 cells is to foster cell-mediated immunity. A virus-infected APC is stimulated by Th1 cells to become an effective APC for CD8 T-cells. Activated CD8 T-cells acquire cytotoxic activity to kill virus-infected target cells. Th2 cells together with antigen activate B-cells to ultimately become antibody-secreting plasma cells (and memory cells—not shown). Antibody binds to antigen neutralizing the antigen and/or opsonizing the antigen for efficient phagocytosis. *Abbreviations*: APC, antigen-presenting cell; MHC, major histocompatibility complex; TCR, T-cell receptor; IL, interleukin.

and granulysin enter the target-cell cytoplasm through these holes to induce apoptosis. Apoptosis can also be induced in the target cell via membrane contact between the CD8-Tk-cell-expressed cell surface molecule FAS ligand and FAS on the target-cell surface.

Communication Between Cells via Class II MHC Molecules

Class II MHC molecules present extracellular and intravesicular peptides to CD4-positive T helper cells to initiate immune responses that culminate in humoral and/or cell-mediated responses designed to remove the invader from the body (Fig. 3).

These heterodimeric molecules (HLA-DR, HLA-DP, and HLA-DQ in humans) that are composed of α and β chains are restricted in their distributions to specialized antigen-presenting cells (APCs): monocyte-derived cells, B-lymphocytes, and dendritic cells found in lymph nodes. Monocyte-derived cells include tissue macrophages, Kupffer cells that line the liver sinusoids, alveolar macrophages, central nervous system microglia, and glomerular mesangial cells. Via class II MHC molecules, such APCs present (e.g., display) extracellular-derived peptides to the α/β TCRs of CD4 T-cells. Almost all infectious agents in some stage of their life cycle pass through the extracellular space of the cell and thus these

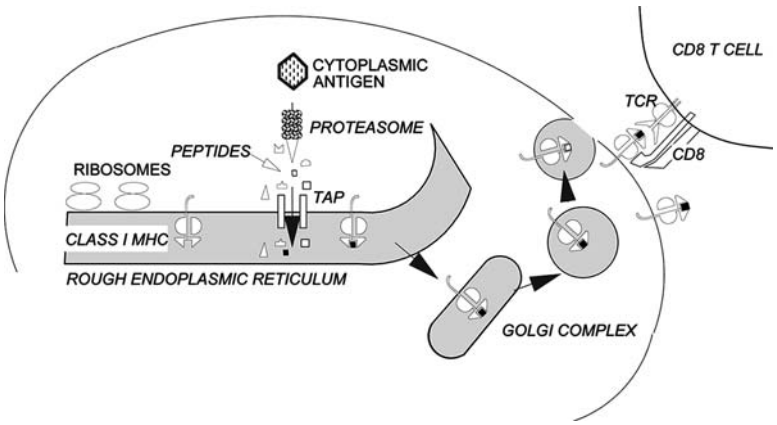

Figure 2 Cytoplasmic antigens, either endogenous or exogenous (e.g., viral infection), are processed by proteasomes to peptides. These peptides are transported into the lumen of the RER by TAP. Once the class I MHC molecule is loaded with peptide, a transport vesicle buds from the RER. This vesicle passes through the Golgi complex to eventually fuse with the plasma membrane where the class I MHC molecules and peptides are oriented to the outside of the cell. In this way, class I MHC molecules display cytoplasmic contents (e.g., peptides) to CD8 T-cells. *Abbreviations*: TAP, transporters associated with antigen processing; MHC, major histocompatibility complex; TCR, T-cell receptor; RER, rough endoplasmic reticulum.

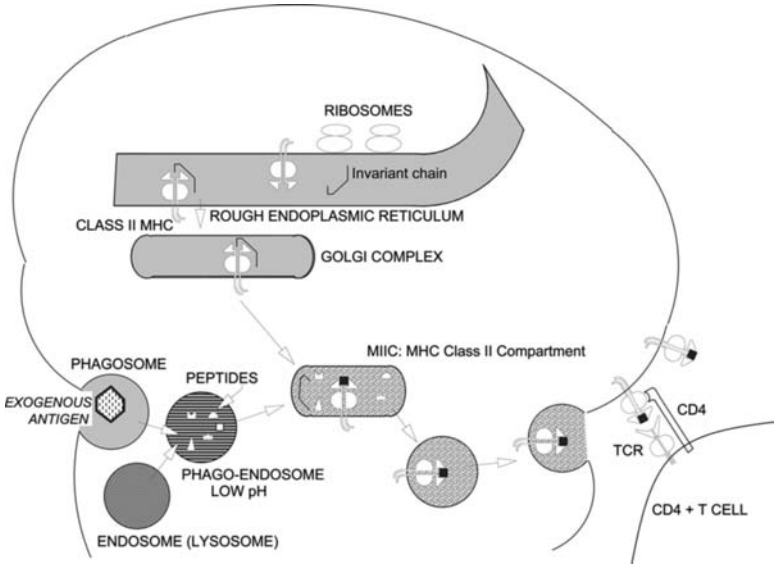

Figure 3 Within the RER, class II MHC molecules bind the invariant chain that protects the class II MHC molecule from inadvertent loading by cytoplasmic peptides. A transport vesicle containing the class II MHC-invariant chain complex buds from the RER and passes through the Golgi complex. Extracellular antigens are taken up by specific APCs via phagocytosis or pinocytosis. The resulting phagosome then fuses with an endosome. Within the phagoendosome, antigen is degraded and peptides result. Fusion of the phagoendosome with the class II MHC transport vesicle forms the MIIC. The low pH of the phagoendosome degrades the invariant chain, allowing peptide loading into the class II MHC molecule. Fusion of the MIIC with the plasma membrane allows the class II MHC molecules to be placed on the plasma membrane displaying peptides to CD4 T-cells derived from extracellular and intravesicular spaces. *Abbreviations*: MHC, major histocompatibility complex; TCR, T-cell receptor; MIIC, MHC class II compartment; RER, rough endoplasmic reticulum; APC, antigen-presenting cell.

agents (or their toxic products) can be presented to CD4 T-cells.

Initial MHC typing (e.g., allele identification and classification) in animals was discovered and accomplished by tissue transplantation. Thus, the naming of these molecules as histocompatibility antigens ensued. Serologic typing of class I and class II MHC molecules followed. In the 1990s, DNA-sequence–based allele typing became a reality using a variety of molecular techniques including allele-specific polymerase chain reactions, allele-specific oligonucleotide probes, and direct sequencing of polymerase chain reaction-amplified gene segments. From classical cellular immunology, the in vitro mixed lymphocyte reaction results primarily from differences in class II MHC molecules.

As a population, humans express a large number of different class I and class II MHC alleles. This ensures that the population as a whole should be able to present peptides from any potential pathogen to T-cells and thus react to that pathogen in a protective manner. In a single individual, up to six different class I MHC molecules can be expressed when the individual is heterozygous at each of the HLA-A, HLA-B, and HLA-C loci.

On any single parental chromosome, the functional class II MHC genes include DPA1 (encoding a DP α chain), DPB1 (encoding a DP β chain), DQA1 (encoding a DQ α chain), and DQB1 (encoding a DQ β chain). For DR molecules, the situation is a bit more complex: there is a single DRA1 locus (encoding the DR α chain) and a DRB1 locus (encoding a DR β chain), and any one of the following three loci: DRB3 (encoding a DR β chain) or DRB4 (encoding a DR β chain) or DRB5 (encoding a DR β chain). In humans, there are only three DRA1 alleles but approximately 99% of the human population share one of these alleles, so for practical purposes DRA1 is nonpolymorphic. Furthermore, while all humans have a DRB1 locus, individuals usually have one additional DRB locus: DRB3, DRB4, or DR5 but not more than one of these three loci. The degree of polymorphism at DRB1 is far greater than the number of alleles at DRB3, DRB4, or DRB5. The DRB2 locus is a pseudogene. Therefore for DR, per chromosome (or haplotype), two DR molecules are expressed: one is the combination of DR α with the β chain product of DRB1 and the second DR molecule is the DR α chain plus the β chain of DRB3, DRB4, or DRB5. Thus, per chromosome, there are usually four class II MHC molecules expressed: DP, DQ, and two types of DR molecules.

Next, let the reader determine how many class II MHC molecules are formed when considering the diploid nature of cells. For DQ, while the α and β chains encoded by a single chromosome will always assemble to form a DQ molecule, on occasion the DQ α chain encoded by chromosome ''A'' will pair with the DQ β chain encoded by the opposite chromosome ''B.'' Likewise, on occasion the DQ α encoded by chromosome ''B'' will pair with the DQ β chain encoded by the opposite chromosome ''A.'' This phenomenon is termed transcomplementation and can also occur for DP chains. This occurrence adds further diversity to the number and type of DP and DQ molecules expressed. Transcomplementation does not apply to DR because DR α chains are essentially nonpolymorphic. If transcomplementation occurs in both ''directions,'' for each DP and DQ, two additional class II MHC molecules are expressed and add to the antigen-presenting diversity of a human. Thus, the maximum number of class II MHC molecules expressed by an individual would be 8 from each individual chromosome and four

Table 1 Numbers of MHC Alleles per Locus

	No
Class I MHC molecules	
HLA-A	303
HLA-B	559
HLA-C	150
Class II MHC chains	
DP β	108
DP α	20
DQ β	56
DQ α	25
DR β	440
DR α	3

Abbreviations: MHC, major histocompatibility complex; HLA, human leukocyte antigen.

molecules formed by transcomplementation. Molecular cloning of the HLA genes and sequence analysis has revealed a tremendous degree of MHC genomic polymorphism in the human population. A recent count of the various MHC alleles is presented in Table 1.

Coordination of the Acquired Immune Response: CD4 Th1 and Th2 Cells

The CD4 T-cells orchestrate (e.g., direct) the immune response. CD4 T-cell function can be described in two polarized functional modes: Th1 cells and Th2 cells. The Th1 cells are responsible for stimulating cell-mediated immunity including activation of CD8 T-cells, macrophages, and natural killer (NK) cells (7). Both Th1 and Th2 cells can play a role in activating B-cells, thereby stimulating humoral (antibody-mediated) immunity. Extracellular invaders such as bacteria and viruses are opsonized by antibody and complement and are then phagocytized by granulocytes or macrophages to clear these pathogens. Fungi are phagocytized and destroyed by Th1-activated macrophages. If the pathogen has reached the cell's cytoplasm, the CD8 T-cell will activate apoptosis in that host cell to destroy the pathogen and its site of reproduction.

The primary difference between Th1 and Th2 cells is the cytokines that they secrete: Th1 cells release interleukin (IL) -2 (T-cell growth factor) and γ interferon (IFN-γ), whereas Th2 cells release IL-4, IL-5, IL-6, IL-10 and transforming growth factor-β (TGF-β). Each subset secretes cytokines that also regulate the other subset: IFN-γ from Th1 cells suppresses Th2 cells, and IL-10 and TGF-β (8) from Th2 cells suppress Th1 cells. A third subset of CD4 T-cells has also been described that are involved in the prevention of autoimmune diseases. These T cells are regulatory T cells.

AUTOIMMUNITY AND AUTOIMMUNE DISEASES

Autoimmunity and Central Tolerance: T-Cells

Autoimmunity is a disorder of self–nonself recognition where self is recognized aberrantly as nonself and an autoreactive process develops (9). If sufficient damage is incurred on the target tissues, the circulating proteins, or the cells, a clinically recognizable autoimmune disease results. Pathologic autoreactivity could result in pathogenic autoantibodies and/or anti-self cell-mediated immune responses that would serve as the effectors of the autoimmune disease. Autoreactivity to self-antigens that could induce autoimmune diseases is normally restricted by the phenomenon of immunological tolerance (10).

Tolerance is the active process where the immune system does not normally develop an effector response to self-antigens. T-cell tolerance is acquired by the time of birth and is necessary to ensure that the body does not mount a destructive immune response to self. Central T-cell tolerance results from self–nonself discrimination that occurs in the thymus during T-cell development (11,12). In the thymus, T-cells must express CD4, CD8, and a TCR during T-cell thymic ontogeny. Developing T-cells, called thymocytes, must be able to recognize self-MHC to avoid apoptosis; however, excessive adherence to self-MHC (which might trigger autoimmunity) also leads to apoptosis. This destruction of anti-self T-cells in the thymus results in "clonal deletion."

In the process of clonal deletion, T-cells that have been "rescued" by their initial interaction with self-MHC will be induced to undergo apoptosis at the thymic corticomedullary junction if their TCR interaction with MHC is excessively strong (e.g., representing a state of possible autoimmunity). Ultimately in the thymus, CD8 T-cells survive because of their "modest" ability to perceive class I MHC molecules, whereas CD4 T-cells survive because of their "modest" ability to perceive class II MHC molecules.

Normally T-cell thymic ontogeny results in T-cells exiting the thymus that recognizes self-MHC molecules and, presumably, foreign peptides, yet these T-cells do not strongly recognize self-peptides. The ability of T-cells to recognize MHC is critical to the antigen-peptide presenting role of MHC molecules. There would be no purpose for TCRs to interact, for example, with self-surface molecules that do not present peptides. The ability to TCRs to perceive peptides presented by one's own MHC molecules is termed MHC restriction. The TCR recognizes the presented peptide plus a portion of the MHC molecule.

Autoimmunity and Central Tolerance: B-Cells

Tolerance toward self-antigens is primarily a function of T-cells. However, developing B-cells can undergo tolerization: IgM-positive-IgD-negative, immature, naive B-cells upon exposure to antigen will either be anergized or be induced to undergo apoptosis. When a B-cell is tolerized but does not die, the B-cell is said to be anergized. If the tolerized B-cell is induced to undergo programmed cell death, the B-cell undergoes apoptosis. Anergy results when few antigenic epitopes interact with the B-cell receptors. On the other hand,

apoptosis is triggered when multiple antigenic epitopes interact with the B-cell receptors providing a more powerful tolerization signal. B-cell tolerance is an ongoing process because B-cells are produced by the bone marrow throughout the individual's lifetime.

Autoimmunity and Peripheral Tolerance: T-Cells

Peripheral tolerance (or anergy) has evolved, presumably, because not all antigens necessarily enter the thymus during T-cell ontogeny (Fig. 4) (13). Initial T-cell activation, regardless of whether the cell is a CD4 or CD8 T-cell, requires two signals. One signal is antigen-peptide specific via an MHC–TCR interaction, whereas the second signal is antigen nonspecific (e.g., CD28 of the T-cell interacting with B7 on the cell expressing the MHC molecule). Peripheral tolerance results when the antigen-specific signal is present (e.g., self-MHC do present self-peptides), but the second activation signal is absent. In fact, once exposed to antigen peptide in the absence of the second signal, the T-cell cannot respond in the future and is thus essentially permanently anergized. This aspect of peripheral tolerance produces "clonal anergy." Defects in T-cell signaling are hypothesized to play a role in loss of tolerance in type 1 diabetes (see Vol. 1; Chap. 5) (14).

Autoimmunity and Peripheral Tolerance: B-Cells

Peripheral tolerance in terms of B-cells occurs via T-cell tolerance. Like T-cells, B-cells require two sets of signals to become fully activated by undergoing affinity maturation and class (isotype) switching. One set of signals comes from the interaction of the B-cell receptor (surface antibody) and the antigen, while the second set of signals comes from helper CD4 T-cells of either the Th1 or the Th2 subclasses. This second set of signals includes Th1- or Th2-secreted cytokines and an interaction between CD40 ligand (CD40L) on the T-cell and CD40 on the B-cell. If the antigen is bound by B-cell surface antibody but there is no T-cell help, the B-cell will not be activated, will not proliferate, will not class switch, and will not undergo affinity maturation. Therefore, naive mature B-cells without T-cell help (because the T-cells are tolerant) should not produce large amounts of high-affinity IgG autoantibodies. If they produce any antibodies, they would be IgM antibodies. However, IgM autoantibodies are not generally found in many autoimmune diseases and are absent in type 1 diabetes.

Autoimmunity and Loss of Tolerance

When a breakdown in tolerance occurs, the immune system recognizes "self" as "foreign" and mounts a humoral and/or cell-mediated immune response that can result in an autoimmune disease (15). Only two autoimmune diseases have been shown to be monogenic [autoimmune polyglandular/polyendocrine syndrome (APS) type 1 (16–19) and the immunodysregulation, polyendocrinopathy, x-linked (IPEX) syndrome (20)], while all other autoimmune diseases are polygenic (21).

Loss of tolerance can occur by many theoretical routes; however, loss of peripheral tolerance is thought to be the most reasonable explanation based upon the highly selective nature of organ-specific autoimmune responses and diseases. Alternatively, tolerance may have never been initially developed (22). Loss of peripheral tolerance most likely results from molecular mimicry (23). Molecular mimicry occurs, theoretically, when an immune response to a foreign antigen cross-reacts with a self-antigen leading to clinical disease. Molecular mimicry is likely in the prototypic autoimmune disease rheumatic fever. Examples of molecular mimicry include similarities in the antigenic structure of group A, β-hemolytic *Streptococcus* and antigens found in the heart (producing carditis), joints (resulting in arthritis), and basal ganglia (eliciting chorea). For example, cardiac myosin shares certain antigenic epitopes with streptococcal M protein. Similarities between HLA antigens and bacterial antigens have been described in reactive arthritides that are associated with intestinal infection (e.g., proteus). For example *Klebsiella nitrogenase* and

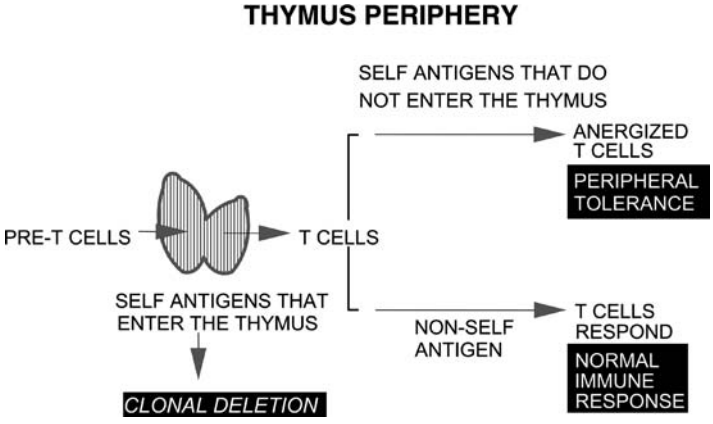

Figure 4 In the thymus, exposure of developing T-cells to antigen peptides produces clonal deletion of such potentially autoreactive T-cells. This process can be described as "central" thymic tolerance. T-cells that leave the thymus that subsequently encounter novel self-antigen not previously seen in the thymus can be anergized producing peripheral tolerance. The remaining T-cells are then available to respond to nonself antigens elaborating a protective immune response.

HLA-B27 are cross-reactive and implicated in the pathogenesis of ankylosing spondylitis and Reiter syndrome (arthritis, conjunctivitis, and urethritis). Further cross-reactivities are described among collagen antigens and mycobacteria. Mycobacterial proteoglycan wall component and cartilage protein cross-reactivity have been described in rheumatoid arthritis. Other theories of how tolerance fails or is broken include failure of thymic clonal deletion (24), sequestered antigen theory, altered self-antigen (25,26), aberrant class II MHC expression, superantigen theory of autoimmunity, and polyclonal B-cell stimulation.

The factors that lead to a disruption in self-tolerance, resulting in autoimmunities, have not been fully identified (27). To a large degree, this phenomenon is genetically programmed because autoimmune diseases are frequently associated with specific immune response gene alleles, e.g., specific HLA types. For example, ankylosing spondylitis is increased in frequency in men that especially carry the HLA-B27 allele. Systemic lupus erythematosus is more common in HLA-DR2–positive individuals. Also environmental influences, including viral infections and diet, are often implicated in the triggering of autoimmune processes. Children suffering from congenital rubella infection frequently develop type 1 diabetes. As noted previously, molecular mimicry between an environmental antigen and an endogenous antigen could lead to autoimmunity. Amino acid homologies have been described between coxsackie virus and the β-cell autoantigen glutamic acid decarboxylase. Whereas eradication of virally infected cells by CD8 T-cell lysis provides an important defense against viral illnesses, this mechanism may expand beyond its immunological defense function and lead to an autoimmune disorder. Th1 cells may play a crucial role in these processes (28,29).

With refinements in laboratory technique, intracellular antigens (i.e., thyroglobulin) can now be found in the circulation of normal subjects. This discovery discredits to some degree the ''sequestered antigen'' theory of autoimmune disease for at least several endocrinopathies. It is also of great interest that many autoantigens are enzymes or receptors whose distribution is often limited to specific tissues (Table 2) (30,31).

During the aging process, there is a progressive breakdown in self-tolerance and an increased appearance of autoimmune phenomena with self-reactive autoantibodies. However, clinically apparent autoimmune disease may not be obvious in older persons because of the decreased efficiency of the immune system with advancing age, and the limited duration of an autoimmune process that begins in an elderly individual. Thus, with aging, a higher prevalence of various autoantibodies will be recorded; however, fewer of those individuals harboring such autoantibodies will actually express disease. In contrast in younger individuals, autoantibodies will be of lower prevalence, yet more of the individuals expressing those autoantibodies will be affected with clinically apparent disease.

Table 2 Target Enzymes or Receptors in Autoimmune Diseases

Disorder	Enzyme or receptor autoantigen
Atrophic thyroiditis	TSH receptor (autoantibodies act as antagonists)
Autoimmune Addison disease	21-Hydroxylase, 17-hydroxylase, $P450_{scc}$
Celiac disease	Transglutaminase
Chronic atrophic gastritis	H^+/K^+-ATPase (proton) pump
Chronic active hepatitis	Cytochrome P450
Graves disease	TSH receptor (autoantibodies act as agonists)
Hashimoto thyroiditis	Thyroperoxidase
Insulinomimetic hypoglycemia	Insulin receptor (autoantibodies act as agonists)
Premature ovarian failure	3 β-hydroxysteroid dehydrogenase, 17-hydroxylase, $P450_{scc}$
Primary biliary cirrhosis	Pyruvate dehydrogenase dihydrolipoamide acetyltransferase
Systemic vasculitis	Myeloperoxidase
Type B insulin resistance	Insulin receptor (autoantibodies act as antagonists)
Type 1 diabetes	Glutamic acid decarboxylase, carboxypeptidase H
Vitiligo	Tyrosinase
Wegner granulomatosis	Elastinolase

Abbreviation: TSH, thyroid-stimulating factor.

CLASSIFICATION AND RECOGNITION OF AUTOIMMUNE DISEASES

Autoimmune diseases can be classified as organ-specific [e.g., autoimmune endocrinopathies (Table 3)] or non–organ-specific or systemic [(e.g., collagen vascular diseases)(Table 4)]. Autoimmune diseases in which an antibody is made to a circulating hormone [e.g., insulin autoantibodies: autoimmune hypoglycemia (32), thyroid hormone autoantibodies (33), and thyroid-stimulating hormone (TSH) autoantibodies (34)], ACTH autoantibodies (35), and testosterone autoantibodies (36) form a subgroup of the organ-specific autoimmunities.

In general, four types of findings support an autoimmune etiology for a disease: (i) evidence of humoral (antibody/B-cell) and/or cell-mediated (T-cell) autoreactivity; (ii) ability to transfer disease with either serum or lymphocytes (this is usually performed in animal models of autoimmune disease); (iii) disease recurrence in transplanted tissue in the absence of immunosuppression; and (iv) Ability to prevent or cure disease with immunotherapy through either immunosuppression or induction of tolerance (37).

Autoantibodies, the hallmark of B-cell autoimmunity, can be identified by several methods (Table 5) (38,39). Their participation in an autoimmune disorder can be assessed by complement fixation and lysis of target cells in tissue culture or by promoting cytolysis of target cells by NK cells

Table 3 Examples of Organ-Specific Autoimmune Disorders

- Antireceptor diseases
  - Atopic diseases involving B_2 adrenergic receptors
  - Atrophic thyroiditis
  - Graves disease
  - Insulin-resistant diabetes/acanthosis nigricans syndrome
  - Hypoglycemia—insulinomimetic autoantibodies
  - Myasthenia gravis
- Autoimmune endocrinopathies
  - Addison disease
  - Autoimmune diabetes insipidus
  - Autoimmune hypoparathyroidism
  - Autoimmune polyglandular syndrome type 1
  - Autoimmune polyglandular syndrome type 2
  - Autoimmune primary gonadal failure
  - Hashimoto thyroiditis
  - Hypophysitis
  - Pancreatic α-cell autoimmunity
  - Pancreatic δ-cell autoimmunity
  - Type 1 diabetes mellitus
- Anticirculating protein disorders
  - Anti-ACTH autoantibodies
  - Anti-TSH autoantibodies
  - Insulin autoantibodies—hypoglycemia
  - Thyroid hormone autoantibodies
  - Glucagon autoantibodies
- Autoimmune cytopenias
  - Immune hemolytic anemia
  - Immune leukopenia
  - Immune thrombocytopenic purpura
- Gastrointestinal autoimmunities
  - Celiac disease
  - Chronic lymphocytic gastritis/pernicious anemia
  - Crohn disease
  - Ulcerative colitis
- Hepatobiliary autoimmunities
  - Chronic active hepatitis
  - Cryptogenic cirrhosis
  - Primary biliary cirrhosis
  - Sclerosing cholangitis
- Dermatologic autoimmunity
  - Autoimmune alopecia totalis or areata
  - Autoimmune vitiligo
  - Bullus pemphigoid (bullus, gestationis, and cicatricial)
  - Chronic bullous disease of childhood
  - Dermatitis herpetiformis
  - Discoid lupus
  - Epidermolysis bullosa acquisita
  - Erythema nodosa
  - Linear IgA disease
  - Pemphigus (vulgaris, foliaceous, and paraneoplastic)
- Neuromuscular
  - Acute disseminated encephalomyelitis
  - Chronic inflammatory demyelinating polyradiculoneuropathy
  - Chronic neuropathy with monoclonal gammopathy
  - Eaton–Lambert syndrome
  - Guillain–Barré syndrome
  - Multifocal motor neuropathy with conduction block
  - Multiple sclerosis
  - Myasthenia gravis (also classified as an antireceptor disorder)
  - Polymyositis
  - Stiff-person syndrome
- Paraneoplastic syndrome
  - Cerebellar degeneration
  - Encephalomyelitis
  - Opsoclonus–myoclonus syndrome
  - Retinopathy
- Basement-membrane autoimmunity
  - Good pasture syndrome

Abbreviation: TSH, thyroid-stimulating factor.

Table 4 Examples of Nonorgan-Specific Autoimmune Disorders

- Connective tissue diseases
  - Ankylosing spondylitis
  - Behçet syndrome
  - Dermatomyositis
  - Mixed connective tissue disease
  - Progressive systemic sclerosis (scleroderma)
  - Psoriasis
  - Reactive arthritides
  - Reiter syndrome
  - Rheumatic fever
  - Rheumatoid arthritis
  - Sjögren (Sicca) syndrome
  - Systemic lupus erythematous
- Vasculopathies (can be classified as a subtype of connective tissue diseases)
  - Hypersensitivity vasculitis
  - Kawasaki disease
  - Polyarteritis nodosa
  - Takayasu arteritis
  - Temporal arteritis
  - Thromboangiitis obliterans
  - Wegener granulomatosis

or macrophages in the process of antibody-dependent cell cytolysis. These events are difficult to demonstrate in research laboratories and are beyond the scope of all commercial laboratories. In other diseases, autoantibodies may bind to membrane receptors stimulating target cells, as in Graves disease or insulinomimetic hypoglycemia, or interfere with receptor functions, as occurs in atrophic thyroiditis or myasthenia gravis. In

Table 5 Identification of Autoantibodies

Methods of autoantibody detection

- Cytoplasmic autoantibodies
  - Indirect immunofluorescence: Ig and complement fixing Ig, using human or animal tissue sections as substrates
  - Immunohistochemical methods
  - Immunoprecipitation: sera or Ig against cell extracts
  - Western blotting
  - Other methods: hemagglutination of tissue-antigen–coated RBCs or latex beads, radioimmunoassay, complement-fixation assays, enzyme-linked immunosorbant assays
- Cell-surface autoantibody determinations
  - By indirect immunofluorescence (using isolated xenogeneic or allogeneic target cells); binding of [125I] protein A, antigen precipitation by sera from an affected individual; or as measured by flow cytometry with a fluorescein-labeled second antibody

Determination of autoantibody effects

- Cell metabolism/products
  - Measurement of changes in cell metabolism (i.e., cAMP production) or cell products (hormones) after exposure to sera, or purified or partially purified Ig serum fractions. Target cells are isolated from tissue, tissue culture, or tissue slices.
- Cytolysis
  - Measurement of cytolysis by 51Cr release or supravital staining after the addition of sera, or purified or partially purified Ig serum fractions
- Transplacental passage
  - Study of the clinical effects of autoantibodies transplacentally passed from mother to fetus
- Passage to animals
  - Purification of Ig [Protein A or $NH_4(SO_4)_2$] and passage to animals

myasthenia gravis, the target autoantigen is the acetylcholine receptor. On the other hand, in Eaton–Lambert syndrome, the autoantigen is the presynaptic voltage-gated calcium channel. Autoantibodies to ganglionic acetylcholine receptors in autoimmune autonomic neuropathies have been described (40).

The finding of lymphocytic infiltration of a target organ or tissue is histopathologic evidence of cell-mediated autoimmunity. Examples of this include insulitis as pathognomonic of type 1 diabetes and the lymphocytic infiltrate, even with lymphoid follicle formation, in Hashimoto thyroiditis. Cell-mediated autoreactivity can also be shown in vivo by positive delayed-type hypersensitivity reactions during skin testing with specific syngeneic, allogenic, or xenogeneic "self" antigens, although, practically speaking, such testing is never carried out in the clinical evaluation of autoimmune endocrinopathies. In vitro, cell-mediated autoimmunity can be assessed by the production of cytokines (e.g., IL-1, IL-2, tumor necrosis factor, or IFN) or by proliferation of T-cells (measured by ^{3}H-thymidine incorporation) in response to autoantigens mixed with patients' APCs and lymphocytes. T-cell cytolysis of target cells in tissue culture can be measured by ^{51}Cr release or supravital staining of damaged cells. Once labeled for a specific length of time, healthy cells retain ^{51}Cr and exclude supravital stains.

Cytokines and reactive oxygen intermediates (O_2^-) are also incriminated as mediators or final effectors of autoimmune cell damage (41). IL-1 is often implicated along with IFN-γ and tumor necrosis factor in damaging β cells. In the case of type 1 diabetes mellitus, in tissue culture, IL-1 is toxic to isolated islets. At low doses, IL-1 inhibits glucose-stimulated insulin release while at higher doses, IL-1 is directly toxic and leads to islet cell death. Certain lymphokins, i.e., IFN-γ, may induce high levels of class I MHC expression, as well as low levels of class II MHC expression, that may propagate autoimmune responses once initiated.

Several other lines of evidence can support an autoimmune etiology for a particular disease: (i) clinical associations with known autoimmune diseases, (ii) disease association with particular HLA alleles, (iii) the ability to induce a similar disease in animals after injection of "self" antigens (often in Freund's adjuvant to exaggerate the response), (iv) increased disease frequency in females over males, and (v) increased disease frequency with advancing age. A wide variety of autoimmune mechanisms have been proposed as outlined in Table 6.

Table 6 Suggested Pathogenic Mechanisms of Autoimmune Endocrinopathies

Autoantibody hormone-receptor binding
Blocking of receptor-hormone activation, e.g., atrophic thyroiditis
Stimulation by autoantibodies with receptor activation, e.g., Graves disease
Autoantibody-induced target-cell destruction/dysfunction
Formation of local immune complexes with subsequent local inflammation
Complement-dependent cytolysis
ADCC by macrophages, natural killer cells, possible role for subsequent IL-1, TNF-mediated cytolysis
Autoantibody binding to a circulating protein with inappropriate levels of circulating free hormone producing excessive or deficient hormone effects, e.g., autoimmune hypoglycemia
Immune complex formation with distal localization and destruction, e.g., immune complex nephritis secondary to autoimmune thyroid disease.
Cell-mediated autoimmune target-cell destruction, e.g., classic CD8 T killer (DTH) responses
Cytokine and/or free radical destruction of target organ
Combinations of the above

Abbreviations: ADCC, Antibody-dependent cell cytolysis; IL, interleukin; TNF, tumor necrosis factor; DTH, delayed-type hypersensitivity.

AUTOIMMUNE INSULIN RESISTANCE AND HYPOGLYCEMIA SYNDROMES

There are several rare disorders of carbohydrate metabolism that have an autoimmune basis: type B insulin resistance, hypoglycemia resulting from insulinomimetic autoantibodies, and autoimmune hypoglycemia (Vol. 1; Chap. 15).

Type B Insulin Resistance

In type B insulin resistance, insulin resistance occurs as a result of autoantibodies directed toward the insulin receptor that interfere with insulin binding (42). A cutaneous manifestation of insulin resistance is acanthosis nigricans. Acanthosis nigricans is recognized as raised, waxy plaques that occur in skin folds especially around the neck and upper trunk, under the arms, breasts, and in the groin where skin abrasion occurs. In severe cases, the elbows, knees, and periumbilical skin can become exceptionally rough.

Type B disease, which has been described in a few adolescents, is more common in females and is associated with other autoimmune disorders. Type B patients may display antinuclear antibodies, anti-DNA antibodies, episodic hypocomplementemia, hypergammaglobulinemia, and leukopenia. A number of type B patients have been reported with systemic lupus erythematosus, scleroderma, Sjögren syndrome, ataxia–telangiectasia (43), and pheochromocytoma (44). In one patient, a dramatic remission was induced with steroid therapy (45).

Because commercially available testing for insulin receptor autoantibodies is not available, the diagnosis of type B insulin–resistant diabetes is suspected in nonketotic diabetic patients with acanthosis nigricans that require large doses of insulin ($>$100–1000 units/day) for treatment of hyperglycemia. The presence of non–organ-specific autoantibodies or non–organ-specific autoimmune diseases increases the likelihood of type B disease.

Hypoglycemia Resulting from Insulinomimetic Autoantibodies

Autoantibodies directed against the insulin receptor usually block insulin action. Patients have been

described with insulin receptor autoantibodies that displayed insulin-like action producing hypoglycemia (46). This disorder has been reported in children. Hypoglycemia secondary to insulinomimetic autoantibodies has been identified in adults with Hodgkin disease (47) and lupus (48,49) as well as children (50). Insulinomimetic autoantibodies, a very rare cause of hypoglycemia, can be considered when the biochemical determinations suggest hyperinsulinism (e.g., nonketotic hypoglycemia), yet insulin concentrations are depressed. Tests for insulin receptor autoantibodies are available generally only from research laboratories; therefore, the diagnosis must be based on clinical grounds. No specific therapy is available.

Autoimmune Hypoglycemia

In this disorder, autoantibodies are produced against circulating insulin (51,52). Whereas insulin antibodies are common in patients who have received exogenous insulin (including human insulin), patients with autoimmune hypoglycemia spontaneously develop such antibodies (53). Pediatric cases have been identified (54). This condition is most often recognized in Japan (55), but it has been reported in Norway and the Netherlands (56). Some subjects have developed this syndrome following exposure to antithyroid medications used to treat Graves disease (57). Thiourea-containing compounds are suspected to be triggers. Nevertheless, a report noted a decline in insulin autoantibodies despite continuation of methimazole therapy in a patient with Graves and autoimmune hypoglycemia (58). The author has familiarity with one unpublished, local case of autoimmune hypoglycemia.

The circulating autoantibody–insulin complexes can release insulin inappropriate to metabolic needs, increasing the free insulin concentration (59). At these times, hypoglycemia results from relative hyperinsulinism. The amount of circulating insulin complexed to autoantibodies may also lead to glucose intolerance or a mild diabetic state as well as hypoglycemia during fasting.

Autoimmune hypoglycemia is suspected when insulin autoantibody (IAA) are detected in non–insulin-treated, nondiabetic individuals who suffer from hypoglycemia. If drug induced, removal of the inciting drug may ameliorate the condition.

THYROID AUTOIMMUNITY

Thyroiditis and Graves Disease

Thyroid inflammation (thyroiditis) can be classified as acute (suppurative) thyroiditis resulting from bacterial thyroid gland infection, subacute (deQuervain) thyroiditis resulting from viral thyroid gland infection, or chronic thyroiditis resulting from an autoimmune lymphocytic infiltration of the gland, e.g., Hashimoto thyroiditis. Hashimoto thyroiditis results from cell-mediated autoimmune destruction of the thyroid follicular cells (60,61). On the other hand, Graves disease results from humoral autoimmunity to the TSH receptor (62). Agonist autoantibodies bind to the TSH receptor stimulating the gland to produce clinical thyrotoxicosis (i.e., hyperthyroidism). Hashimoto thyroiditis is the most common cause of goiter and acquired hypothyroidism in childhood (63) (Vol. 2; Chaps. 17 and 18).

Autoimmune thyroid disease (AITD) is a term that encompasses the spectrum of disorders spanning Hashimoto thyroiditis to Graves disease (Table 7). The two conditions are highly interrelated. Graves disease and Hashimoto thyroiditis can be observed in the same family and susceptibility to these diseases appears to be inherited in an autosomal-dominant pattern. AITD is commonly seen in both children and adults and can even rarely develop prenatally (64).

With an increasing frequency of adolescent girls becoming mothers (e.g., intended or unintended teenage pregnancy), pediatricians and pediatric endocrinologists must also be aware of postpartum thyroid disease that occurs in 5% to 10% of women following delivery (65). AITD in the postpartum period is termed postpartum thyroiditis. Postpartum thyroiditis follows approximately 5% of all pregnancies and follows approximately 10% of pregnancies of women with type 1 diabetes. The disorder can be manifested as hypo- or hyperthyroidism or combinations of sequential hypo- and hyperthyroidism. The presence of thyroid autoantibodies confirms the diagnosis. As well, the presence of thyroid autoantibodies predicts an increased risk for postpartum thyroiditis. Usually, euthyroidism returns by one year postpartum. However, later in life, there is a high risk for a recurrence of thyroid disease.

Clinically available tests of thyroid autoimmunity include thyroid microsomal (TMA) and thyroglobulin autoantibodies (TGA). Both assays are available clinically in a variety of formats including agglutation and enzyme immunoassays. The TMA assay has been updated and replaced in many institutions by the thyroperoxidase autoantibody assay (TPOA) (66,67). TPO is the autoantigen recognized in the TMA assay.

Table 7 Autoimmune Thyroid Disease: Spectrum of Disorders

Disorder	Possible manifestations
Hashimoto thyroiditis	Goiter
	Exophthalmos (less common than in Graves disease)
	Hypothyroidism (waxing–waning then permanent)
	Hashitoxicosis (transient)
	Postpartum thyroiditis (transient by nature)
	Thyroid fibrosis (late; with complete destruction)
Atrophic thyroiditis	Hypothyroidism (without goiter)
Graves disease	Goiter
	Exophthalmos
	Pretibial myxedema
	Hyperthyroidism
	Hypothyroidism (possible with long-term disease)
	Thyroid fibrosis (possible late manifestation; with complete destruction)

TPOA/TMA and TGA are detected in both Hashimoto thyroiditis and Graves disease. However, TGA are not as common as TMA/TPOA in all forms of AITD. In children, TMA/TPOA correlates best with the presence of Hashimoto thyroiditis, and virtually all children with Hashimoto thyroiditis will have TMA/TPOA at some time in their course, although some children also have TGA (67,68).

The TMA/TPOA and TGA assays do not distinguish Hashimoto thyroiditis and Graves disease although the autoantibodies are more common in Hashimoto thyroiditis than in Graves disease. Hashimoto thyroiditis and Graves disease are usually distinguished on the clinical and laboratory bases of hypo- versus hyperthyroidism. One variant of autoimmune thyroiditis is atrophic thyroiditis where TSH receptor autoantibodies bind to the receptor but do not stimulate the receptor (69,70). Such blocking (antagonist) autoantibodies induce thyroid atrophy and hypothyroidism. About 20% to 25% of individuals with atrophic thyroiditis exhibit antagonistic TSH receptor autoantibodies versus 10% of individuals with goitrous thyroiditis. Goiter is not present in the patients with atrophic thyroiditis, unlike patients with CLT. With disappearance of antagonistic TSH receptor autoantibodies, hypothyroidism can spontaneously remit (71).

Two new autoantigens have been recognized in AITD: megalin (GP330) and the thyroid Na^+/I^- symporter. Megalin is a multiligand receptor found on the apical surface of selected epithelial cells, including the thyroid gland. Antibodies to megalin were found in 50% of subjects with autoimmune thyroiditis and 10% of Graves disease subjects (72). Megalin is also referred to as a polyspecific receptor protein. Na^+/I^- symporter is not believed to be a major thyroid autoantigen because autoantibodies to the symporter are found in only 10% of subjects with Hashimoto thyroiditis and 20% of Graves disease subjects (73). Clinical assays are not available for autoimmunity to either megalin (GP330) or thyroid Na^+/I^- symporter.

TSH receptor autoantibody (TRAb) assays can be of value if the diagnosis of Graves disease is in question (74). TRAbs are more common in Graves disease than Hashimoto thyroiditis. It is important to recall that patients with Hashimoto thyroiditis can experience episodes of transient hyperthyroidism with accelerated gland destruction and release of thyroid hormone inducing a state of hyperthyroidism. This condition is termed Hashitoxicosis. Whereas radioiodine uptake is elevated in Graves disease, in Hashitoxicosis radioiodine uptake is not increased. As well, the pattern of radioiodine uptake is patchy throughout the gland in Hashitoxicosis, while radioiodine uptake in Graves disease is more homogeneous throughout the gland.

There are two main types of TRAb assays: thyroid-stimulating immunoglobulins (TSIs)(Fig. 5) and thyrotropin-binding inhibitory immunglobulins (TBIIs) (Fig. 6).

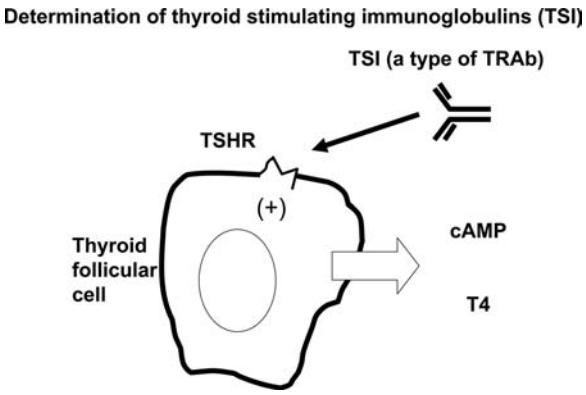

Figure 5 There are two main types of TRAb assays: TSIs and thyrotropin-binding inhibitory immunoglobulins. The TSI assay is carried out by incubating patient sera with thyroid cells, thyroid cell lines, or thyroid gland slices in tissue culture observing for the release of thyroxine or cAMP into the media. *Abbreviations*: TRAb, thyroid-stimulating hormone receptor autoantibody; TSI, thyroid-stimulating immunoglobulin; TSHR; cAMP.

The TSI assay is carried out by incubating patient sera with thyroid cells, thyroid cell lines, or thyroid gland slices in tissue culture observing for the release of thyroxine or cAMP into the media. Recall that the TSH receptor signals intracellullary through adenyl cyclase and cAMP generation. In many assays, stimulation must exceed 25% of baseline to represent a ''positive'' TSI assay. In the TBII assay, patient serum is added to a system that includes TSH receptors and radiolabeled TSH. If the patient's serum interferes with the binding of the radiolabeled TSH to the TSH receptors, TBII are detected. The TBII assay does not distinguish whether the TRAbs are agonists or antagonists (Vol. 2; Chap. 17).

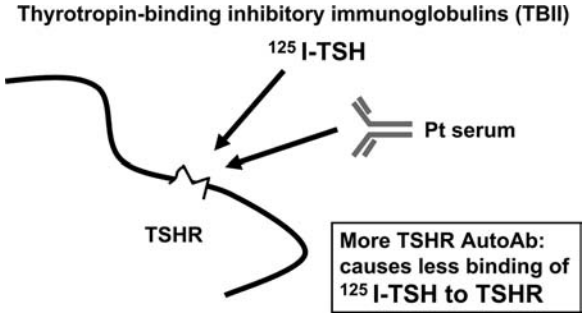

Figure 6 In the TBII assay, patient serum is added to a system that includes TSH receptors and radiolabeled TSH. If the patient's serum interferes with the binding of the radiolabeled TSH to the TSH receptors, TBII are detected. The assay does not distinguish whether the TRAbs are agonists or antagonists. *Abbreviations*: TBII, thyrotropin-binding inhibitory immunoglobulin; TSH, thyroid-stimulating hormone; TSHR, thyroid-stimulating hormone receptor autoantibody.

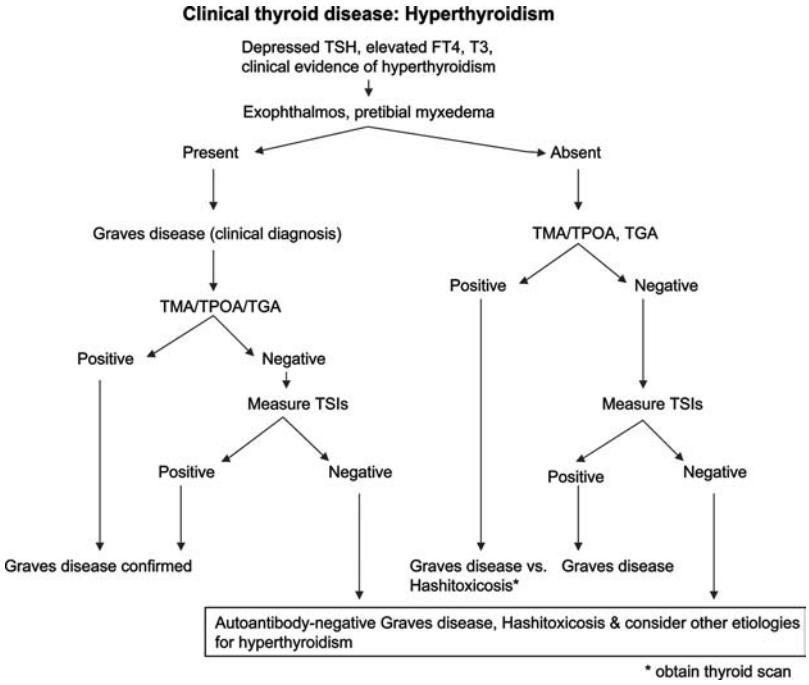

Figure 7 Clinical algorithm for the diagnosis of hyperthyroidism. *Abbreviations:* TSH, thyroid-stimulating hormone; TMA, thyroid microsomal autoantibodies; TPOA, thyroperoxidase autoantibody assay; TGA, thyroglobulin autoantibodies; TSI, thyroid-stimulating immunoglobulin.

TSI and TBII are detected in Graves disease; however, in atrophic thyroiditis, TSI is negative while TBII is positive. If specific serologic evidence of Graves disease is sought, TSI testing is superior to TBII testing. If specific serologic testing for atrophic thyroiditis is desired, both TSI and TBII analyses are recommended. TRAbs may uncommonly be detected in Hashimoto thyroiditis. This illustrates how many of the endocrine autoantibody assays are not 100% sensitive or specific for selected AITD disorders.

Whereas hypothyroidism and hyperthyroidism can be readily diagnosed on clinical grounds coupled with thyroid function testing [e.g., TSH, free T4 (FT4), and T3], the etiology of such disorders can be sought through autoantibody testing (Figs. 7 and 8). The recognition of AITD in patients has important implications for their medical care and the care of their family. For patients with AITD, the natural history of Hashimoto thyroiditis and, to a lesser extent, Graves disease should be recognized as

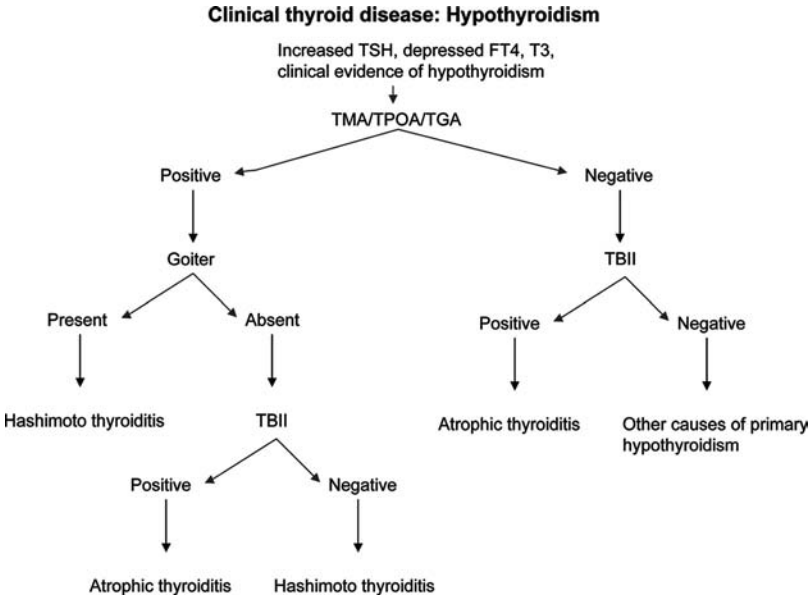

Figure 8 Clinical algorithm for the diagnosis of hypothyroidism. *Abbreviations:* TSH, thyroid-stimulating hormone; TMA, thyroid microsomal autoantibodies; TPOA, thyroperoxidase autoantibody assay; TGA, thyroglobulin autoantibodies; TBII, thyrotropin-binding inhibitory immunoglobulin.

waxing–waning conditions. With accelerated thyroid gland destruction in Hashimoto thyroiditis, transient hyperthyroidism can result (Hashitoxicosis), which should not be treated with antithyroid drugs. After one to two years approximately 50% of patients with Graves disease treated with antithyroid drugs will go into remission on a transient or permanent basis. The usual long-term outcome of Hashimoto thyroiditis is complete destruction of the thyroid gland with consequent clinical hypothyroidism and a small, sometimes firm, thyroid gland on palpation.

Importantly, recognition of the autoimmune etiology for thyroid dysfunction should initiate a search for associated autoimmune conditions in the patient and their family members (75,76). In probands with AITD, their first-degree relatives should be screened for thyroid autoantibodies. Also, gastric parietal autoantibodies (PCA) are common in patients with AITD (77) and their first-degree relatives. PCA are detected by indirect immunofluorescence using human stomach as substrate.

It is also strongly advised that all children with type 1 diabetes should be screened for thyroid autoimmunity (78,79). Approximately 25% of girls with type 1 diabetes will display TMA. Half of these girls will exhibit thyroid dysfunction. Hashimoto thyroiditis is seen in 80% of clinically evident cases, and in the remaining 20% of cases Graves disease occurs. Boys express TMA at half the frequency as girls, and half of TMA-positive boys will display a clinical thyroid disorder. In type 1 diabetes, TMA are usually present at the time of diagnosis. Therefore, it may be most prudent to screen for TMA once in type 1 diabetes patients at the time of diagnosis of type 1 diabetes. If TMA are detected, yearly TSH testing is advised. Once the TSH is outside the reference range, FT4 is measured. A persistent elevation in TSH with a FT4 within the reference interval suggests subclinical hypothyroidism. Clinical observation for the development of symptoms is reasonable clinical management, although thyroxine treatment to prevent symptoms is an acceptable alternative. If the FT4 is low, thyroxine replacement should definitely be initiated to treat hypothyroidism.

A persistent decrease in TSH and a normal FT4 and T3 are consistent with subclinical hyperthyroidism. Therapy would be instituted if there are cardiac arrhythmias or osteopenia; however, the frequency of these events in children is rare or unknown. If the FT4 is high or the T3 is elevated in the face of a normal FT4, antithyroid medications (e.g., propylthiouracil or methimazole) should be initiated. Suppressed TSH, normal FT4, and elevated T3 identify T3 toxicosis, which is a subtype of hyperthyroidism.

AITD and the Fetus

The question may arise as to whether maternal Hashimoto thyroiditis in pregnancy has an adverse effect on the fetus. Certainly, hypothyroid women who are untreated or inadequately treated during pregnancy can give birth to infants whose IQs will be lower than their peers. However, although TMA/TPOA and TGA do cross the placenta, by themselves they have no adverse consequences (80). On the other hand, TRAbs can influence the fetal thyroid.

Transient primary hypothyroidism in newborns can result from antagonist autoantibodies that bind to the TSH receptor and block endogenous TSH binding (81–83). While TRAbs may be antagonistic, such TRAbs may less commonly serve as agonists to the TSH receptor (84). TBIIs have been identified in infant and maternal sera (85,86). Finding thyroid-binding inhibitory immunoglobulins in mothers of infants with congenital hypothyroidism suggests that the hypothyroidism will be transient. However, thyroid replacement therapy in such infants should be continued until age three or four years when a trial off hormone replacement can be carried out to confirm or deny that the hypothyroidism is persistent (87). About 1 in 80 infants born to women with Graves disease displays transient neonatal hyperthyroidism.

ADRENAL AUTOIMMUNITY

Most cases of Addison disease result from autoimmune adrenalitis: a cell-mediated destruction of the adrenal cortex (88–90). While biochemical evidence of glucocorticoid and mineralocorticoid deficiency establishes the endocrinologic diagnosis of primary adrenal insufficiency (Vol. 2; Chap. 8), the diagnosis of adrenal autoimmunity requires adrenal autoantibody (ACA) testing (91,92).

Autoimmune Addison disease can exist as an isolated autoimmune endocrine disorder or it can be part of an APS (Table 8) (17,93–95).

APS type 1 is the association of mucocutaneous candidiasis, hypoparathyroidism, and Addison disease (or ACA). Two out of three of these disorders must be present to diagnose APS type 1. Because associated disorders include dental enamel hypoplasia and nail dystrophy, APS type 1 has also been termed the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome. Other associated conditions in APS type 1 patients include gonaditis (producing primary gonadal failure in women) and autoimmune hepatitis (Table 9). Less commonly

Table 8 Diagnostic Criteria for the APS

APS type 1	At least two of the following: Mucocutaneous candidiasis Hypoparathyroidism Addison disease or adrenal autoantibodies
APS type 2	Addison disease (or adrenal autoantibodies) plus Autoimmune thyroid disease (Schmidt syndrome) Type 1 diabetes, or Autoimmune thyroid disease and Type 1 diabetes (carpenter syndrome)

Abbreviation: APS, autoimmune polyglandular syndromes.

Table 9 Spectrum of Disease in the APS

	Type 1	Type 2
Adrenalitis/Addison disease	+++	+++
Hypoparathyroidism	+++	−
Mucocutaneous candidiasis	+++	−
Dental enamel hypoplasia/nail dystrophy	++	−
AITD	+	+++
Type 1 diabetes	+	+++
Gonaditis	++	+
Hypophysitis	−	+
Autoimmune hepatitis	++	+
Vitiligo/alopecia	+	+
Dermatitis herpetiformis	−	+
Fat malabsorption	+	−
IgA deficiency	+	+
Celiac disease	−	+
Autoimmune pernicious anemia	+	+
Pure red-cell aplasia	+	−
Immune thrombocytopenic purpura	−	+
Progressive myopathy	+	−
Myasthenia gravis	−	+
Stiff person syndrome	−	+
Parkinson disease	−	+

−, unobserved or rare; +, observed; ++, common; +++, pathognomonic.
Abbreviations: APS, autoimmune polyglandular syndromes; AITD, autoimmune thyroid disease.

associated conditions are type 1 diabetes mellitus, AITD, vitiligo, alopecia, fat malabsorption, IgA deficiency, pernicious anemia, red cell aplasia, and progressive myopathy. APS type 1 results from mutations in the autoimmune regulator (AIRE) gene whose predicted protein product appears to function as a transcription factor (18,19). Being homozygous for AIRE mutations produces APS type 1. APS type 1 and the IPEX syndrome are the only recessive autoimmune diseases: APS type 1 is an autosomal-recessive disorder whereas IPEX is X-linked recessive. APS type 1 usually has its onset in childhood and affects boys and girls equally. Although ACA have been reported in the IPEX syndrome, adrenal insufficiency has not been reported as of yet.

The association of Addison disease/ACA with either AITD or type 1 diabetes supports the diagnosis of APS type 2 (96). Other associated autoimmune conditions in APS type 2 include gonaditis, hypophysitis, autoimmune hepatitis, vitiligo, alopecia, dermatitis herpetiformis, IgA deficiency, celiac disease, pernicious anemia, immune thrombocytopenia, myasthenia gravis, stiff person syndrome, and Parkinson disease (Table 9). Similar to most other autoimmune endocrine disorders, APS type 2 is polygenic in etiology with typical onset in adulthood and affects women more often than men.

The classic assay for ACA involves indirect immunofluorescence using human adrenal gland as substrate. The fluorescence of the cortex indicates the presence of ACA in the patient's serum (97). Commonly all layers of the adrenal cortex fluoresce, whereas the medulla rarely fluoresces (98). The adrenal cortical cytoplasmic autoantigens were localized to the microsomes of the adrenal cortical cells similar to TMA/TPOA (99).

In new-onset patients with Addison disease, 60% to 70% or more of patients will express ACA (100). Studies in the 1990s established that target adrenal autoantigens were enzymes whose expression was limited to steroid-producing endocrine glands: 17-hydroxylase and 21-hydroxylase (101–104).

Expressed in the adrenal fasiculata and adrenal reticularis, 17-hydroxylase catalyzes the conversion of pregnanelone to 17-hydroxypregnenolone and progesterone to 17-hydroxyprogesterone. The lack of 17-hydroxylase expression in the adrenal glomerulosa permits the ultimate synthesis of aldosterone in that layer of the adrenal cortex. 17-Hydroxylase activity is part of the P450 enzyme encoded by CYP17, which also contains 17,20-ketosteroid reductase activity that converts 17-hydroxpregnenolone to dehydroepiandrosterone and 17-hydroxyprogesterone to androstenedione. 21-Hydroxylase is expressed in the glomerulosa where progesterone is converted to desoxycorticosterone and in the fasiculata where 17-hydroxyprogesterone is converted to desoxycortisol. Antibodies to 17- and 21-hydroxylase can be detected by immunoprecipitation or enzyme-linked immunosorbant assay (ELISA) techniques. At least one commercial vendor has developed assays for the detection of autoantibodies to 21-hydroxylase by radioisotopic and nonradioisotopic ELISA methodologies. Importantly, in many comparison studies, the ACA and 21-hydroxylase assays performed similarly in identifying humoral adrenal autoimmunity in patients with Addison disease.

The detection of either ACA or adrenal-enzyme autoantibodies in otherwise asymptomatic individuals predicts the latter development of Addison disease (105). Similar to the natural history of type 1 diabetes, the development of Addison disease passes through various sequential, cumulative stages: (i) elevated renin and normal to low aldosterone, (ii) deficient cortisol response to ACTH injection, (iii) elevated basal ACTH concentrations, and (iv) deficient basal aldosterone and cortisol secretion (106). Also similar to the predictive function of islet autoantibodies for the development of type 1 diabetes and TMA/TPOA/TGA predicting AITD, autoantibodies directed against the adrenal cortical cytoplasm or steroidogenic enzymes precede the first appearance of the clinical manifestations of Addison disease (107); however, their predictive power is stronger in children than in adults (108,109). Higher titer autoantibodies are also more predictive of progression to clinical disease (110,111).

Patients with type 1 diabetes who are seropositive for TPOA/TMA or TGA have an approximate 6% chance of expressing ACA (112). Without TPOA/TMA/TGA, the risk is only 2%. In ACA or 21-hydroxylase autoantibody-positive, yet asymptomatic, individuals, because there is a high risk of subsequently developing clinical Addison disease, such individuals should have yearly measurements of supine renin (sought as an indication of mineralocorticoid insufficiency)

and cortrosyn-stimulated cortisol (sought as an indication of glucocorticoid insufficiency: cortisol at baseline, 30 and 60 minutes, or baseline and 30- or 45-minute samples).

Elevated renin and/or deficient cortisol response to stimulation (e.g., peak cortisol $< 20\,\mu g/dL$ with a change over baseline of $< 7\,\mu g/dL$) suggests incipient adrenal insufficiency and the need for close scrutiny. Initiating replacement doses of cortisol should be considered. At the least, patients must be warned of the dangers of acute adrenal insufficiency (e.g., Addisonian crisis) and educated on the administration of parenteral steroids [e.g, hydrocortisone (hydrocortone phosphate or solucortef) or methylprednisolone (medrolacetate or solumedrol)] in cases of crisis (113). Therefore, a prescription for glucocorticoids and education on injection techniques should be provided along with instructions that the patient should obtain a medic-alert bracelet for Addison disease/adrenal insufficiency.

PARATHYROID AUTOIMMUNITY

Unfortunately, there are no reliable clinical tests for parathyroid autoantibodies despite their description almost 40 years ago (114). There is Western blot evidence for autoantibodies to the calcium sensing receptor; however, this finding has not been widely confirmed and testing for such autoantibodies is certainly not available on a routine clinical basis (115). Of questionable clinical significance, autoantibodies to circulating parathyroid hormone (116,117) and the renal tubular parathyroid hormone receptor have been observed but have not so far been described in children. It is prudent to state that autoantibodies to many circulating hormones (e.g., T4, T3, and ACTH) have been recognized, but only IAA have major clinical significance. Sometimes antihormone autoantibodies may interfere with the measurement of the hormone.

Autoimmunity to the parathyroid glands is part of APS type 1. In isolation, parathyroiditis (e.g., chronic lymphocytic inflammatory destruction of the parathyroid glands), however, cannot be diagnosed serologically. If a patient has hypocalcemia, hyperphosphatemia, an inappropriately low parathyroid hormone level (e.g., $< 65\,pg/mL$), and other endocrine autoimmunities are present, such as AITD, adrenalitis (e.g., positive ACA or adrenal-enzyme autoantibody), or type 1 diabetes, it is likely that the patient's hypoparathyroidism is also autoimmune in etiology.

GONADITIS

An autoimmune cell-mediated attack on the ovary can cause primary ovarian failure (118). The histologic appearance of a chronic inflammatory lymphocytic infiltrate is termed ''gonaditis.'' In the case of the ovary, the disorder can specifically be termed ''oophoritis.'' While males can express autoantibodies directed against gonadal cells, males do not suffer primary gonadal failure. Hypothetically, males may be less susceptible to such a disorder because spermatogenesis occurs throughout postpubertal life. In contrast, the ovary has a limited number of ova. Once these ova are exhausted, irreparable hypogonadism occurs. As well, spermatogenesis occurs in the seminiferous tubules of the testes, which might represent an immunologically privileged site where serum does not have direct access to spermatogonium, etc.; a basement membrane separates the developing sperm from the interstitium where antibody could be present. Probably of even more importance, lymphocytes do not have access to the lumen of the tubule in the absence of inflammation.

Primary ovarian failure due to autoimmune destruction of the ovary is a clinical consideration when primary or secondary amenorrhea is ''idiopathic'' in nature or occurs in the setting either APS type 1 or type 2. Approximately one in four women with autoimmune Addison disease will exhibit amenorrhea and 10% will develop premature ovarian failure (119). The presence of any autoimmune endocrinopathy coincident with ''idiopathic'' primary hypogonadism suggests that ovarian failure may be autoimmune in etiology.

A variety of autoantibodies to steroid-producing cells and tissues have been described (120,121). Steroidal-cell autoantibodies (SCA) are a collective for autoantibodies detected by indirect immunofluorescence against any of the following targets: the theca interna/granulosa layer of Graafian follicles, cells of the corpus luteum, the placental syncytiotrophoblast, and the Leydig cells of the testes. Ovaries from pregnant rabbits are used as substrate for indirect immunofluorescence for the detection of autoantibodies to the theca interna/granulosa layer of Graafian follicles and cells of the corpus luteum. Human placenta is employed as substrate for the identification of autoantibodies to placental syncytiotrophoblast, whereas human testis is utilized as substrate for assay of autoantibodies to the Leydig cells. If any or all of these tissues display fluorescence, SCA are positive. For completeness, testing for reactivity to all three tissues (testes, ovary, and placenta) should be requested if an autoimmune etiology for oophoritis is sought. Because ACA will also react with the testes, placenta, and ovary, in the presence of ACA it is not possible to determine whether SCA are absent or present. On the other hand, SCA do not react with the adrenal gland. In the absence of associated autoimmune disorders, SCA, specifically against the ovary, appear to be rare (122). On the other hand, gonadal autoantibodies have been observed in approximately 60% of cases of idiopathic premature ovarian failure (123).

In addition to demonstrating an etiology for primary ovarian failure (e.g., primary or secondary amenorrhea and SCA positivity), SCA in a menstruating woman predict the later development of ovarian

failure. Over 12 years of follow-up, ovarian failure occurred in 100% of SCA-positive women with APS type 1 (124). If a woman with SCA wishes to have children, storage of ova for use in a future pregnancy can theoretically be considered.

HYPOPHYSITIS

There a numerous case reports of individuals with suspected anterior pituitary tumors, where a chronic lymphocytic infiltrate has been observed in the surgically removed pituitary tissue (125–127). This has been termed ''autoimmune hypophysitis'' or ''lymphocytic hypophysitis.'' The inflammatory process can also involve the infundibular stem and the posterior pituitary. Such autoimmune hypophysitis is more common than either granulomatous or xanthomatous hypophysitis. Rarer causes of pituitary inflammation include sarcoidosis, Wegner granulomatosis, histiocytosis, tuberculosis, and syphilis (Vol. 2; Chap. 3).

Many of these affected individuals have displayed coexistent endocrine autoimmunities. However, there are no clinically available serologic tests for pituitary autoantibodies although work is progressing in this field (128). In some patients with type 1 diabetes, as well as their immediate relatives, autoantibodies reactive with prolactin and growth-hormone-secreting cells have been visualized by indirect immunofluorescence (129). No associations between such autoantibodies and clinical disease were recognized in these individuals. By Western blotting, pituitary autoantigens of 40 and 49 kDa have been identified, although expression of these proteins was not restricted to the pituitary gland (130). For the 49 kDa autoantibody, positive sera were most common in subjects with lymphocytic hypophysitis (70%) with lesser frequencies in subjects with Addison disease (42%), thyroid autoimmunity (15%), rheumatoid arthritis (13%), and in normal subjects (10%). In a Swedish group of patients with hypopituitarism, 28% had 49 kDa autoantibodies (131).

The important issues are (i) to recognize that hypophysitis is a neuropathologic diagnosis that can definitively be made only on surgically removed pituitary tissue, and (ii) hypophysitis is associated with other immunoendocrinopathies. Other than producing a mass effect in the pituitary that can be associated with various forms of potentially serious hypopituitarism (presumably through compression of adjacent cells), there appear to be no other adverse consequences of hypophysitis. Diabetes insipidus (DI) would appear to occur uncommonly in patients with hypophysitis, but it has been reported (132,133). When a pituitary mass occurs in a patient with an autoimmune endocrinopathy, hypophysitis must be in the differential diagnosis of the pituitary mass. Nonetheless, the diagnosis of hypophysitis is dependent on histologic examination of the tissue. The coexistence of an anterior pituitary adenoma or a craniopharyngioma is still very possible. Overall, idiopathic hypopituitarism in children is believed to be rarely caused by autoimmunity (134).

AUTOIMMUNE DI

Most cases of central DI result from disorders of the hypothalamus or the posterior pituitary, which is an anatomic and functional extension of the hypothalamus. Hypothalamic damage can follow from tumors (e.g., dysgerminomas), hemorrhage, trauma, malformations, and infections (e.g., meningitis or encephalitis). Similar processes can affect the pituitary. When central DI occurs in the absence of an anatomic lesion, autoimmune DI can be considered. On a research basis, pertinent autoantibodies have been described (135). Up to one-third of children with otherwise idiopathic DI may have an autoimmune process responsible for their condition. An adult has been reported with scleroderma and DI (136). Suspected autoimmune DI has also been observed in cases of APS type 1 (137) and other autoimmune conditions (100,138,139).

If there are associated immunoendocrinopathies, DI may very well be autoimmune in etiology. Because there are no clinically available tests for autoantibodies to the hypothalamic neurons that produce antidiuretic hormone, the diagnosis of autoimmune DI can only be made on clinical grounds and anatomic hypothalamic lesions must be thoroughly excluded.

NONENDOCRINE AUTOIMMUNITIES ASSOCIATED WITH IMMUNOENDOCRINOPATHIES

Nonendocrine autoimmunities can be frequently observed in association with various autoimmune endocrine disorders. The association of pernicious anemia and its serologic marker PCA with AITD was highlighted previously and is reviewed below.

Gastric Parietal Cell Autoimmunity

Because of the frequent coexistence of AITD and pernicious anemia in both individuals and families, the term thyrogastric autoimmunity was coined over 20 years ago. An extension of this concept is the strong association of thyrogastric autoimmunity with type 1 diabetes.

PCA were noted above to be detected by indirect immunofluorescence using human stomach as substrate. Another autoantibody indicative of gastric parietal cell autoimmunity is the intrinsic factor blocking autoantibody. A transporter specific to parietal cells is a target antigen: gastric $H^+/K^{(+)}$-ATPase pump (140,141). There are no clinically available assays for autoantibodies to the gastric $H^+/K^{(+)}$-ATPase pump although research assays have been developed (142).

The importance of PCA is that PCA predict an increased risk of developing pernicious anemia. PCA themselves do not appear to be pathogenic for the destruction of gastric parietal cells. However, chronic lymphocytic gastritis can destroy the gastric parietal cells leading to intrinsic factor deficiency. Because intrinsic factor is necessary for the absorption of vitamin B12, deficiency of intrinsic factor leads to vitamin B12 malabsorption and eventual vitamin B12 deficiency. Vitamin B12 deficiency can affect all rapidly dividing cells such as orogastrointestinal cells in addition to blood cells (i.e., red blood cells, platelets, and white blood cells). Together with a macrocytic anemia, leukopenia with hypersegmented neutrophil nuclei, and thrombocytopenia are consistent with vitamin B12 deficiency. Anemia is manifested clinically in dyspnea with exertion, faintness, and pallor. Neurologically, pernicious anemia is characterized by numbness, tingling, and weakness, which are evidence of spinal cord dysfunction. Other findings of pernicious anemia include anorexia, a sore smooth tongue, diarrhea, weight loss, and fever.

Because gastric parietal cells are also responsible for gastric hydrochloric acid production, gastric parietal cell destruction produces hypo- or achlorhydria that can interfere with normal iron absorption. It can be argued that all children with AITD should be screened for PCA (143,144). If PCA are present, yearly measurements of vitamin B12 and ferritin should be undertaken. Iron supplementation can treat iron deficiency noted in a declining or frankly low ferritin concentration. If the vitamin B12 level is less than 100 pg/mL, vitamin B12 deficiency is present and should be treated with vitamin B12 injections of 1 mg monthly. If the vitamin B12 level is between 100 and 299 pg/mL (the lower limit of the reference interval being 300 pg/mL), an elevated methylmalonic acid level in the blood or urine supports the diagnosis of vitamin B12 deficiency.

Celiac Disease

Besides pernicious anemia, another nonendocrine autoimmunity that is common in individuals with type 1 diabetes is celiac disease (a.k.a., gluten enteropathy and celiac sprue) (145,146). Occurring in both children and adults, celiac disease results from a cell-mediated immune response to gluten that is found in wheat (147). The result of this sensitivity is chronic inflammation with consequent atrophy of the upper small intestine mucosa. Clinical manifestations of celiac disease include diarrhea, steatorrhea, malabsorption, and vitamin and nutritional deficiencies.

While intestinal biopsy showing a flat mucosa with loss of villi, crypt hyperplasia, and intraepithelial lymphocytosis is histologically characteristic for celiac disease, intestinal biopsy is not a reasonable screening procedure. However, similar to PCA, screening for susceptibility to pernicious anemia and tissue transglutaminase autoantibodies can be carried out as a serologic screen for celiac disease. IgA autoantibodies to tissue transglutaminase are more sensitive and specific for celiac disease than the IgG tissue transglutaminase autoantibodies. While IgA autoantibodies against gliadin (antigliadin antibodies), reticulin, and endomysium (antiendomysial antibodies) have been described in subjects with celiac disease, the major autoantigen in celiac disease does appear to be the enzyme transglutaminase (148). The specific target autoantigen recognized by the antiendomysial antibodies is tissue transglutaminase. Individuals with transglutaminase autoantibodies (149) should undergo periodic small bowel biopsy. If the histologic findings of celiac disease are identified, wheat and wheat products should be eliminated from the diet. Transglutaminase autoantibodies have been detected in approximately 2% to 5% of subjects with type 1 diabetes.

There is considerable controversy concerning whether or not patients with type 1 diabetes should be screened for celiac disease in the absence of symptoms or signs of celiac disease (150). This issue remains unresolved.

Table 10 Non-APS-non-IPEX Autoimmune Endocrinopathy Associations

Presenting condition	Associated endocrinopathies
Type 1 diabetes	AITD Pernicious anemia
Genetic syndromes (Down, Turner, Kleinfelter)	Type 1 diabetes AITD Pernicious anemia
Congenital rubella infection	Type 1 diabetes Hashimoto thyroiditis

Abbreviations: APS, autoimmune polyglandular syndromes; AITD, autoimmune thyroid disease.

Table 11 Endocrine Autoantibody Tests that are Available from Hospital or Reference Laboratories and their Methodologies

Autoantibody	Methodology(s)
Islet cell cytoplasmic autoantibody	IFA
Insulin autoantibody	RBA
GAD autoantibody	RBA
IA-2 autoantibody	RBA
Thyroid microsomal autoantibody	HA, IFA, RIA, CF, latex agglutination
Thyroperoxidase autoantibody	RIA, ELISA, immunochemiluminometric
Thyroglobulin autoantibody	HA, IFA, RIA, CF, latex agglutination, immunochemiluminometric
Thyrotropin-binding inhibitory immunoglobulin	Radioreceptor assay
Thyroid-stimulating immunoglobulin	In vitro bioassay, immunoassay measurement of cAMP or T4
Adrenal cytoplasmic autoantibody	IFA
21-Hydroxylase autoantibody	RBA, RIA, ELISA
Steroidal-cell autoantibody	IFA

Abbreviations: CF, complement fixation; ELISA, enzyme-linked immunosorbant assay; HA, hemagglutination; IFA, indirect immunofluorescence assay; RBA, radio-binding assay; RIA, radioimmunoassay; GAD, glutamic acid decarboxylase.

Autoimmune Hepatitis

Chronic autoimmune hepatitis can be observed in children and adults with APS type 1 or type 2. A detailed discussion of the serology of this disorder goes beyond the scope of this chapter. However, it is worth noting that the following autoantibodies are commonly detected in individuals with autoimmune hepatitis: smooth muscle (SMA; approximately 50% prevalence), mitochondria [antimitochondrial antibodies (AMA); approximately 15% prevalence], and liver–kidney-mitochondrion-1 (LKM-1) (151). Although nonspecific, antinuclear antibodies can also be observed in approximately 80% of cases of autoimmune hepatitis. Nine different mitochondrial antigens have been described, termed M1 through M9. The E2 subunit of the pyruvate dehydrogenase complex (the M2 AMA antigen), the asialoglycoprotein receptor (152), cytochrome P450, UDP-glucuronosyl transferases (153), and F-actin (the SMA autoantigen) are autoantigens described in chronic hepatitis.

CONCLUDING REMARKS

Awareness of the conditions associated with an initially recognized autoimmune disorder will guide the type of autoantibody testing and screening, subsequent functional laboratory testing, and family screening and testing. APS type 1 and type 2 and the IPEX syndrome have been described in some detail above. The associations of type 1 diabetes with AITD, pernicious anemia, and adrenalitis bear emphasis (Table 10).

As well, autoimmune endocrinopathies are common in certain individuals with an autosomal (Down syndrome) or sex chromosome (Turner syndrome and Klinefelter syndrome) aneuploidy (Table 10). Children

Table 12 Antibody Testing for Autoimmunity and Appropriate Secondary Screening Tests

Disease	Autoantibody(s)[a] diagnostic for an autoimmune etiology	Indicated secondary laboratory screening or autoantibody testing
Type 1 diabetes	ICA, GADA, IA-2, IAA[b]	TMA/TPOA/TGA[c] and PCA; if either TMA/TPOA/TGA or PCA positive: ACA or 21-hydroxylase autoantibodies (evaluation for APS type 1); consider: IgA transglutaminase (controversial)
Thyroiditis •Hashimoto (goitrous) •Atrophic (nongoitrous)	TMA, TPOA, TGA	PCA (evaluation for thyrogastric autoimmunity); if PCA positive: ACA or 21-hydroxylase autoantibodies
Graves disease	TMA, TPOA, TGA; TSI (run TSI if above autoantibodies negative)	PCA (evaluation for thyrogastric autoimmunity); if PCA positive: ACA or 21-hydroxylase autoantibodies
Addison disease	ACA, 21-hydroxylase	Ca^{2+}, PO_4^{3-}, PTH[d] (evaluation for APS type 1), TMA/TPOA/TGA (evaluation for APS type 2), PCA, SMA, AMA
Primary ovarian failure	SCA	TMA/TPOA/TGA, PCA, SMA, AMA, Ca^{2+}, PO_4^{3-}, PTH[d]
Primary hypoparathyroidism	None	ACA, TMA/TPOA/TGA, PCA, SMA, AMA
Celiac disease	IgA transglutaminase	Controversial
Mucocutaneous candidiasis	None	ACA or 21-hydroxylase autoantibodies, Ca^{2+}, PO_4^{3-}, PTH[d] (evaluation for APS type 1)
Pernicious anemia	PCA (+/− intrinsic factor blocking autoantibody)	TMA/TPOA/TGA

+/−, with or without.

[a]Single autoantibody positivity is sufficient to indicate an autoimmune etiology in a patient with a clinically recognized disease; more than one autoantibody is ordered to increase the sensitivity of serologic detection of autoimmunity.

[b]Although type 1 diabetes is associated with many of the initially detected diseases, islet autoantibody screening to predict type 1 diabetes is not recommended outside of research settings because there is no currently available effective preventative therapy for type 1 diabetes.

[c]TMA/TPOA/TGA: measure TMA or TPOA and TGA; consider measuring TGA only if TMA and TPOA are negative.

[d]Measure PTH if calcium is low or phosphate is high.

Abbreviations: ICA, islet cell cytoplasmic autoantibody; GADA, glutamic acid decarboxylase autoantibody; IAA, insulin autoantibody; TMA, thyroid microsomal autoantibodies; TPOA, thyroperoxidase autoantibody assay; TGA, thyroglobulin autoantibodies; PCA, parietal cell autoantibodies; ACA, adrenal autoantibodies; APS, autoimmune polyglandular syndrome; TSI, thyroid-stimulating immunoglobulin; SMA, smooth muscle antibodies; AMA, antimitochondrial antibodies.

Table 13 Clinical Evaluation and Management of Individuals Positive for Various Autoantibodies or Presenting with Disorders Associated with Endocrine Autoimmunities

Condition/autoantibody(s)	Yearly Clinical Evaluation/ Measurement and Management
ICA, GADA, IA-2A, and/or IAA[a]	Frequently sampled intravenous glucose tolerance test; if first phase insulin response is repeatedly $< 1\%$ percentile: enter into research trial to prevent type 1 diabetes[a]
TMA, TPOA, and/or TGA	TSH measurement; if abnormal: measure FT4; treat hypo- or hyperthyroidism
ACA (or) 21-hydroxylase autoantibody	Cortrosyn stimulation test; supine renin; treat glucocorticoid and/or mineralocorticoid deficiency (Addison disease)
Steroidal-cell autoantibody	Estradiol in women, LH and FSH; treat hypogonadism; also evaluate adrenal function (see ACA/21-hydroxylase autoantibody-positive recommendations)
Gastric parietal cell autoantibody or intrinsic factor blocking autoantibody	Vitamin B12 (if > 100 and < 300 pg/mL: measure plasma or urine methylmalonic acid); ferritin; treat vitamin B12 and/or iron deficiency
Transglutaminase autoantibodies	Small bowel biopsy (consult pediatric gastroenterology for appropriate follow-up and management and frequency of rebiopsy if initial biopsy is normal)
APS type 1 without apparent hypoparathyroidism	Calcium and phosphate; if hypocalcemia and/or hyperphosphatemia detected: measure intact PTH; treat hypoparathyroidism
APS type 1 without apparent hypoadrenalism	ACA (or) 21-hydroxylase autoantibody; if positive: cortrosyn stimulation test and supine renin; manage as above per ACA or 21-hydroxylase autoantibody positive
Mucocutaneous candidiasis and/or isolated hypoparathyroidism	ACA (or) 21-hydroxylase autoantibody; if positive: cortrosyn stimulation test and supine renin; manage as above per ACA or 21-hydroxylase autoantibody positive

[a]For research purposes only.

Abbreviations: ICA, islet cell cytoplasmic autoantibody; GADA, glutamic acid decarboxylase autoantibody; IA-2A, IA-2 autoantibody; IAA, insulin autoantibody; TMA, thyroid microsomal autoantibodies; TPOA, thyroperoxidase autoantibody assay; TGA, thyroglobulin autoantibodies; TSH, thyroid-stimulating hormone; ACA, adrenal autoantibodies; APS, autoimmune polyglandular syndrome.

with congenital rubella infection are also at risk for certain autoimmune endocrinopathies (154).

Table 11 lists the autoantibody determinations and their methodologies that are commonly clinically available in hospital or reference laboratories that can be ordered by clinicians.

Autoantibody testing in type 1 diabetes is further discussed in Vol. 1; Chap. 5. Table 12 summarizes testing for diagnosis and appropriate secondary screening tests. Several sections of this chapter have more elaborate discussions of these topics (see above).

Table 14 Autoantibody Testing in First-Degree Relatives of Probands Presenting with Autoimmune Disorders

Disorder in the proband	First-degree relative should be screened for	Indicated testing[a]
Type 1 diabetes	Type 1 diabetes	In research settings only ICA, GADA, IA-2A, IAA[b]
	AITD	TMA/TPOA/TGA[c]
	Pernicious anemia	PCA (with or without intrinsic factor blocking autoantibody testing)
AITD	Pernicious anemia	PCA (with or without intrinsic factor blocking autoantibody testing)
Addison disease (or) APS	Type 1 diabetes	In research settings only ICA, GADA, IA-2A, IAA[b]
	AITD	TMA/TPOA/TGA[c]
	Pernicious anemia	PCA (with or without intrinsic factor blocking autoantibody testing)
	Hypoparathyroidism	Ca^{2+}, PO_4^{3-}, PTH[d]
	Primary ovarian failure	SCA
	Autoimmune hepatitis	SMA, AMA
Pernicious anemia	AITD	TMA/TPOA/TGA[c]

[a]Single autoantibody positivity is sufficient to pursue a functional laboratory evaluation as indicated in Table 9.

[b]Although type 1 diabetes is associated with many of the initially detected diseases in probands, islet autoantibody screening to predict type 1 diabetes in relatives is not recommended outside of research settings because there is no currently available effective preventative therapy for type 1 diabetes.

[c]TMA/TPOA/TGA: Measure TMA or TPOA and TGA; consider measuring TGA only if TMA and TPOA are negative.

[d]Measure PTH if calcium is low or phosphate is high.

Abbreviations: ICA, islet cell cytoplasmic autoantibody; GADA, glutamic acid decarboxylase autoantibody; IA-2A, IA-2 autoantibody; IAA, insulin autoantibody; AITD, autoimmune thyroid disease; TMA, thyroid microsomal autoantibodies; TPOA, thyroperoxidase autoantibody assay; TGA, thyroglobulin autoantibodies; PCA, parietal all autoantibodies; APS, autoimmune polyglandular syndrome; SCA, steroidal-cell autoantibodies; SMA, smooth muscle autoantibodies; AMA, antimitochondrial antibodies.

The clinical evaluation of endocrine autoanti body–positive, asymptomatic individuals is depicted in Table 13. Specific information about thyroid function testing can be found in Vol. 2; Chaps. 17–19, whereas adrenal function testing is addressed in Vol. 2; Chaps. 8 and 9, and gonadal function testing is reviewed in Vol. 2; Chaps. 11, 13, and 14.

Appropriate autoantibody testing in first-degree relatives of individuals with autoimmune diseases discussed in this chapter is summarized in Table 14.

Considering the high relative frequency of type 1 diabetes and AITD, the autoimmune endocrinopathies are among the most common collective types of disorders that are seen in general pediatric practice, as well as in the subspecialty practice of pediatric endocrinology. Knowledge of the associations highlighted in this chapter will guide appropriate diagnostic strategies and interventions in probands and their families.

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Multiple Endocrine Neoplasias

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INTRODUCTION

The multiple endocrine neoplasias (MEN) are inherited diseases characterized by the occurrence of hormone-secreting/hormone producing tumors in two or more endocrine glands in an individual patient and/or in the patient and one or more family members. Although they are relatively rare, their importance is due to their risk of malignancies, which can be reduced by early diagnosis and treatment, especially among family members. There are two types of MEN syndromes: type-1 and type-2. Multiple endocrine neoplasias type-2 (MEN-2) has been further subcategorized into two clinical forms called MEN-2a and MEN-2b (formerly MEN-3) with distinct patterns of tissue involvement (Table 1). MEN-1 and MEN-2 share certain characteristics: both of these syndromes have dominant patterns of inheritance. They are hormone producing endocrine tumors, which are often benign in nature but have a potential for malignancy. They occur relatively early in life which allows for the screening of asymptomatic family members at risk.

MULTIPLE ENDOCRINE NEOPLASIAS-1

Multiple endocrine neoplasias-1 (MEN-1) was first described more than 50 years ago as Wermer's syndrome (1). MEN-1 is a dominant autosomal disorder characterized by tumors of the parathyroid glands, anterior pituitary (AP), and pancreatic islets. Foregut carcinoid tumors are less common while nonendocrine tumors such as lipomas, facial angio-fibromas, and skin collagenomas are relatively frequent. Endocrine and nonendocrine features of MEN-1 and their relative frequencies are summarized in Table 2 (2,3). The prevalence of MEN-1 as estimated by autopsy studies approximates 2.5:1000. However, 1% to 18% of patients with primary hyperparathyroidism have been variously reported to have underlying MEN-1 (the prevalence is age dependent) while among patients with a gastrinoma, the prevalence has been reported from 16% to 38%. Some 3% of patients with pituitary tumors have been reported to have MEN-1 (2). All age groups may be affected, but in 80% of patients, the clinical manifestations will have appeared by the fifth decade of life (3). Parathyroid hormone (PTH) secreting tumors are rare in children, but they are increasingly common through late midlife.

Genetics and Pathogenesis

The gene responsible for MEN-1 is located at chromosome 11q13 (5). Loss of heterozygosity at this locus in MEN-1 tumors suggested that MEN-1 gene was likely a tumor suppressor gene (TSG) (6). A decade later, the MEN-1 gene was identified by positional cloning and many of the germ-line and somatic mutations in the MEN-1 gene have been reported (6,7). The human MEN-1 gene consists of 10 exons with an 1830 base-pair coding region that encodes a novel protein, named menin. Menin is a 610-amino-acid protein that has no homology to any other known protein. The menin protein has a nuclear location, with two nuclear binding domains located in the C-terminus (8). The first documented interactive binding domain was the activated protein 1 (AP 1) transcription factor junD but not other members of the AP 1 family. Menin binds to JunD, a jun/fos transcription factor and inhibits tumor-suppressor transcriptions stimulated by JunD (9). Other proteins that interact with menin are yet to be discovered. All the truncate menin proteins seen in MEN-1 lack at least one of these nuclear localization signals resulting from nonsense and frameshift mutations. MEN-1 mutations are scattered in and around the open reading frame of menin and are diverse in their types. The majority of mutations (75%) in the MEN-1 gene are functionally inactivating,

Table 1 Genetics and Clinical Features of MEN Syndromes

	Locus	Gene	Mutation distribution	Mutation effect	Encoded protein	Mode of inheritance	Age of onset (yrs)	Key endocrine features	Age to perform gene analysis in family members (yrs)
MEN-1	11q13	MEN1	Spread over nine coding exons	Gene inactivation	Nuclear protein, binding junD	Autosomal dominant	15–25	Primary hyperparathyroidism Pituitary tumors Pancreatic tumors	15
MEN-2	10q11.2	Ret		Gene activation	Transmembrane glycoprotein, tyrosine kinase	Autosomal Dominant	30–40		
MEN-2a			Exons 10,11					Medullary thyroid carcinoma Pheochromocytoma	5
MEN-2b			Exon 16				1–18	Primary hyperparathyroidism	Neonatal
FMTC			Exons 10,11,13,14				30–40	Medullary thyroid carcinoma Pheochromocytoma Medullary thyroid carcinoma	5

Abbreviations: MEN, multiple endocrine neoplasias; FMTC, familial medullary thyroid carcinoma.

consistent with MEN-1 being a TSG (2,10,11). In the 5% to 10% of mutations, which are not in the coding region of the MEN-1 gene are often found in the promoter or enhancer regions that affect MEN-1 transcription rates (12). The importance of MEN-1 gene inactivation in tumorigenesis is further substantiated by the fact that MEN-1 is one of the most common mutated genes in sporadic endocrine tumors, including parathyroid adenoma, gastrinoma, insulinoma, VIPoma, and bronchial carcinoids. About 80% to 90% of MEN-1 families carry germline, MEN-1 mutations whereas some of MEN families have no identifiable mutations despite having typical clinical features. Unlike in MEN-2, no correlations have been reported between the underlying MEN-1 genotype and phenotype (13). MEN-1 families demonstrate age related differences in the penetrance of MEN-1 mediated phenotype, with nearly 100% of the carriers

Table 2 Clinical Features of MEN 1 Syndrome and Their Approximate Penetrance

Endocrine features	Penetrance (%)	Nonendocrine features	Penetrance (%)
Parathyroid adenoma	90	Facial angiofibromas	85
Pituitary Tumors		Collagenomas	70
Prolactinoma	20	Lipomas	30
Other: GH + PRL	5	Leiomyoma	5
GH	5	Meningiomas	5
ACTH	2	Ependymoma	1
TSH	Rare		
Nonfunctioning	5		
Adrenal tumors			
Nonfunctioning adrenal cortex	20		
Pheochromocytoma	<1		
Entero-Pancreatic Tumors			
Gastrinoma	40		
Insulinoma	10		
Nonfunctioning including pancreatic polypeptide	20		
Other (glucagonoma, VIPoma, somatostatinoma, etc.)	2		
Foregut Carcinoids			
Gastric enterochromaffin-like tumor	10		
Thymic carcinoid nonfunctioning	2		
Bronchial carcinoid nonfunctioning	4		

Abbreviations: GH, growth hormone; PRL, prolactin; ACTH, adenocorticotropic hormone; TSH, thyroid-stimulating hormone.

of the MEN-1 mutations being affected clinically by the age of 60 years (14).

Clinical Features

The clinical phenotype in MEN-1 results from the hypersecretion of the hormones by the glands affected and the effects of malignancy when it occurs.

Primary Hyperparathyroidism

Primary hyperparathyroidism is the most common (95% of patients) component and is usually the first endocrine manifestation of MEN-1 (15). In contrast, MEN-1 is rare in the population accounting for only a small number (1–3%) of patients who present with primary hyperparathyroidism (16). The typical age of onset of hyperparathyroidism in MEN-1 is 15 to 20 years (17). The disease usually presents with a long asymptomatic stage characterized by hypercalcemia, clinically manifest by polyuria, polydipsia, muscular weakness, constipation, nephrolithiasis, back and limb pains from demineralized osteoporotic bone, and osteitis fibrosa cystica. Older patients may develop characteristic subperiosteal absorptions of the second phalanges and ''salt and pepper'' changes resulting from alternating hypo- and hyperdensities in skull radiographs. Hypercalcemia in elderly patients may lead to marked changes in affect and problems of mentation that can have them erroneously diagnosed with senility or psychiatric disorders. A hypercalcemic storm or crisis is rarely encountered these days while the development of parathyroid cancer almost never occurs (18). Laboratory findings document elevated total (serum) calcium and ionized calcium (plasma) levels plus low serum phosphate levels in the face of elevated and nonsuppressed concentrations of PTH. Whereas, measurement of PTH by certain immunoassays may detect inert PTH fragments, those that measure more intact molecules or the midportion of the molecule have lower false positive rates. Replicate elevations of serum calcium over 10.2 mg/dl are consistent with the diagnosis while levels over 13 mg/dl can be life threatening and require urgent treatment. Patients often have hypercalciuria exceeding 200 to 300 mg/day of calcium on normal diets. In patients with acute symptoms of hypercalcemia, hydration with saline infusions, and diuretics to force natriuresis is helpful in the emergency situation. Oral phosphate can be given with more chronic effects, however, the dosages given should be initially low (1–2 g daily) to avoid precipitation of calcium phosphate in cardiac tissues. Later, the dose can be increased to 2–4 g daily if necessary. Mithramycin is a cytotoxic antibiotic that can inhibit bone resorption, but is toxic and best reserved for cancer-related hypercalcemias. Biphosphonate compounds have been used increasingly to control both acute and chronic hypercalcemias (19). Calcimimetics or calcium-sensing receptor agonists represent a new class of drugs for the management of hyperparathyroidism, which act directly on the parathyroid gland to decrease PTH secretion and perhaps to inhibit parathyroid tumor growth also. While the latter agents were still under investigation at the time of writing, clinical trials have already demonstrated their effectiveness in controlling hyperparathyroidism (20).

MEN-1 can be differentiated from sporadic parathyroid adenoma by the earlier age of onset of the hypercalcemia (typically 15–25 vs. 50–60 years), lack of a female bias (the male/female ratio of MEN-1 is 1:1 vs. 1:3), different parathyroid pathology with asymmetric enlargement of multiple parathyroid glands in MEN-1, high recurrence risk, no malignant changes, autosomal dominant pattern of inheritance in pedigrees, and the additional presence of pituitary or pancreatic tumors, or skin manifestations. MEN-1 should also be differentiated from familial hypocalcuric hypercalcemia that has its onset from birth and is characterized by low levels ($<$100 mg in adults) of calcium in the urine and normal level of PTH in the serum (21).

Surgical removal of the hyperfunctioning tumors is the treatment of choice, which is best accomplished by a good preoperative work-up involving ultrasound and/or magnetic resonance imaging (MRI) studies, and sometimes preoperative venous sampling for PTH, whereas, 20% of parathyroid tumors are located in the upper mediastinum. The Tc^{99m} sestamibi (methoxyisobutylisonitrile) scan is the most useful among several methods that may be used alone or in combination (22). The necessity for surgery in hyperparathyroidism, especially in children, remains somewhat controversial, especially with the recent availability of biphosphonates and calcimimetics to treat it. Surgery may be delayed if the patient has only a mildly elevated serum calcium level, no renal calculi and normal bone density, no previous hypercalcemic crisis. In addition, the patients need to be monitored periodically for serum calcium, PTH levels, bone density, and urinary calcium. Surgical intervention is, however, indicated when the calcium level increases to more than 12.0 mg/dl after adjusting serum albumin levels, PTH levels rise, and/or renal stones, or decreased bone densities occur. Early parathyroid surgery also decreases gastrin release, because high calcium levels in MEN-1 increases the secretion of gastrin from gastrinomas. Parathyroidectomy lowers calcium levels and therefore decreases gastrin release in MEN-1 (23). However, medical treatment of gastric acid oversecretion, Zollinger–Ellison Syndrome (ZES), is so successful currently, that coexistence of hyperparathyroidism and ZES is not sufficient indication for parathyroidectomy in MEN-1.

Entero-Pancreatic Tumors

The second most common clinical manifestation of MEN-1 is an entero-pancreatic tumor. The incidence of pancreatic islet cell or gastrointestinal tumors in

MEN-1 is age dependent but varies from 30% to 80% (24). The occurrence of pancreatic tumors in MEN-1 is important because of their malignant potential. Gastrinoma is the most common cause of severe symptoms and signs in MEN-1 and they are usually malignant with half having metastasized before diagnosis (25). The ZES is the most frequent syndrome detected in MEN-1 leading to persistent peptic ulcerations and hyperacidity due to the hypersecretion of gastrin, often from a pancreatic islet cell tumor. The hypercalcemia from parathyroid adenoma can elevate further serum gastrin levels and worsen symptoms. Patients with ZES may also present with diarrhea or steatorrhea. The diagnosis is made by an elevated fasting serum gastrin concentration in association with an increased basal gastric acid secretion. Whereas H2 receptor histamine antagonists such as cimetidine or (Tagamet), ranitidine (Zantac), or famotidine (Pepcid) usually reduce gastric hyperacidity, they often fail to do so adequately in this condition. However, reduction of basal acid output may be achieved by the substituted benzimidazoles (e.g., Omeprazol), which are potent inhibitors of parietal cell H^+/K^+-ATPase (23). In addition, somatostatin analogs are also effective reducing both gastric acid hypersecretion and serum gastrin levels (24).

Because of the multifocal nature of the tumors in MEN-1 syndrome, surgical therapy is controversial and often not successful. Only 16% of patients become free from symptoms after surgical intervention (26). In some islet tumors, watery diarrhea hypokalemia and hypochloremia can be seen in the absence of increased gastrin levels, due to hypersecretion of vasoactive intestinal peptide (Vipoma) and prostaglandins, while hypersecretion of glucagon can induce weight loss, hyperglycemia, venous thromboses, and a characteristics necrotizing migratory erythema of the skin.

Insulinoma is the second most common pancreatic islet cell tumor in patients with MEN-1 which presents more often in patients under 40 years of age (3). The tumors are typically multicentric and may become malignant. Patients present with hypoglycemia especially after fasting. Biochemical investigations reveal inappropriately raised plasma insulin concentrations in association with hypoglycemia. Elevated plasma *C*-peptide and proinsulin levels are useful to confirm the diagnosis, since proinsulin levels may be disproportionately high. Surgery is the optimal treatment and is usually curative when a discrete tumor is found (27).

Besides α and β islet cell tumors, others (δ cell) may secrete pancreatic polypeptide, or less commonly adenocorticotropic hormone (ACTH), serotonin, or somatostatin. However, surgical removal of the tumor may be difficult when metastases have already occurred by the time of diagnosis. In some patients the administration of Octreotide may be helpful.

Pituitary Tumors

Pituitary tumors occur in 15% to 20% of patients with MEN-1 (28). Some 60% of these tumors are prolactin secreting fewer than 25% are growth hormone (GH) secreting, whereas, about 5% secrete ACTH. Nonfunctioning pituitary tumors also occur. The clinical features of pituitary adenomas depend upon the hormone(s) secreted and the size of the tumor. Women can present with amenorrhea, infertility, and galactorrhea and men with impotence, whereas, acromegaly or Cushing disease can occur with either gender. Pituitary tumors can also compress the optic chiasm, causing bilateral peripheral hemianopsia. The diagnostic and therapeutic approach to pituitary tumors in the context of MEN-1 is not different from that in sporadic cases and consists of medical therapy with dopamine agonists such as cabergoline, bromocriptine, or the long- acting somatostatin analog (Octreotide) selective hypophysectomy for larger tumors, and radiotherapy for residual unresectable tumors. However, pituitary tumor screening should continue after an apparently successful resection, as pituitary tumors may recur from residual tumor cells.

Foregut Carcinoid Tumors and Adrenal Tumors

Thymic carcinoid tumors develop in about 5% of the patients, especially in men who are heavy smokers (29). In contrast, bronchial carcinoid tumors are found mainly in females (30). The tumors are usually nonfunctioning and are not often recognized before the age of 40 years. Thymic carcinoid tumors appear more aggressive in MEN-1. Gastric entero-chromaffin-like cell carcinoids are usually multiple, smaller than 1.5 cm and mainly diagnosed incidentally during gastric endoscopy for ZES in MEN-1 (31,32). Other tumors seen on occasion in MEN-1 include adrenal adenomas (sometimes ACTH secreting) and carcinomas, and thyroid tumors that are never of the medullary C-cell type.

Both cutaneous and visceral lipomas, angiofibromas, and collagenomas are nonendocrine features of MEN-1. They have been suggested to be helpful for the diagnosis of nonsymptomatic MEN-1 carriers (33).

Clinical Variants of Multiple Endocrine Neoplasias-1

Hyperparathroidism is the most common and usually the first presenting clinical feature of MEN-1. Identifying isolated hyperparathyroidism with a MEN-1 mutation can be a variant of MEN-1. Another variant of MEN-1 in some families is associated with a high incidence of prolactinoma but lower incidence of gastrinoma than typical MEN-1.

Screening of Family Members

Family members of patients with MEN-1 are at high risk for carrying the defective gene. The closer the relationship to the proband, the higher the risk. Screening for the MEN-1 gene is of great importance because early diagnosis and treatment of these tumors can reduce morbidity and mortality rates. Genetic screening of MEN-1 is labor intensive due to scattered mutations in and around the open reading frame

of the menin gene and the diversity of mutants described (34). Whereas, the proper role of genetic testing in affected pedigrees still need to be defined, individuals found to be carriers of a mutant MEN-1 gene should nevertheless undergo periodic hormonal screening. Screening for tumor signs in MEN-1 can be difficult and expensive because of the large number of potentially useful tests. Biochemical screening (serum concentration of calcium, gastrin, and prolactin) is recommended at least once a year for the earliest presenting and potentially treatable conditions of MEN-1. Those found to have the mutation from the age of five years onwards, should be screened every three to five years by pituitary and abdominal imaging.

MULTIPLE ENDOCRINE NEOPLASIAS-2

MEN-2 (Sipple's syndrome) is inherited in an autosomal dominant pattern as is MEN-1 and with a high degree of penetrance. Some 500 to 1000 kindreds affected by MEN-2 have been identified to date (35). All variants of MEN-2 show high penetrance for medullary thyroid carcinoma (MTC), a calcitonin-secreting tumor of C-cells. Virtually all patients show evidence for MTC as palpable nodule and/or elevated calcitonin levels by age 40 (36). Clinical variants of MEN-2 are summarized in Table 3. MEN-2a which accounts for over 75% of MEN-2 as described by Sipple (37) and others (38), consists of bilateral and multicentric MTC in 90%, unilateral or bilateral pheochromocytoma in 50%, and parathyroid hyperplasia or adenomas in 20% to 30% of affected individuals (39–41). Rare forms of MEN-2 include familial medullary thyroid carcinoma (FMTC) in which the tendency to develop thyroid carcinoma is not associated with other clinical manifestations (42). MEN-2a is also associated with cutaneous lichen amyloidosis (43) and Hirschsprung's disease (44). In MEN-2b, patients present with MTC and pheochromocytoma, without development of hyperparathyroidism (45). In addition, affected individuals have a marfanoid habitus and multiple mucosal, and intestinal ganglioneuromatosis (46–48). In all reported cases, the mucosal neuromas are associated with bilateral and multicentric C-cell hyperplasia, or MTC, or both. The MTC in MEN-2b is more aggressive than in MEN-2a, and occurs at an earlier age (49). MEN-2 tumors result from germline mutations in the *RET* protooncogene (35,50). There are important correlations between the MEN-2a and MEN-2b clinical phenotype and individual *RET* codon mutations (51,52).

Genetics and Pathogenesis

The gene responsible for MEN-2 has been identified as the REarranged during Transfection (*RET*) gene located on the chromosome 10q11.2 (51,53). The coding sequence of *RET* consists of 21 exons extending over more than 60 kb of genomic DNA (54). *RET* encodes a cell surface tyrosine kinase receptor composed of a large extracellular domain, a single transmembrane region, and an intracellular tyrosine kinase domain. The extracellular domain contains a cadherine ligand-binding site, which may be important for cell–cell signaling, and a cysteine-rich extracellular site that is important for receptor dimerization (55). The *RET* protein is a member of the receptor tyrosine-kinase family, which transduces growth and proliferation signals in tissues derived from the neuronal crest, from which all of the tissues involved (thyroid C cells, adrenal medulla, and autonomic ganglia), and urogenital tract (56,57) in MEN-2 are derived. Glial cell-line-derived neurotrophic factor (GDNF) is a ligand for the *RET* receptors (58). *RET* mediates the signals of GDNF family including GDNF, neurturin, persephin, and artemin (59–61). They are expressed in the nervous system and promote the survival of neurons during neuronal development. The GDNF receptor-α members (GFRα-1, GFRα-2, GFRα-3, and GFRα-4) are glycosyl-phosphatidylinositol cell surface proteins expressed in GDNF responsive cells and interact with GDNF family members to activate *RET* receptors (62,63). *RET* mutations can lead to gain of function in two ways. The first, is a nonhereditary somatic rearrangement in which genomic DNA from *RET* kinase region is fused to a

Table 3 MEN-2 Syndromes

Syndrome	Characteristic features
MEN-2a	MTC Pheochromocytoma Parathyorid hyperplasia/adenoma
MEN-2a variants	
FMTC	MTC
MEN-2a with cutaneous lichen amyloidosis	MEN-2a and pruritic skin lesions located over the upper back
MEN-2a or FMTC with Hirschsprung's disease	MEN-2a or FMTC with Hirschsprung's disease
MEN-2b	MTC Pheochromocytoma Mucosal neuromas (conjuctiva, eye lids, buccal mucosa, lips, tongue) and intestinal ganglioneuromatosis Marfanoid habitus

Abbreviations: MEN, multiple endocrine neoplasias; MTC, medullary thyroid carcinoma; FMTC, familial MTC.

promoter sequence of another gene, creating a RET-PTC gene which underlies many papillary thyroid cancers (64). The second is where missense or stop codon mutations of *RET* occur in MEN-2. The most common mutations in MEN-2 affect codons encoding extracelluar cysteine residues (609,611,618,620,630,634) and are in exons 10 and 11 with a few mutations located within the intracellular domain of *RET* in exons 13, 14, and 15 (35,65,66). The most common mutation is cysteine to arginine substitution at codon 634 (35). In contrast to MEN-1 where there is no relationship between any specific mutation (genotype) and clinical phenotype, there is a strong correlation between the *RET* genotype and phenotype in MEN-2. Any amino acid substitution at codon 634 most commonly results classic MEN-2a with MTC, pheochromocytoma, and hyperparathyroidism, or one of its variants. Most cases of MEN-2b cases are caused by a codon 918 mutation located on exon 16 with the substitution of methionine with threonine, which alters the substrate-recognition pocket in the tyrosine kinase protein (67). All the mutations both in the MEN-2a and MEN-2b lead to activation of the protein. This is different from almost all other inherited predispositions to neoplasia, which are due to mutations that inactivate tumor suppressor protein (Table 1).

Clinical Features of Multiple Endocrine Neoplasias-2

Medullary Thyroid Carcinoma

MTC is usually preceded by multicentric hyperplasias of the parafollicular or C cells of the thyroid gland (49,68). The clinical manifestation of this tumor is in MEN-2a is no different from that in the sporadic case. The earliest abnormality in the thyroid gland in MEN-2 is hyperplasia of C-cells (68). The time for progression to multicentric neoplasia is not known but such changes have been noted as early as three years of age in MEN-2a and during the first month of life in MEN-2b (69–71). Metastasis can unfortunately occur at the earliest stage of disease being common when the tumor becomes larger than 1 cm (72,73), when it presents as a thyroid nodule and/or cervical lymphadenopathy. Sometimes MTC can cause Cushing's syndrome because of ectopic production of ACTH. Serum levels of calcitonin, the primary product of MTC, are nearly always high, especially if the tumor is palpable (74). In patients with C-cell hyperplasia or small C-cell tumors, the resting serum levels of calcitonin can be normal but will rise after pentagastrin and/or calcium infusions (75,76). The calcitonin level after surgery is a good marker to detect persistent or recurrent disease. The mandatory treatment of MTC in any MEN-2 syndrome is total thyroidectomy because of the high risk of malignancy. This should be performed as early as possible, especially in MEN-2b, in which the MTC tends to be more aggressive and have undergone malignant transformation by the time of diagnosis. Radiation therapy can be useful to reduce the tumor dimension and prevent local recurrence, however, the tumors are not very sensitive to x-ray or thermal radiation (77).

Pheochromocytoma

Some 40% of patients with MEN-2a and MEN-2b develop pheochromocytomas, usually presenting 10 years or so later than MTC (78). Most of pheochromocytomas in MEN-2 are intra-adrenal, and are generally nonmalignant. The clinical presentations of pheochromocytoma have changed over the past decades. Severe tension type headaches, and cardiac arrythmias associated with large pheochromocytomas are rarely seen today. Early symptoms include anxiety, intermittent headaches, variable hypertension, diaphoresis, and palpitations. Death from pheochromocytoma is uncommon. Routine surveillance and detection of pheochromocytomas coupled with α-adrenergic and β-adrenergic antagonist usage and improved surgical management have resulted in improved outcome. Laboratory evaluations reveal raised urinary excretions of epinephrine and norepinephrine or metanephrines; elevations of serum metanehrines (40). The 24 hour urine excretion of epinephrine, norepinephrine, and their metabolites (metanephrine and normetanephrine) is increased in large pheochromocytomas or in the late stage of the disease. Imaging techniques such as computed tomography (CT) or MRI and scanning with ^{131}I-MIBG confirm the diagnosis and localize biochemically proven tumors. Whereas in MEN-2a there is an increased risk of bilateral pheochromocytomas, MRI studies should be performed to detect possible bilateral disease, before surgery is undertaken. Laparascopic adrenalectomy is the treatment of choice in cases of unilateral tumor (79). Bilateral adrenalectomy should be limited to patients with bilateral tumors, or more specifically, in those with a family history of aggressive bilateral adrenal medullary disease. Adrenal insufficiency remains a significant problem in patients who underwent bilateral adrenalectomies. Adrenal cortical-sparing adrenalectomy is a promising technique for preventing such adrenal insufficiency (80), but there is a limited experience for long-term surveillance.

Sporadic pheochromocytoma can be associated with MEN-2, von Hippel–Lindau (VHL) disease and neurofibromatosis (NF) type 1. Germline mutations of six genes in sporadic pheochromocytoma have been found; *RET*, VHL, NF-1, and the genes for mitochondrial succinate dehydrogenase subunits SDHB, SDHC, and SDHD for familial pheochromocytoma-paraganglioma syndrome (81–83). VHL disease is an autosomal dominant neoplastic disorder characterized by renal cell carcinoma, retinal and cerebellar hemangioblastomas, pheochromocytomas, and islet cell tumors. VHL type 1 is characterized by the absence and VHL type 2 by the presence of pheochromocytoma. Germline VHL mutations in sporadic pheochromocytoma is infrequent (84–86). The VHL gene is a TSG mapped to chromosome 3p25 (87).

Primary Hyperparathyroidism

Involvement of the parathyroid glands in this syndrome is always benign. It had been reported in 10% to 25% of MEN-2a patients (41). Hyperparathyroidism is much less common in MEN-2b. Hyperplasias or benign adenomas can occur and are often clinically silent. Otherwise symptoms of hypercalcemia, renal stones, or osteitis fibrosa cystica have been described in earlier reports (41). The diagnosis can be achieved finding high or normal level of PTH in the presence of hypercalcemia, as discussed above under MEN-1. The indications for surgery are similar to those in patients with sporadic primary hyperparathyriodism.

Screening in Family Members for Multiple Endocrine Neoplasias-2

The early detection of affected family members is essential so that thyroidectomy can be performed as soon as possible. Before DNA testing was available, biochemical screening was performed using intravenous pentagastrin or calcium infusions to stimulate the secretions of calcitonin from malignant or hyperplastic C cells, however there were some false-positive responses (88). In family members, such tests, if found to be negative, should be performed yearly. However, *RET* mutation testing, rather than calcitonin testing is optimal for further management of the MEN-2 syndrome. In a MEN-2 family, the affected patient should be first analyzed to determine the specific mutation responsible for the disease. The number of possible mutations in the *RET* gene are modest and a single mutation can be detected in more than 90% of the index cases. There is a strong correlation between specifically mutated codon of *RET* and the MEN-2 phenotype as mentioned, including the aggressiveness of MTC. Once the mutation in the proband has been found, all other family members should be tested for the same mutation. If found, screening for familial MTCs before the age of four to five years in case of MEN-2a and in the neonatal period in the case of MEN-2b (Table 1) should follow in those found to be carriers. The MTC risk and thyroid management in children is based on *RET* mutations (89) as follows: (i). Children with MEN-2b and/or *RET* codon 883, 918, or 922 mutations are classified as level three or as having the highest risk from aggressive MTC. All of these infants should have thyroidectomy within the first six months and preferably within the first month of life; (*ii*). Children with any *RET* codon 611, 618, 620, or 634 mutations are classified as level two or as having a high risk for MTC. All of these children should have thyroidectomy performed before age of five years; (*iii*). Children with *RET* codon 609, 768, 790, 791, 804, and 891 mutations are classified as level 1 or as having the least risk of thyroid medullary cancer among the three *RET* codon mutation stratification categories. The latter should also have a total thyroidectomy although MTCs in patients with these mutations grow more slowly and develop at an older age than with the high risk mutations.

ADRENAL ADENOMAS

Benign adenomas are the most common cause of an adrenal mass affecting 3% to 7% of the population (90). The majority of cases are clinically silent, being usually found incidentally during a CT scan of the abdomen performed for an unrelated reason. The prevalence of so-called incidentalomas gradually increases from three years of age, to be as high as 7% in adults over the age of 50 years (91). Primary adrenal carcinomas are much rarer.

Genetics

The molecular mechanisms of the adrenal tumorigenesis have been studied extensively to understand the underlying biology. Evidence has accumulated to show that tumorigenesis in the adrenal gland is different from that in other endocrine tissues.

Cyclic AMP is a second messenger involved in the proliferation and hypersecretion of many endocrine tissues. The activation of G-protein-coupled receptors and guanosine triphosphate (GTP) -binding proteins, and the regulatory proteins of cAMP are involved in the pathogenesis of acromegaly and toxic thyroid adenomas. However, the activation of cAMP/protein kinase A pathway seems not to be important in the development of neoplasias of the adrenal cortex (92). Cytogenetic studies have shown that adrenal tumorogenesis is characterized by the amplification of chromosomes not commonly affected in other tumors, suggesting the possible presence of adrenal-specific oncogenes.

Adrenal adenoma or carcinoma can occur in several hereditary tumor syndromes such as Li-Fraumeni syndrome, Beckwith–Wiedemann syndrome (BWS), MEN-1, Carney complex, and familial adenomatosis polyposis. The discovery of the genetic defects underlying these disorders increases the potential understanding of adrenal tumorigenesis.

Li–Fraumeni syndrome is a rare familial tumor syndrome that is characterized by breast cancer, leukemias, soft tissue sarcomas, gliomas, and adrenocortical carcinomas. The underlying genetic defect has been identified as germ-line point mutations in the *p*53 TSG. The other nonaffected *p*53 allele is inactivated in tumor tissue by deletion of the short arm of chromosome 17, thus eliminating all wild-type *p*53 activity (93).

BWS is a rare disease characterized by macroglossia, gigantism, earlobe pits, increased risk of Wilm's tumor of the kidney, hepatoblastoma, rhabdomyosarcoma, and adrenal carcinoma, resulting from the allelic loss of a TSG mapped to chromosome 11p15 (94).

The Carney complex is a rare autosomal dominant disorder characterized by myxomas of the heart, tumors involving the peripheral nervous system,

spotty pigmentation of the skin (lentiginosis), and endocrine neoplasias (e.g., adrenocortical tumors). The molecular defect has been identified in mutations of the protein kinase A type 1-α regulatory subunit on chromosome 2p16 (95).

Familial adenomatous polyposis is a dominant autosomal disorder in which patients develop multiple (often >100) adenomatous polyps in the large intestine with a strong tendency to undergo malignant degeneration. Adrenocortical neoplasms also occur with an abnormally high prevalence (96). The disease is caused by a mutation in the adenomatous polyposis coli gene located at 5q21.

The discovery of the genetic defects underlying the familial syndrome and the fact that most of the benign and malignant neoplasias of adrenal are monoclonal, suggest that genetic changes at a specific locus are necessary for the development of such tumors.

Progression from adrenal adenoma to carcinoma involves a monoclonal proliferation of cells and involves several candidate genes. Several factors have been associated with malignant transformation including genes encoding *p*53, *p*57 cyclin-dependent kinase, menin, insulin-like growth factor (IGF) -II, MC2R, and inhibin-α (97). For aldosterone-producing tumors, the aldosterone synthase receptor (the CYP11B2 gene), the corticotropin-regulated promoter (the CYP11B1), and the angiotensin II type-1 receptor have been studied but they seem not to be associated with sporadic adrenal adenoma (98). In cortisol producing tumors, the loss of heterozygosity of the corticotropin receptor (MC2R) has been discovered to occur frequently in adrenocortical carcinomas but not in adenomas (99). Because there is the loss of heterozygosity on chromosome 11q13 in 20% of sporadic adenomas, some authors studied the expression of the MEN-1 gene, located on 11q13, in sporadic adenomas but found mutations in less than 10% of the tumors. This suggests that another TSG is important for the predisposition to adrenal tumors encoded by the 11q13 region (100).

Clinical Features

Adenomas that can develop in the adrenal gland can be subclassified as glucocorticoid secreting, aldosterone secreting, and nonfunctioning adenomas.

Aldosterone-Producing Adenomas

One of the most common cause of primary aldosteronism is aldosterone-producing adenoma (APA) accounting for approximately 30% of cases (101). These lesions are commonly small, usually being 1–3 cm in diameter and therefore difficult to image. They are benign solitary tumors composed of lipid-rich adrenocortical cells surrounded by a well-defined capsule (101). Patients can present with hypertension, urinary frequency, muscular weakness, paraesthesias, cramps, and tetany. Determination of plasma aldosterone (high), plasma renin activity (low), and 24 hour urinary aldosterone excretion (high) and sodium (low) should be performed in newly diagnosed hypertensive patients with hypokalemia and/or patients with a young age of onset of hypertension. A ratio of plasma aldosterone to plasma renin activity of 20 ng/l or more indicates the presence of a primary hyperaldosteronism. The relationship is best demonstrated by obtaining a renin level with the patient standing up which greatly stimulates the release of renin.

Once primary hyperaldosteronism is diagnosed, the differential diagnosis lies between an aldosterone-producing adrenal tumor, bilateral idiopathic hyperaldosteronism, and glucocorticoid remediable aldosteronism (GRA) in which aldosterone biosynthesis is regulated by ACTH rather than by renin. A postural test can differentiate between adenoma and idiopathic hyperaldosteronism (100). GRA is autosomal dominant disorder and is also known as familial hyperaldosteronism type 1. If plasma aldosterone and renin activity are measured in the morning after overnight recumbency and two hours of ambulation, aldosterone levels decrease in patients with adenoma, because of the diurnal fall of the ACTH, which is a minor stimulus to aldosterone production. In the case of idiopathic hyperaldosteronism, the level of plasma aldosterone will rise during the test under the stimulus of angiotensin II that increases in the upright position. Moreover, the level of 18-hydroxycorticosterone is elevated in patients with APA but is lower in patients with idiopathic hyperaldosteronism. The diagnosis of glucorticoid-suppressible hyperaldosteronism can be obtained by measuring the plasma level of 18-hydroxycortisol and 18-oxocortisol, which are products of the methyloxidation of cortisol by the aldosterone synthase that is typically elevated in this condition. The enzyme is the product of a chimeric gene that arose from an unequal crossover event. The hybrid gene can catalyze the reaction but is regulated by ACTH instead of the usual renin-angiotensin system. The administration of dexamethasone for four days inhibits the secretion of ACTH and suppresses the production of aldosterone. Dexamethasone can also be given as long term treatment. DNA analyses for the identification of the hybrid gene mutation responsible for the glucocorticoid-suppressible hyperaldosteronism have recently become available (102).

CT and MRI are the best imaging techniques to identify adrenal mass (103). CT can identify adrenal masses of up to 10 mm, and gives information about size, homogeneity, presence of calcifications or areas of necrosis, and eventual local infiltration. MRIs are best for detecting adrenal medullary tumors.

Glucocorticoid-Producing Adenomas

Glucocorticiod-producing adenomas are responsible 10% to 15% of instances of Cushing's syndrome that is ACTH-independent. Hyperproduction of cortisol determines the severity of symptoms such as hypertension, obesity, and hirsutism in affected females. Symptoms gradually develop in patients

with adenomas compared to those with carcinomas. The tumor may secrete other steroids, such as androgens, estrogens, or mineralocorticoids. Malignant tumors in young children tend to secrete multiple hormones. To detect hormonal hypersecretion by the adrenal gland, the best test is the 24 hours urinary free cortisol level, which, in a patient with adrenal adenoma and subclinical Cushing's disease, will be more than 550 mmol/day. A dexamethasone suppression test can also be performed.

Treatment

If the tumor is hormone-secreting, or is clinically silent but larger than 5 cm, the patient should undergo surgery. The treatment of choice for benign adrenal adenoma is a laparoscopic adrenalectomy from the preferred retroperitoneal approach. If the tumor is suspected to be malignant, then classical transperitoneal surgery should be performed (104). Patients with APA must have their blood pressure carefully controlled and their potassium level monitored before surgery. Hypertension can still be present after the intervention because of the long-term vascular damage resulting from prolonged hyperaldosteronism (105). In the case of aldosteronomas moreover, the patient can have a period of hyponatremia and hyperkalemia after the surgery due to continued suppression of the renin-angiotensin-aldosterone system, when a high-salt diet and mineralocorticoid therapy may have to be given temporally. Patients with corticoid-secreting adenomas may likewise need hormonal replacement therapy because of atrophy of the hypothalamic corticotropin-releasing hormone-secreting cells.

THYROID ADENOMAS

Thyroid adenomas represent 90% of the nodular lesions of the thyroid. Single nodules are more common in women than in men, but the incidence of thyroid nodules increases throughout life in patients of both genders. The typical thyroid adenoma is a solitary encapsulated lesion demarcated from the surrounding parenchyma. Adenomas derived from follicular epithelia form uniform follicles that contain colloid. On histological examination the adenomas can be classified into subtypes based on the degree of follicle formation and their colloid content as: macro-follicular, micro-follicular, Hurthle cell adenoma, atypical adenoma, and adenoma with papillary changes.

Genetics

Most thyroid tumors are sporadic and non-familial, suggesting that the underlying genetic abnormality is likely to be a somatic, acquired mutation. Several studies have documented that multiple factors are involved, including hormones, cellular proteins, and oncogenes. At the cellular level, these factors promote the progression from normal to benign, to well-differentiated tumor and to thyroid carcinoma (106–108). Segev et al.(109), divided the factors involved into three categories; (i) factors that promote tumor proliferations, such as hormones [thyroid-stimulating hormone (TSH)], oncogenes, (RAS, MYC, RET/PTC, etc.), and corresponding signal transduction pathways; alterations of those believed to initiate the progression towards tumorigenesis, (ii) factors that delay tumor proliferation, such as regulators of differentiation and cell-cycle regulation (cyclins, tumor growth factor-β, etc.), and (iii) factors controlling cellular immortalization and death, such as telomerase and regulators of apoptosis (Fas/Fas-L, Bcl, PTEN, MDM2). These factors are summarized in Table 4.

Gene transfer studies suggest that the early stages of thyroid tumor development may be the consequence of the activation or 'de novo' expression of protooncogenes or growth factor receptors, such as RAS, *RET*, NTRK, MET, Gs protein (Gsp), and the thyrotropin receptor (109,110). Alterations in the expression of these genes are associated with the development of neoplasms, ranging from benign toxic adenomas (Gs-α and TSH receptor), to follicular (RAS) and papillary (RET/PTC, NTRK, MET) carcinomas. In contrast, alterations in TSG (*p*53, Rb) are observed in aggressive and poorly differentiated forms of thyroid cancer, suggesting that they represent relatively late genetic events (109). Pituitary TSH is the main stimulator of growth and function of normal follicular thyroid cells. When TSH binds to its membrane receptor, it activates guanine-nucleotide-binding protein Gs and stimulates the adenylate cyclase cyclic adenosine monophosphate (cAMP) pathway (106). Increases in the intracellular levels of cAMP activate protein kinase, which in turn stimulates thyroid hormone production and induces the proliferation of thyroid epithelial cells. Adenylate cyclase activity decreases when the GTP is hydrolyzed by the intrinsic GTPase activity of Gs-α. Many thyroid adenomas are caused by activating somatic mutations in genes that encode for the TSH receptor, or the α subunit of the guanyl-nucleotide stimulatory protein (Gs) that results in constitutive activation of thyroid epithelial cells in the absence of TSH (111,112). Gs-α mutations are found mostly in hyperfunctioning adenomas (112).

Most of the mutations causing adenomas have been found in the third cytoplasmic loop on the sixth transmembrane segment of the TSH receptor, and in the C-terminus of Gs-α that is critical to the receptor interaction (so-called hot spot sequence) (113,114). These mutations do not explain all the tumors, or toxic adenomas that are not secondary to TSH-R mutations. Other genes encoding for proteins involved in the TSH-R pathway could be activated and responsible for the tumorgenesis. Mutations of the RAS oncogenes have been found in both benign and malignant thyroid tumors and represent the early mutational events able to enhance the cell proliferation. There are three RAS proteins: H-RAS, K-RAS,

Table 4 Summary of Molecular Factors Involved in Thyroid Tumorigenesis

Factor that promote tumor proliferation	Description
TSH	Stimulaetes thyrocyte growth, hormone production
Gsp	Facilitates binding of TSH receptor ligand binding and downstream increase of camp
EGF	Inhibits differentiation, upregulates proto-oncogene
VEGF	Promote tumor growth through angiogenesis
IGF-1	Promote thyroid cell proliferation
MET	Important thyroid tyrosine kinase binds HGF and stimulates thyrocyte proliferation
MYC	Nuclear transcription factor involved in control of cell growth and differentiation
RAS	Membrane linked, conveys signals from tyrosine kinase receptor to MAP kinase cascade
BRAF	Cytosolic serine/threonine kinase, binds to RAS and activates MAP kinase cascade
RET/PTC	Tyrosine kinase receptor, normally binds TGF-beta related neurotrophic factors
TRK-T	Peripheral nervous system tyrosine kinase which normally binds nerve growth factor
Cyclin D1	Cell cycle progression mediator, activates CDK, phosphorylates Rb
TPO	Catalyzes iodide oxidation, thyroglobulin iodination, and iodothyronine coupling
CP/LF	Bind copper and iron, leading to hydroxyl radical damage and malignant behavior
DAP4	Exopeptidase described in T-cell activation, increased activity in many malignancies
HMGI	Nuclear protein family of chromatin structure and formation regulators
Factors that hinder tumor proliferation	**Description**
TGF-beta	Blocks cAMP dependent thyrocyte proliferation through CDK and apoptosis
P21	CDK inhibitor, effector of p53-mediated G1 arrest of the cell cycle
P27	CDK inhibitor, controls G1 to S phase progression
Rb	Allows cell cycle progression in phosphorylated form, controls E2F transcription factors
P53	Transcription factor, essential mediator of cell cycle arrest in response to genetic damage
PAX-8/TTF-1	Paired domain transcription factor, controls thyroid-specific gene expression
E-Cadherin	Glycoprotein facilitates cell adhesion and maintains epithelial integrity, blocks invasion
Galectins	Carbohydrate binding protein which facilitate cell–cell and cell–matrix interactions
CD44	Membrane glycoprotein associated with cell–matrix adhesion, hyaluronic acid receptor
Factors affecting cellular immortalization and death	**Description**
Telomerase	Reverse transcriptase, extends cell life by maintaining length of chromosomal telomeres
Bcl	Family of homologous proteins which are pro- or anti-apoptotic
Fas/Fas-L	Ligand/receptor system, promotes apoptosis through activation of caspases
PTEN	Lipid phosphatase, through PI-3K or Akt/PKB, causes apoptosis or G1 arrest
MDM2	Oncogene, interacts with p53, E2F1, other gene products, ability to transform cells

Abbreviations: TSH, thyroid stimulating hormone; IGF, insulin-like growth factor; EGF, epidermal growth factor; VEGF, vascular endothelial growth factor; Gsp, Gs protein; MAP kinase, mitogen activated protein kinase; CDK, cyclin dependent kinase.
Source: From Ref. 109.

and N-RAS, encode 21-kDa monomeric proteins (*p*21) that possess GTPase activity and are structurally related to G proteins. All of them are membrane-anchored and they mediate signaling through many tyrosine kinases (mitogen activated protein kinases), including those of hormone receptors. The hydrolysis of the GTP to guanosine diphosphate that is bound to the RAS protein determines the inactivation of the signal. Mutations in the GTP-binding domain (codons 12,13) or the GTP-ase domain (codon 61) cause activation of the RAS oncogenes and are commonly identified in a variety of different human cancers and benign and malignant endocrine tumors (108,115). In thyroid tumors, point mutations or amplifications of all three RAS oncogenes can occur (116). There is a general agreement on the role of the RAS oncogenes in the early phases of the tumorigenesis, but the real prevalence of these mutations still needs to be

determined. The angiogenic and the soluble factors that stimulate them have been recently studied in their pathogenesis. Vascular endothelial growth factor (VEGF), the principal cytokine that mediates angiogenesis, has been associated with tumorigenic potential of thyroid cancer cell lines (117). Other authors found overexpression of VEGF in follicular adenomas, Hurthle cell neoplasia, and papillary carcinomas. In follicular carcinoma and anaplastic carcinomas, VEGF was suppressed, suggesting an important role for the cytokine in the early stages of the thyroid carcinogenesis (118). Epidermal growth factor aberrations have also been reported.

Clinical Features

Thyroid adenomas often present as a mass without any other clinical sign or symptoms, discovered during an examination or radiological procedure. In a minority of cases, adenomas can be hyperfunctional and cause clinical features of hyperthyroidism. Neoplasia must be strongly considered when nodules are "cold" and do not take up ^{131}I on radio isotope scanning.

PITUITARY ADENOMAS

Pituitary adenomas are common, mostly benign monoclonal neoplasms derived from adenohypophyseal cells. They usually produce and secrete pituitary hormones autonomously, that can cause clinical syndromes such as hyperprolactinemia, acromegaly or Cushing's disease. Alternatively, they can be small, nonfunctioning lesions that are discovered casually, similarly to radiological "incidentalomas" or during autopsy. They are typically benign lesions with a very small possibility (0.1%) of becoming malignant. However, they can present from the effects of aggressive local growth invading the brain parenchyma, the cavernous and paranasal sinuses, the bony clivius, and cause symptoms of intracranial mass, hypopituitarism, and/or peripheral visual field disturbances, and headaches from pressure effects on the optic chiasm (119). Based on X-chromosome inactivation analysis, functional and non-functional pituitary adenomas are of monoclonal origins (120). This pathogenesis should give rise to five well-defined tumor types, derived from each one of the normal AP cell types. Contrary to this expectation, pituitary adenomas are extremely heterogeneous, and have been difficult at times to subtype. They are classified according to morphometric and secretory characteristics (121) as prolactinomas [excess prolactin (PRL) secretion], somatotrophinomas (excess GH secretion leading to gigantism or acromegaly), ACTH-secreting adenomas (resulting in pituitary Cushing's disease), nonfunctioning adenomas, and the least frequent adenomas secreting either TSH or gonadotropins. Although less common, some pituitary tumors produce more than one pituitary hormone and also frequently express multiple hypothalamic-releasing hormone receptors (multiresponsive). Multifunctional cells seem to be involved in plasticity processes such as trans-differentiation or paradoxical secretion. In one study, more than 80% of the cells derived from various pituitary tumors showed a multifunctional phenotype (122). This may explain the occurrence of their paradoxical hormonal secretions.

Although the true incidence of pituitary adenomas is unknown, recent data suggest that pituitary adenomas occur in as many as 20% of the general population (123). At autopsy, careful histological assessment identifies pituitary adenoma in 22.5% to 27% of patients, with no gender-based differences in incidence (124). The incidence increases with aging with more than 30% of people 60 years of age having clinically silent tumors. Pituitary adenomas are, however, rare in children, with only 3.5% to 8.5% of these tumors being diagnosed during childhood. At such time they manifest clinically especially in girls, and it has been reported that they are less aggressive and invasive than tumors of adults (125). Pituitary adenomas represent approximately 15% of all intracranial tumors (126). Prolactinomas are the most common type of adenoma; GH- and ACTH-producing adenomas each represent 10% to 15% (often they are mixed), while TSH-secreting tumors are rare (127).

Genetics and Pathogenesis

For many years, there has been controversy regarding the molecular basis of pituitary tumorigeneisis. Several animal studies have suggested a fundamental role for hypothalamic hormones in the development of pituitary tumors. Recently the question was resolved when it became clear that all the pituitary tumors arise from a single cell. This monoclonality implies that intrinsic genetic alterations account for the initiating event. Furthermore, the tissue surrounding the pituitary adenoma is usually normal, suggesting that independent cellular events do not necessarily precede adenoma formation. It is likely that the majority of pituitary adenomas develop from transformed cells that are dependent on hormonal stimulation for tumor progression (128). The dysregulation of cell proliferation and differentiation may occur by the activation of oncogenes or inactivation of TSG. Oncogene activation may occur as a result of an activating single-point mutation. Because the gain of function is a dominant event, a single altered allele may be sufficient to produce the phenotypic change. In contrast, the TSG are recessive oncogenes that require the inactivation of both alleles (by deletion, rearrangement, or silencing through methylation) to cause neoplastic proliferation of the cell in a clonal fashion. Oncogenes involved in pituitary tumorigenesis are pituitary tumor-transforming gene (*PTTG*), Gs protein (gsp) and cyclin D1 gene (*CCND1*) (129). Heterozygous activating somatic point mutations in the α-subunit of the stimulatory Gs protein were the earliest dominant activating mutations described in endocrine tumors (130). Activation results when a missense mutation replaces residue 201 or 227. The resulting oncogene encodes for

mutated G protein called *gsps*, and the mutation results in a ligand-independent, constitutively elevated cAMP and hormone hypersecretion. The *gsps* mutations are described in up to 40% of somatotroph adenomas, but rarely in nonfunctioning adenomas (<10%), and absent in prolactinomas and TSH-secreting adenomas (131–133). Recently, *gsps* mutations have been identified in 6% of ACTH-secreting adenomas (134).

Pei and Melmed (135) showed increased levels of PTTG mRNA in GH-PRL-secreting, and nonfunctioning pituitary tumors. In vitro and in vivo experiments showed the strong transforming potential of PTTG. Because of its widespread and abundant expression in pituitary tumors, PTTG most likely has a key role in the early induction of pituitary cell transformation. It potentially induces the expression of fibroblast growth factor, a known mediator of cell growth and angiogenesis. Another oncogene involved in pituitary tumorigenesis seems to be the cyclin D1 gene (*CCND1*) located on 11q13. Gene amplification leads to overexpression of CCN. Habbers et al., found an allelic imbalance of the *CCND1* gene in the pituitary tumors. They studied the expression of *CCND1* by an immunohistochemical assay and found that 25% of the tumor cells showed expression of *CCND1*. Both nuclear and cytoplasmic staining was found more frequently in nonfunctional tumors than in somatotropinomas (136). The loss of TSG function has also been implicated. MEN-1 gene, the TSG located on the long arm of the chromosome 11 (11q13) responsible for the MEN-1 syndrome, has been studied, but menin expression is not downregulated in the majority of the sporadic pituitary tumors (137). However, these tumors present loss of heterozygosity at the same locus 11q13 in 20% of cases, suggesting that an additional TSG at this locus is involved in the pathogenesis of pituitary adenomas.

The retinoblastoma gene (*RB1*) is another TSG implicated in several neoplasms. Adenocaricinomas of intermediate lobe corticotroph differentiation have been found in RB1 transgenic knockout mice (138) induced in order to study this gene in the humans, but no mutations have been found in the pituitary adenoma (139). However, preliminary data show loss of heterozygosity at sites telomeric and centromeric to the RB locus in some aggressive pituitary adenomas. These data suggest that presence of another TSG located at 13q that is closely linked to RB1 (140). The frequent loss of heterozygosity of 9p21 also has been found in pituitary adenomas (141). The *CDKN2A* gene maps to this locus and its protein product *p*16 is a cell cycle regulator that is often disrupted in human neoplasias. It prevents the phosphorylation of RB and is responsible for inhibiting progress through the G1/S cell cycle checkpoint. Loss of *p*16 results in RB remaining in its hyperphosphorylated form, negating its ability to inhibit the progression of the cell cycle.

Several growth factors have been implicated in the pathway of the tumorigenesis. They are polypeptides that can regulate cell replication and differentiation, altering the expression of specific genes. The pituitary is a site of synthesis and action of growth factors including IGF-I and -II (IGF-I, IGF-II), nerve growth factor (142), transforming growth factor-α and -β, and basic fibroblast growth factor. Some studies suggest that peptides derived from human pituitary tumor cells can stimulate rat adenohypophysial cell replication in vitro (143). The role of these growth factors in pituitary tumorigenesis remains to be established. The understanding of the exact mechanism involved in pituitary adenoma will have relevance in clinical practice. The identification of specific molecular markers of tumor invasiveness and recurrence will permit the selection of appropriate follow-up protocols and earlier subcellular therapies.

Clinical Features

Prolactinoma

PRL-secreting adenoma (prolactinoma) is the most common hormonally active pituitary adenoma. When the tumor is less than 1 cm in diameter, it is defined as a micro-adenoma. If it is 1 cm or larger, then it is classified as a macro-adenoma.

In women the first clinical manifestations usually are galactorrhea, and ovulatory disorders due to inhibition of luteinizing hormone (LH) and perhaps follicle-stimulation hormone (FSH) secretion because of inhibition of gonadotropin-releasing hormone. In men, hyperprolactinemia results in decreased libido, infertility, impotence with occasional galatorrhea. Women usually present with symptoms at a younger age and tend to have micro-adenoma. Men tend to present at older ages with larger adenoma that can cause visual field abnormalities and hypopituitarism owing to pituitary tissue destruction (144). Some patients with a lactrotoph adenoma are noted to have subtle acromegalic features. Even if the hypersecretion of GH cannot be documented, the tumors contain both PRL and GH. This kind of tumor is known as acidophil stem cell adenoma, which can be differentiated from the usual lactotroph adenomas because of their aggressivity and tendency to recur (145).

In children, prolactinomas are rare, but their clinical presentations have been reported in small numbers of studies. However, the frequency of such tumors is likely underestimated because symptoms tend to occur later in life. As reported in adults, prolactinomas in children occur mostly in girls, causing menstrual disturbances (146). Delayed puberty can also be associated with prolactinomas because of the effect of hyperprolactinemia on the hypothalamic-gonadotropic activity. The symptoms of hyperprolactinemia correlate with its severity. Serum PRL concentrations greater than 20 ng/mL are abnormal. The measurement can be performed at any time, since usual daily activities have little effect on PRL secretion. However,

physical stress and high-protein meals can increase the PRL concentrations; therefore, a slightly high value should be confirmed. In case of persistent slightly elevated serum PRL values or when levels are clearly pathological, an MRI to search for a mass lesion in the hypothalamic-pituitary region is required. It is important to exclude pregnancy in older females when PRL levels are physiologically elevated.

Dopamine agonists are the first-line treatment for patients with micro- or macro-adenomas because they decrease the size and secretion of the tumors. Bromocriptine is a relatively toxic ergot derivate that has been used for decades for treatment of hyperprolactinemia. Preferred dopamine agonists are pergolide or carbegoline (Dostinex). Bromocriptine must be given twice a day and the principal side effects are nausea, postural hypotension, and mental dulling (147) while carbegoline is given orally twice weekly. If the patient cannot tolerate the dopamine agonists, or if the adenoma does not respond to agonist therapy, trans-sphenoidal surgery should be performed. If a significant amount of hormonally secreting tissue remains after surgery, radiation therapy should follow.

Growth Hormone-Secreting Adenoma

GH-secreting adenoma is the most common cause of acromegaly or gigantism. These adenomas account for about one-third of all hormone-secreting pituitary adenomas. The hyperproduction of GH by the tumor stimulates the hepatic secretion of IGF-I, which in turn causes most of the clinical features. In adults the onset of acromegaly is insidious and progresses slowly. The characteristic findings are enlarged jaw and enlarged swollen soft tissues of the hands and feet. The facial features become coarse, with enlargement of the nose and frontal bones (148). Bone density can be increased and cardiovascular disease including hypertension, left ventricular hypertrophy, and cardiomyopathy can occur (149). In children GH excess results in rapid and excessive growth and attainment of adult height beyond the genetic potential. Manifestations of acromegaly can also appear. The diagnosis of acromegaly can be confirmed by measurement of both serum GH concentrations that do not suppress after a glucose load and elevations in GH-dependent growth factors such as IGF-I and IGFBP-3. IGF-I levels are not influenced by food intake, exercise, or sleep. The result must be interpreted relative to age, gender, and developmental stage, because IGF-I concentrations are highest during puberty when they decline. In contrast, GH levels indicate pulsatile secretion and are stimulated by a variety of factors including short-term fasting, exercise, stress, and sleep. To obviate this problem, it is best not to obtain random measurements of serum GH.

The most specific dynamic test for establishing the diagnosis of acromegaly is an oral glucose tolerance test in which postglucose GH values remain greater than 2 ng/mL in 85% of patients with GH hypersecretion (150). Additional hyperprolactinemia occurs in 30% of the patients because the co-secretion of PRL and GH by a somammotroph adenoma. Once GH hypersecretion has been confirmed, the next step is an MRI study of the pituitary with contrast. Pituitary tumors as small as 2 mm in diameter can be detected by this technique and the dimension and the exact extension of the tumor can be identified.

Selective trans-sphenoidal surgical resection is the treatment of choice for patients with somatotroph adenoma that are small enough to be removed surgically (151). Medical therapy is indicated in patients with adenomas that are too large to be completely removed, or when resection has failed. Somatostatin analogs such like Octreotide inhibit GH secretion by binding to specific receptors for somatostatin (152). The long-acting form of Octreotide, which is administrated intramuscularly every two or four weeks, is now available in the United States.

Somatostatin analogs are usually well tolerated but about one-third of patients may experience nausea, fat malabsorption, and decreased gallbladder postprandial contractility (153). Dopamine agonists such as bromocryptine, pergolide, and cabergoline inhibit GH secretion in patients with sommamotroph adenoma. They have a limited efficacy but combined therapy with Octreotide and a dopamine antagonist may be successful when either alone is not. If GH hypersecretion cannot be controlled by surgery or medical therapy, external radiation can be used. Pegvisomant is a GH-receptor antagonist is a recently available adjunctive therapy which blocks GH stimulation of IGF-1 and control symptoms but does not induce tumor regression (154).

Thyroid-Stimulating Hormone-Secreting Adenoma

This kind of adenoma accounts for less than 1% of all hormone-secreting pituitary adenomas and less than 1% of all cases of hyperthyroidism. Adenomas secreting TSH are equally common in men and women, whereas tumors secreting both TSH and PRL are about five times more common in women. Most patients have the typical symptoms and signs of hyperthyroidism, but a few patients have mild or even no symptoms (155). Other clinical features in addition to hyperthyroidism are visual field defect, menstrual disturbances, and galactorrhea. In childhood, these tumors are very rare. Patients have an enlarged gland and clinical symptoms and signs of thyrotoxicosis but without exophthalmos.

The characteristic biochemical abnormalities in patients with TSH-secreting adenoma are normal or high serum TSH concentrations and high serum total and free thyroxine (T4) and triiodothyronine (T3) concentrations. The autonomous TSH secretion usually does not increase in response to thyrotropin-releasing hormone and does not decrease in response to exogenous thyroid hormone administration. Any patients with hyperthyroidism and elevated serum

TSH should undergo a CT or MRI study of the pituitary. MRI is the most sensitive test for the detection of pituitary tumors.

The treatment of choice is trans-sphenoidal resection of the tumor, even if the surgery will result in no change in one-third of patients. Therefore, many patients also require medical therapy. Dopamine agonists (bromocryptine, carbegoline) have proven to be effective but the somatostatin analog Octreotide is effective in almost all patients.

Gonadotropin-Secreting Adenoma

Gonadotroph adenomas are the most common clinically nonfunctioning pituitary macro-adenoma. They usually inefficiently secrete noneffective hormones. Thus they are often identified only when they are large enough to produce neurological symptoms. The most common symptom is impaired vision due to compression of the optic chiasm (156). The patient will complain of diminished vision in the temporal fields. Headache and diplopia due to the compression of the oculomotor nerves can occur. Sometimes gonadotroph adenomas became clinically manifest because of excess hormone secretion. The hypersecretion of FSH determines ovarian hyperstimulation in premenopausal women. In men the hypersecretion of LH can cause elevated testosterone concentrations, which in children will lead to premature puberty (157).

Adrenocorticotropic Hormone-Secreting Adenoma

Pituitary-dependent hypercortisolism is responsible for approximately two-thirds of cases of Cushing's syndrome. The majority of these cases are attributable to basophilic micro-adenomas. In children and adolescents the clinical manifestation are somewhat different from those seen in adults. Younger patients usually present with weight gains that tends to be generalized with skeletal growth failure as direct effect of hypercortisolemia. Red striae are common and blood pressure rise may be found. Compulsive behavior and overachievement in school in contrast with the typical adult emotional liability and depression may be seen (158). Menstrual irregularity or amenorrhea is a common symptom in adolescent girls.

Confirmation of Cushing's disease can be obtained from results of the 24 hours urine free cortisol measurement. In children the value should be corrected by reference to the body surface area. To establish that Cushing's syndrome is due to a pituitary adenoma, stimulation of ACTH, and cortisol following injection of CRH and suppression of cortisol by administration of dexamethasone should be shown. All patients should undergo MRI with the administration of gadolinium. If the MRI is negative, CRH-stimulated bilateral inferior petrosal sinus sampling can be used to confirm that the excessive ACTH is coming from the pituitary. Surgical excision by trans-sphenoidal adenomectomy is the treatment of choice. The remission rate after surgery is 85% to 95% in both children and adults (159).

PANCREATIC ADENOMAS

The pancreatic islet contains α cells (glucagon), β cells (insulin), and δ cells (somatostatin), as well as enterochromaffin cells (serotonin) and pancreatic polypeptide cells. These cells are part of the APUD system, and tumors so derived secrete a wide variety of polypeptides. Gastrinoma and insulinoma are the most common forms. According to a Mayo Clinic Study, the incidence of insulinoma is 4:1,000,000 person-years, and the average age of patients at presentation of insulinoma is in the mid-40s with very low incidence during childhood or adolescence (160). The adenomas are usually small and solitary; the lesion is generally well-encapsulated and highly vascular. Malignant insulinomas occur only in the 5% of cases of adenomas, tend to be larger, and metastasize to the liver and regional lymph nodes. Malignant transformation of glucagonomas is common.

Genetics and Pathogenesis

The molecular mechanism leading to pancreatic tumor is still unclear but several TSGs have been implicated. The TSG Smad/DPC4 has been found mutated in nonfunctional pancreatic tumors (161). Loss of heterozygosity in chromosomes 1, 3q, 3p, 11p, 16p, 17p, and 22q has been noted in several studies (162). Guo et al. (163) found overexpression of $p27^{Kip1}$ in sporadic pancreatic endocrine tumors without differences between benign and malignant tumors. $p27^{Kip1}$ is a universal cyclin-dependent kinase inhibitor, which acts as a tumor suppressor and a negative regulator of cell cycle. In various types of human cancers, the suppression of p^{27Kip1} expression is linked to aggressive behavior. However, the overexpression of p^{27Kip1} in the pancreatic endocrine tumor can be secondary to the other primary molecular disregulations or be a unique molecular pathway leading to endocrine tumorigenesis. Another molecule involved in pancreatic tumorigenesis is the paired-homeodomain transcription factor PAX4. The PAX gene family encodes highly conserved paired-box-containing transcription factors that control the tissue-specific expression of genes during embryogenesis. Because of this important role in the differentiation and development of pancreatic beta-cells, Miyamoto et al. studied PAX 4 expression of PAX 4 mRNA in the tumors, but little or none in the normal islets, suggesting a fundamental role in the development of insulinoma (164).

Clinical Features

A patient with insulinoma will characteristically present with fasting hypoglycemia with neurological symptoms such as confusion, personality change, or seizures (165). The release of catecholamine due to the

hypoglycemia will result in anxiety, palpitations, weakness, tremor, and sweating. Weight gains may occur.

Diagnosis is based on demonstrating Whipple's triad: hypoglycemic symptoms, blood glucose level less than 50 mg/dL, and relief of symptoms after glucose ingestion. Unfortunately, these symptoms are not specific for insulinoma. Because a single overnight fasting blood sugar level combined with a simultaneous plasma insulin level fails to establish the presence of fasting organic hypoglycemia in more than 35% of patients, a 72 hours fast is usually done with blood glucose and insulin levels determined at two to four hours intervals. Recently a study based on 127 patients with insulinoma demonstrated that a 48 hours test is as effective as the 72 hours one (166).

Measurement of *C*-peptide at the end of the fast can help to differentiate endogenous from exogenous (factitious) hyperinsulinemia. *C*-peptide will be proportionally elevated with insulin in patients with insulinoma, and low or normal in patients who abuse insulin or hypoglycemic agents. Nesidioblastosis (islet cell hypertrophy) and familial hyperinsulinemic hypoglycemia simulate the insulinoma's biochemical findings however these causes of hyperinsulinemia present in infancy.

The treatment of choice is surgery, but preoperative localization of the tumor is necessary because of the small tumor size. The imaging techniques available are spiral CT, arteriography, and trans-abdominal and endoscopic ultrasonography. Patients with metastatic insulinoma or those who are not candidates for or refuse surgery, require medical therapy. Diazoxide, verapamil, phenytoin, and Octreotide have all been used to prevent symptomatic hypoglycemia. Diazoxide, which diminishes insulin secretion, is the most effective and should be given in divided doses. However, Diazoxide causes unwanted hair growth, gum hyperplasia and edema.

SUMMARY

The MEN are most often the province of adult endocrinologists, however with increased awareness, many will be found to have their first manifestations during childhood. Whenever an endocrine tumor is discovered in a child or adolescent, an underlying MEN should be considered since early age at onset is a feature of MEN. When an MEN especially MEN-2 is discovered in a family member, genetic testing of the children with follow-up of endocrine evaluation in those positive for the mutation should follow, especially in the highly malignant C-cell tumors of the thyroid.

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Endocrine Tumors in Children

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INTRODUCTION

Endocrine tumors in children pose a number of fundamental questions: Is the tumor a nonfunctioning, functioning, or hyperfunctioning entity? Is it a malignant or a benign process or a hyperplasia responsive to physiological regulations? Apart from clinical presentation, the answers to these questions often depend on specific hormonal biochemical, radiological, and histopathologic findings. Consequently, the optimal assessment and management of these patients remains a challenging task and must include the efforts of endocrinologists, oncologists, surgeons, and other support personnel.

This chapter reviews the clinical approach to children with adrenal, gonadal, and pancreatic tumors.

ADRENAL TUMORS

The incidence of adrenal tumors in children is not known (Vol. 2; Chap. 8 for adrenal cortex and Vol. 2; Chap. 9 for adrenal medulla). Of 58 patients reported by Bertagna and Orth (1), 11 (19%) were between 0.8 and 15 years of age. Adrenal carcinoma represented about 10% of the carcinomas in childhood, according to a registry-based data from the United Kingdom (2). The age of appearance is usually during the first decade of life (3). Girls are more frequently affected than boys, with ratio of 2.5:1 (4). While familial cases are reported (5), occurrence of adrenal carcinoma in patients with Li–Fraumeni syndrome (6), Beckwith syndrome (7), hernihypertrophy (8), and congenital malformations of the genitourinary tract is well known.

Incidence of nonmalignant tumors increases with age. Malignant tumors show a bimodal distribution with the first peak below age five years and the second peak in the fourth to fifth decade (9). Industrial pollutants may play a causative role, as reported from Brazil; however, there is a genetic predisposition to adrenal tumors in this population (10,11).

On a pathological basis, corticotrophin-independent cortisol overproduction represents a spectrum ranging form benign nodular hyperplasia to malignant adrenal tumors. In the 1960s, Meador et al. (12) described primary adrenocortical nodular dysplasia, characterized by nonmalignant, autonomously secreting lesions. In another review (13), a similar condition predominantly affecting children and young adults has been described with bilateral nodular disease, internodular cortical atrophy, and varying degrees of pigmentation. This condition has also been described in association with the Carney complex, which includes myxomas, pigmented skin lesions, peripheral nerve tumors, and various endocrine tumors (14).

The cause of the nodule formation or dysplasia remains to be established. An adrenal-stimulating immunoglobulin has been implicated in the pathogenesis (15). In McCune–Albright syndrome, nodular hyperplasia of the adrenal glands has been reported as the cause of hypercortisolism (Vol. 2; Chap. 8) (16). In these patients, somatic mutation of the α subunit of the G-protein occurs during fetal development, creating a mosaicism of normal and mutant-bearing cells. In the latter, G-protein activation of adenylate cyclase increases cyclic adenosine monophosphate, and there is formation of multiple nodules and overproduction of cortisol. Inverted diurnal rhythm, subnormal morning cortisol concentration, and low corticotrophin in association with gastric inhibitory peptide (GIP) recently were described. Although the causative role of GIP in cortisol overproduction remains undetermined, data support the hypothesis of abnormal expression of receptors in the adrenal (17,18).

Clinical Features

Adrenal tumors in children are usually functional, giving rise to a constellation of symptoms or signs. Depending on the duration of the disease and the action of the metabolic, androgenic, and salt-retaining hormones, the clinical spectrum may include Cushing syndrome and virilization to hypertension. Patients in the younger age group are more likely to produce androgen than cortisol. Therefore, virilization is a more common clinical presentation than Cushing's

syndrome (19). However, in all age groups, a majority of the tumors secrete cortisol, while androgen and aldosterone secretions follow in decreasing frequency. Occasionally, there is alteration of the clinical course with evidence of initial glucocorticoid predominance being overlapped by the androgenic effects or vice versa, as the disease progresses. An incidental adrenal tumor without any clinical manifestation is a distinct entity in adults; this represents only about 5% of all adrenal tumors in children.

Weight gain, truncal obesity, moon face, and buffalo hump are observed in 40% to 60% of children with functional adrenal tumors (Table 1). Obesity and short stature are common presenting features (21,22). In pubertal-age children with androgen-producing tumor, acne, hirsutism, hypertrichosis of the face and trunk, deepening of the voice, and clitoromegaly in females are the distinguishable physical signs. In prepubertal children, excess androgen will lead to virilization with excess body hair, adrenarche, acne, clitoromegaly or abnormal phallic growth, and rapid skeletal growth, along with excess weight gain (23,24). The disorder tends to be more severe and the clinical findings more flagrant in infants than when the onset occurs in older children (25,26).

Premature adrenarche, a common problem in clinical practice, may be the initial presentation, although the severity of the signs and symptoms will help to differentiate the adrenal tumor from benign adrenarche. In post-menarcheal girls, rapid weight gain and menstrual irregularity often results from increased androgens (Vol. 2; Chap. 11). Estrogen-secreting tumors of the adrenals are rare in childhood. In prepubertal boys, these may lead to gynecomastia along with enhancement of growth and skeletal maturation; in girls, sexual precocity, characterized by premature thelarche and advanced growth, may occur (27). If there is evidence of virilization or elevation of blood pressure, the concomitant secretion of androgens or mineralocorticoids should be suspected.

Hypertension, plethora, and fluid retention are also common in children with adrenal tumor, being present in up to 70% (Vol. 2; Chap. 8). In aldosterone-producing tumor, elevated blood pressure is one of the most common manifestations. Both systolic and diastolic blood pressure is abnormally elevated. Muscle weakness, cramping, paresthesia, polydipsia, and polyuria may occur. Despite fluid retention and increase in the intravascular volume, there is no clinical evidence of edema in these patients.

Sodium retention is only mild to moderate. Hypokalemia is the most reliable laboratory abnormality, with electrocardiographic signs of prolonged ST segment and inverted T-wave. Alkalosis is also a frequent finding, causing tetany and Trousseau's sign in untreated patients.

A small percentage of patients are known to have psychiatric symptoms, ranging from acute psychosis and depression to manic-depressive behavior. Asymptomatic hypercortisolemia with normal blood pressure has been reported in children. Diffuse osteoporosis, more noticeable in the vertebral column, is also common in these patients. Impaired glucose tolerance is more frequent than overt diabetes, and the incidence of renal stones is higher than in the general population.

Preoperative differentiation of an adenoma from carcinoma is difficult. Although Cushing syndrome is frequently caused by adenoma, while virilization is associated with carcinoma, benign and malignant tumors may be functionally identical and, thus, clinically inseparable. A normal or exaggerated response to exogenous ACTH stimulation is more often encountered in adenoma than carcinoma but lack of dexamethasone suppression is observed in both (1). Size of the tumor has been noted to be a predictor, with tumor size greater than 75 g being more likely to be malignant (21). Histopathological criteria such as mitoses, necrosis, and capsular and vascular invasions are not reliable predictors of a malignant tumor, as demonstrated by the presence of these findings in patients with benign adenomas (28,29).

Table 1 Clinical Features of Hypercortisolemia in Children and Adults

Symptoms/signs	Children (CS)	Children (CS)	Adult (CD)
General			
Obesity (moon faces)	25	100	85
Growth failure	0	85	NR
Hypertension	3	77	75
Cutaneous			
Plethora	13	77	80
Striae	0	54	50
Acne	88	85	35
Hirsutism	75	85	75
Bruising	0	38	35
Hyperpigmentation	0	38	5
Musculoskeletal			
Osteoporosis	NR	54	80
Weakness	0	46	50
Metabolic			
Glucose intolerance	NR	38	75
Renal stones	NR	15	15
Neuropsychiatric symptoms			
Fatigue, weakness	50	46	85

Abbreviations: CS, Cushing syndrome; CD, Cushing's disease; NR, not reported.
Source: From Ref. 20.

Diagnosis

Because of the anatomical location deep inside the abdomen as well as nonfunctioning nature of the tumor, adrenal adenoma or carcinoma often remains undiagnosed for an average duration of five months. The size attained by these tumors is therefore enormous in many instances. During evaluation of nonspecific complaints or routine physical examination, abdominal mass may be detected in such patients.

Hormonal studies are of vital importance in the diagnosis of adrenal tumors. Levels of urinary

free cortisol, 17-KS, and 17-OHCS are significantly elevated in patients with a functioning adrenal tumor (30). Plasma cortisol is elevated, along with loss of diurnal rhythm. Complete androgen profile including DHEA, androstenedione, and testosterone levels should be studied in patients with clinical evidence of virilization (Vol. 2; Chaps. 11 and 12). Differentiation from congenital adrenal hyperplasia is an important but difficult task. In the hypercortisolemic state, lack of suppression of plasma cortisol following administration of dexamethasone is characteristic of adrenal tumors. Therefore, performance of a low- or high-dose dexamethasone test should be a priority in these patients (Vol. 2; Chap. 8).

Differentiation of pituitary disease from adrenal disease is challenging. Apart from providing further understanding about the functional relations between the pituitary and the adrenal, the metyrapone test may be useful in differentiating adrenal adenomas from carcinomas. In about 50% of cases, adrenal adenoma is responsive to metyrapone while carcinomas are usually nonresponsive. Because the tumors do not respond and the normal adrenal cortices are atrophic, ACTH stimulation test has very limited use in the diagnosis of adrenal tumors. Differentiation of central precocious puberty from estrogen-secreting adrenal tumors in girls may be necessary. Urinary estrogens and 17-KS and plasma DHEA, DHEAS, and estrogens are elevated along with absent gonadotropin response following GnRH stimulation in adrenal tumors. Measurement of serum and 24-hour urinary aldosterone levels as well as plasma renin activity is useful for initial evaluation of suspected aldosterone-producing tumors. To differentiate secondary hyperaldosteronism and avoid false-positive results, all medications, particularly diuretics, should be discontinued prior to laboratory studies. In patients with elevated serum aldosterone levels, complete suppression of aldosterone secretion by administration of dexamethasone suppressible hyperaldosteronism from primary hyperaldosteronism. Elevated aldosterone levels, low renin activity, and high urinary aldosterone with lack of dexamethasone suppression establish a diagnosis of hyperaldosteronism (Vol. 2; Chap. 8). Further diagnostic studies, including the imaging studies, should be performed in these patients.

Radiological studies are an important component in the diagnosis of adrenal tumors. Computed tomographic (CT) scan and magnetic resonance imaging aim to localize the tumor and define the extent of the disease. Intravenous pyelography is useful to delineate the relationship of the kidney to the tumor mass. Ultrasonography shows an adrenal mass in the majority of cases. CT scan is, however, the ideal method because it allows visualization of other abdominal organs. Angiography is often required to provide the surgeon with a map of the tumor's blood supply.

Treatment

Complete surgical excision with replacement steroid therapy provides the best choice for treatment of these patients (31). Preoperative, operative, and postoperative managements are of critical importance. For primary pigmented nodular adrenocortical disease, bilateral adrenalectomy with steroid replacement is preferred (28). For adrenal adenoma, unilateral adrenalectomy or resection of the tumor followed by replacement steroid therapy is the treatment of choice. Replacement therapy with glucocorticoid is necessary until normal function in the contralateral gland is restored. This is usually for six to 12 months, although suppression from the tumor can persist for up to two years.

For adrenal carcinoma, surgical therapy aims to excise the tumor and local metastasis completely to enhance the chance of cure. For inoperable or partially resectable carcinoma, combination chemotherapy may offer an alternative management approach (31–35). However, experience with pediatric patients is largely anecdotal. Mitotane therapy has been successful in patients with intrauterine adrenal carcinoma and metastasis (33). In addition, medical therapy with metyrapone and aminoglutethimide in combination is useful for control of symptoms. Ketoconazole, an inhibitor of steroid biosynthesis, is the preferred drug to decrease cortisol secretion in selected cases. RU 486, a glucocorticoid antagonist, has also been employed to control the symptoms secondary to hypercortisolism.

Prognosis and Follow-Up

Early diagnosis and surgery offer the best hope for long-term survival, with adenoma exhibiting an extremely good outcome. Adrenal carcinoma in children, on the other hand, is an extremely progressive disease. Final adult stature is stunted in most of these patients. Replacement therapy with glucocorticoid is required for an indefinite period of time. Careful follow-up at a three- to six-month interval is mandatory. Clinical assessment and serum levels of cortisol, DHEA, androstenedione, testosterone, and plasma renin activity are necessary to detect recurrent or metastatic disease. The 24-hour urinary 17-KS, 17-OHCS, and free cortisol, along with the other hormones that were initially abnormal, should be measured. A repeat dexamethasone stimulation test should be done to assess the suppressibility of cortisol production.

GONADAL TUMORS

Testicular Tumors

Causes and Presentation

Constituting about 1% of all cancers in men, testicular tumors occur most commonly during the third and fourth decades of life. Germ-cell tumors, accounting for about 90% of all testicular tumors in the pediatric

age group, include embryonal carcinoma, endodermal sinus tumor, and teratoma, representing a spectrum of progressive histological differentiation. In young adults, however, the spectrum includes seminoma, which, represent 30% to 50% of all germ-cell tumors (36,37).

The best-documented risk factor for the development of germ-cell tumors is cryptorchidism, which is associated with about 10% of germ-cell tumors (Vol. 2; Chap. 15) (36–38). The degree of lack of descent of the testes correlates with the likelihood of tumor formation, with abdominal testes more at risk than those in the inguinal area. Orchidopexy performed before the age of six, however, reduces this risk significantly. The pathogenesis of these tumors is not clear. Incidence of tumor in the contralateral normally descended testis is higher than that of controls (38–40). Although several factors including higher temperature, higher gonadotropin levels, and congenitally abnormal germ cells have been proposed, none has provided compelling evidence to attain wide acceptance.

Dysgenetic gonads associated with androgen insensitivity, persistent mullerian syndrome, and true hermaphroditism and Klinefelter syndrome have a higher incidence of germ-cell tumors (41,42). Down syndrome and cutaneous icthyosis (43) with steroid sulfatase deficiency also have been reported to be associated with occurrence of testicular tumors. One of the strongest risk factors for the development of a germ-cell tumor is a history of prior contralateral tumor (44), or other alterations (45,46). Familial occurrence of tumor has been reported, with six-fold increase for a son whose father had a germ-cell tumor (47).

Inadequately treated congenital adrenal hyperplasia has been observed to be associated with testicular tumors (48,49). It is presumed that the development and progression of these tumors is enhanced by the chronic stimulatory effect of elevated corticotropin.

Painless mass is the common mode of presentation; however, pain and tenderness are found in half the cases. Symptoms or signs due to metastasis to the retroperitoneal lymph nodes or lungs are the initial findings in a small proportion of patients. Tumors of Leydig-cell origin may secrete testosterone, producing signs of sexual development. Unilateral or bilateral gynecomastia may occur as a result of secretion of estrogen or of chorionic gonadotropin by the stromal or germ-cell tumors (50,51).

Diagnosis

Careful examination is crucial to the diagnosis testicular tumors. An area of hardness, nodularity, or altered consistency should be determined. Localization of the tumor and differentiation of simple hydrocele from reactive hydrocele with testicular tumor can be reliably performed by sonographic study. Patients suspected of having germ-cell tumor should undergo radiographic or imaging studies of the chest, abdomen, and skeletal system to detect metastastic disease. The functional and histological behavior of various testicular tumors is detailed in Table 2.

Malignant behavior of Sertoli-cell tumors correlates directly with large size (53). Preoperatively, as well as during the follow-up, measurement of biomarkers such as serum human chorionic gonadotropin (hCG) and α-fetoprotein (AFP) (52) is useful, particularly for monitoring these patients.

Treatment

Following diagnosis of testicular tumor, immediate surgery is indicated. For teratoma, surgery alone is usually sufficient, while localized germ-cell tumor requires radical surgery (involving excision of the spermatic cord structures and the testicle). Periodic evaluation of the chest and abdomen and measurement of serum AFP level allow identification of tumor recurrence. For malignant tumors with metastatic or recurrent disease, radical surgery and chemotherapy (cisplatin, vinblastine, and bleomycin) offer the best outcome. However, controversy remains about the need for retroperitoneal lymph node resection in pediatric patients (54).

Ovarian Tumors

Ovarian tumors, which represent approximately 1% of childhood malignancies (55), are classified into two categories on the basis of their cells of origin: germ-cell tumor, originating from primordial germ cells, are more common than non–germ-cell tumors

Table 2 Classification, Median Age, Frequency, Secretory, and Histological Characteristics of Childhood Testicular Tumors

Classification	Median age (yr)	Frequency (%)	Secretory activity	Tumor characteristics
Germ-cell tumors				
Endodermal sinus tumor	2	26	AFP	Malignant
Teratoma	3	24	None	Usually benign
Embryonal carcinoma	Late teens	20	AFP, hCG	Both
Teratocarcinoma	Late teens	13	None	Both
Gonadoblastoma	5–10	<1	None	Both
Non–germ-cell tumors				
Leydig-cell tumors	5	6	Androgen	Benign
Sertoli-cell tumor	1	4	Androgen, estrogen	Benign

Abbreviations: AFP, α-fetoprotein; hCG, human chorionic gonadotropin.
Source: From Ref. 52.

in all age groups and account for 90% of ovarian tumors in pre-menarcheal girls. The majority of these germ-cell tumors are teratoma, having a histologically benign and functionally inactive nature. Non–germ-cell tumors, originating from stromal cells, such as granulosa-, theca-, Sertoli-, and Leydig-cell tumors secrete androgens (56–58).

Classification, median age, distribution, secretory and histological characteristics, and the common presentation of ovarian tumors are provided in Table 3. Dysgerminoma, the most common germ-cell tumor of the ovary, presents with painless abdominal mass (57). Endodermal sinus tumor, the most aggressive type of the germ-cell tumors, presents as a painful mass with rapid metastasis to distant sites. Teratomas, the most common and benign tumor of germ-cell origin, usually remain hormonally inactive. In contrast, embryonal carcinomas, which are typically found as an admixture of dysgerminoma, endodermal sinus tumor, or teratoma, may undergo differentiation, become hormonally active, and manifest with effect of hormone production. Gonadoblastomas are uncommon tumors, yet an important ovarian tumor in girls for two reasons. First, these tumors occur more in phenotypic females with abnormal karyotype containing components of Y chromosomes (46, XY; 45, X/46, XY; 45, X/46, X fra). A recent report has shown the occurrence of gonadoblastoma in 7% to 10% of Y chromosome–positive Turner's syndrome patients (56). Second, these tumors, which occur in dysgenetic gonads where differentiation into testis or ovary has been absent or incomplete, usually contain both germ- and stromal-cell components and frequently exhibit a tendency to recur. Because all the gonadal tissue is potentially involved and carcinoma in situ is always a possibility, removal of the gonads is recommended at early stage.

About 5% of granulosa-cell tumors develop in prepubertal girls. In these girls, with a median age of eight years, presenting symptoms are attributed to the hormones produced by the tumors (57). Precocious sexual development characterized by premature breast development, pubic and axillary hair, white vaginal discharge, or irregular uterine bleeding may be the mode of presentation. Excessive weight gain with or without acceleration of linear growth may also occur with advancement in the skeletal age (Vol. 2; Chap. 11). On rare occasions, androgen production may lead to irregular bleeding or amenorrhea. Although hormonally inactive tumors may remain asymptomatic, acute abdominal symptoms may be the presentation in a small proportion of these patients. This is largely the result of torsion or rupture of the tumor. Adequate clinical evaluation of these girls should include pelvic examination, to be performed under sedation or anesthesia, because palpable mass is almost always a diagnostic of tumor.

To complete the endocrine evaluation, the pituitary–ovarian axis and estrogen profile should be studied. Estradiol level is usually elevated while luteinizing hormone and follicle-stimulating hormone levels are suppressed, thereby excluding the differential diagnosis of central precocious puberty. Vaginal cytology reveals maturation of squamous cells, reflecting the effect of estrogen. Sonographic study helps in localizing the mass in the ovary, although it is not a useful method to exclude adrenal disease. Laparoscopy and biopsy are often necessary. Surgical resection of the lesion (i.e., unilateral salpingoophorectomy) usually yields good outcome. Recurrence of the disease is unusual. Compared to adult granulosa-cell tumors, juvenile granulosa-cell tumors have distinct histological features characterized by luteinized cells, irregular follicles, and fibroblast-like cells, and the absence of Call–Exner bodies. The tumors may be cystic, solid, or both. Theca-cell tumors are often hormonally active and manifest by premature breast development, which is similar to that of granulosa-cell tumors. They are usually slow growing and lack the acuteness often encountered in patients with granulosa-cell tumor. Diagnostic studies and management approach are, however, identical in both of these conditions.

Classic virilizing ovarian neoplasms often called arrhenoblastoma, Sertoli-Leydig-cell tumors occur

Table 3 Classification, Median Age, Frequency, Secretory, and Histological Characteristics and Presentation of Ovarian Tumors in Childhood

Classification	Median age (yr)	Frequency (%)	Secretory activity	Tumor and presentation
Germ-cell tumors				
Dysgerminoma	16	17	hCG	M[a]/mass
EST	18	11	AFP	H/mass, pain
Embryonal carcinoma	14	4	AFP, hCG	M/sexual precocity
Choriocarcinoma	–	Rare	hCG	M/sexual precocity
Teratoma	10–15	29	None	B/mass
Gonadoblastoma	8–10	Rare	A, hCG	M/virilization
Carcinoid	–	Rare	Serotonin	Nonspecific
Struma ovarii	–	Rare	Thyroxine	Hyperthyroidism
Non–germ-cell tumors				
Granulosa–theca cell	8	13	E, P	L/sexual precocity
Sertoli–Leydig cell	8	17	A, P	L/virilization

[a]Indicates degree of malignant behavior: L, low-grade malignancy; M, moderately malignant; H, highly malignant.
Abbreviations: EST, endodermal sinus tumor; A, androgens; E, estrogens; P, progesterone; AFP, α-fetoprotein; hCG, human chorionic gonadotroin.
Source: From Refs. 52, 59.

most commonly during the teenage or early adult years (58,60). Due to the effect of androgens, the early symptoms and signs consist of weight gain, amenorrhea, hirsutism, acne, deepening of the voice, and clitoromegaly. Abdominal mass and nonspecific gastrointestinal and urinary symptoms may be present concurrently. Although symptoms and signs are more intense and the progression of the course is more rapid compared to congenital adrenal hyperplasia, androgen-producing adrenal tumor, and polycystic ovarian disease, clinical differentiation may be difficult (61). ACTH-stimulated adrenal study, GnRH-stimulated gonadotropin profile, and estrogen levels are essential to confirm or exclude these differential diagnoses. Sonographic study of the ovary often provides adequate information to detect cystic lesions. However, CT scan is often required to assess the adrenal disease in noncystic ovarian lesions. Histologically, these tumors show an intermediate-to-poor degree of differentiation (62).

Identification of the cellular origin of such tumors is possible; the presence of mRNA for P450c11 and P450c21 and ACTH receptor will indicate adrenal tissue (60). Prediction of prognosis based on the cells of origin is a possibility. Because of patients' relatively young age, conservative surgical management with preservation of the uterus and contralateral ovary is the goal of therapy (63). In advanced or recurrent disease, chemotherapy and irradiation remain the alternative (59,60); the benefits of such therapy are not proven.

TUMORS OF ENDOCRINE PANCREAS

Hyperinsulinemias of Infancy and Nesidioblastosis

Neonatal hypoglycemia with concurrent hyperinsulinemia is a common diagnostic entity (Vol. 1; Chaps. 16 and 17). Hyperinsulinemia due to abnormal KATP channel, mutations of the glutamate dehydrogenase and glucokinase genes have emerged as the more specific entities in the recent years. Diagnosis is based on hypoglycemia, hyperinsulinemia, increased C-peptide, and response to glucagons; histopathologic studies often reveal focal or diffuse disease. Medical treatment with diazoxide and octreotode and diets are effective. Surgery is indicated in selected patients (64,65).

Nesidioblastosis, a term used in the past, has been defined as the diffuse proliferation of islet cells budding off from the pancreatic duct, leading to the formation of numerous small clusters of the B-cells (66,67). Male and female are equally affected and familial occurrence has been reported. Clinically, the condition is encountered in neonates and infants with persistent symptomatic hypoglycemia (Vol. 1; Chap. 16). Almost exclusively, neonatal hypoglycemia is attributed to impaired hepatic glucose output due to hyperinsulinemia. Severe persistent hypoglycemia determines the clinical picture (68–71). Seizures, apnea, respiratory distress, listlessness, and cyanosis are the common manifestations. Neonates are usually macrosomic, and infants frequently weigh above the 97th percentile. Physical examinations are otherwise unremarkable in these patients. Demonstration of hyperinsulinemila in the face of hypoglycemia, normal liver function, as well as other glucoregulatory hormones confirms diagnosis.

While diagnostic imaging studies are performed and the patient awaits surgical treatment, medical therapy should be instituted. This includes diazoxide, corticosteroids, and epinephrine. However, partial pancreatectomy is recommended in these patients. Surgery should be performed at the earliest opportunity to minimize episodes of hypoglycemia and the risk of consequent neurologic impairment (72–74).

Terms such as nesidioblastoma, multifocal ductuloinsular proliferation, microadenomatosis, nesidiodysplasia, and islet-cell dysmaturation syndrome have been used to describe the morphological variants of nesidioblastosis (66,75). Clinical and biochemical means are not helpful to characterize the morphological patterns in these patients (Table 4). At the functional level, further controversy exists about the relationship between hypoglycemia and nesidioblastosis: pathological characteristics similar to nesidioblastosis are known to exist in patients with normoglycemia, while normal pancreatic morphology has been described in patients with hypoglycemia (76–78).

Insulinoma

Functioning β-cell tumors have been found in patients from birth to old age, with approximately 10% of all cases occurring in individuals below 20 years of age (79–81). There is slight preponderance to females, with adequate reasons for this observation being unclear (82).

Although insulinomas may belong to a spectrum that includes islet-cell tumors, nesidioblastosis, and multiple endocrine neoplasia (MEN) type 1, it is important to differentiate this entity from the rest because of therapeutic implications (Vol. 2; Chap. 27). If it is part of MEN type 1, long-term follow-up has to focus on the detection of other tumors.

Clinical Features

In patients with insulinoma, the hypoglycemia due to hyperinsulinemia determines the clinical pictures. Combinations of adrenergic and neuroglycopenic symptoms, as shown in Table 5, are present in vast

Table 4 Distribution (%) of Morphological Patterns in Nesidioblastosis

Lesions	Ref. 64	Ref. 65
Hyperplasia	33	29
Nesidioblastosis	17	34
Discrete adenomas	16	29
Normal pancreas	30	8

[a]Report was not available in 4% of the patients.

majority of patients (81,82), although the latter tends to predominate in individuals with organic hyperinsulinism. The time of the day when symptoms occur and the relationship of these symptoms to meals are important. If the symptoms are present in the morning during fasting state, hyperinsulinemic hypoglycemia remains a strong possibility; if symptoms are reported during the post-meal period, excessive insulin response due to leucine sensitivity becomes a possibility. Presence or absence of other diseases, such as pituitary, adrenal, hepatic, renal, or autoimmune diseases, should likewise be ascertained. Family history of MEN type 1, possible access to hypoglycemic agents, history of ethanol ingestion, and the nutritional status of the patient should be specifically determined.

Apart from the symptoms being nonspecific, physical examinations are also noncontributory. Thus, clinical diagnosis of insulin-producing tumor is a difficult task. As shown in Table 6, initial diagnosis in patients with proven insulinomas is extremely variable, with 50% of patients being diagnosed inappropriately. The duration of symptoms in patients with islet-cell tumor is also variable and may be as short as two weeks or as long as 20 years. Because they learn to avoid symptoms by eating frequently throughout the day and night, some patients with long-standing disease may present with obesity and increased linear growth, which may also be the direct consequence of the anabolic and growth-promoting effect of insulin.

Laboratory Tests

Although normoglycemia and normal serum insulin levels have been documented in a small percentage of patients with insulinoma (84), this condition is usually suspected in nondiabetic individuals with hyperinsulinemic hypoglycemia during the fasting state. Indeed, absolute values of blood glucose and serum insulin, as well as their ratio, normally up to 0.3, at the fasting state require documentation in these patients before one undertakes more definitive and expensive tests (85). Lack of ketonemia, ketonuria, and acidosis in the presence of fasting hypoglycemia is also strongly supportive of a diagnosis of hyperinsulinemic hypoglycemia.

Table 5 Symptoms of Hypoglycemia

Adrenergic	Neuroglycopenic
Anxiety	Headache
Nervousness	Blurred vision
Tremulousness	Paresthesias
Sweating	Weakness
Hunger	Tiredness
Palpitation	Confusion
Irritability	Dizziness
Pallor	Amnesia
Nausea	Incoordination
Flushing	Behavioral change
Angina	Seizures, coma

Source: From Ref. 81.

Table 6 Initial Diagnosis in 46 of 91 Patients with Proven Insulinoma

Diagnosis	Number of patients
Epilepsy	14
Nervous exhaustion	6
Psychoses	6
Stroke	4
Hysteria	4
Menopause	3
Tetany	2
Brain tumor	2
Diabetes	2
Inebriation	2
Heart attack	1

Source: From Ref. 83.

An amended insulin glucose ratio, calculated by the multiplying the insulin level by 100 and then dividing by the blood glucose minus 30, is considered a better discriminator between normal and abnormal insulin secretion. This ratio of normally 50 or less measures the degree of suppression of the pancreatic insulin secretion and reduces false-negative results.

Measurement of plasma C-peptide and proinsulin levels are also useful in differentiating factitious hypoglycemia from islet-cell tumor (86–88). Normally, the pancreatic insulin secretion parallels the plasma level of peptides. In children with suspected hypoglycemia due to insulinoma, a limited fast of variable duration (6–72 hours) is often necessary (89). This is especially the case when hypoglycemia is observed in the absence of inappropriate elevation of insulin levels.

Duration of the fast should be determined on the basis of age, concurrent conditions, and severity of symptoms. Infants and young children should fast 4 to 12 hours under close observation in the hospital. In adults, prolonged fast of 72 hours has been used (83). However, with the availability of insulin, proinsulin, and C-peptide assays, the 72-hour fast is often not necessary.

For detection of symptoms of hypoglycemia and measurements of blood glucose, insulin, and urine ketones at the time of the symptoms, hospitalization is necessary. In patients with insulinoma, the time of symptom development during the fast has been variable, ranging from 7 to 60 hours. Spontaneous increase in blood glucose levels has also been reported in such patients.

Provocative tests for assessment of insulin secretion are sometimes necessary (90). This is particularly advantageous when the clinical evidence is compelling, yet time limitations do not allow hospitalization for prolonged fasting. Glucagon, leucine, and tolbutamide are β-cell stimulatory agents used as a stimulus to insulin secretion. However, the tolbutamide test should not be used because it may be dangerous.

Depressed glucosylated hemoglobin and fructosamine levels may be present in patients with insulinoma, supporting the presence of hypoglycemia during the preceding six to weeks.

Differential Diagnosis

Hypoglycemia due to various systemic diseases is frequently encountered in clinical practice. Hyperinsulinemia is the primary differentiating feature between these patients and those with insulinoma. Among hyperinsulinemic patients, KATP channel hyerinsulinism, hyperinsulinism–hyperammonemia syndrome (91), glucokinase hyperinsulinism, and nesidioblastosis should be of additional considerations. Specific diagnostic tests include IGFBPI, free fatty acids, serum ketones, and ammonia levels. The most difficult differential diagnosis is nesidioblastosis (92–94).

Localization of the Lesion

Once clinical and biochemical evidence of hyperinsulinism is established, anatomical localization of the insulin-secreting lesion is indicated. This is accomplished by ultrasonography, CT scan, highly selective arteriography, or percutaneous transhepatic pancreatic venous sampling (95–99).

Of all the methods of study, preoperative ultrasonography is the most inexpensive and least invasive technique to localize pancreatic tumors. However, the accuracy is low, with detection of 25% of pancreatic lesions (98). On the other hand, this will avoid the false-negative results of laparotomy that can occur in patients with nesidioblastosis where no identifiable tumor is present. Preoperative endoscopic ultrasonography increases the accuracy of diagnosis and has been the choice for many surgeons (98).

Using intraoperative ultrasonography by applying the probe on the surface of the pancreas, lesions too small to be palpable have been detected. CT scan performed with contrast enhancement can improve the sensitivity of detection up to 40% (95). Detection of the lesion also depends on its size and location: lesions measuring less than 2 cm or located on the head or tail of the pancreas are most likely to be missed. Although ultrasonography and CT scan have low accuracy, these are reasonable first choices for tumor staging and detection of metastasis. Selective arteriography is useful to demonstrate insulinomas, with a success rate ranging from 30% to 90%; lesions as small as 0.5 cm in diameter have been detected. Preoperative angiography is utilized to determine the number, size, and location of the tumors. Tumors located in the head or tail of the pancreas are most likely to be missed.

Transhepatic venous sampling with simultaneous arterial blood sampling has been useful in detecting small tumors that were missed during preoperative imaging studies (99). Apart from obtaining blood samples from different points in the portal, splenic, and mesenteric venous system, it is important to draw simultaneous peripheral blood samples to allow for changing during the course of the procedure. During this study, venous insulin concentration at least 50% higher than the arterial level is considered to be diagnostic of insulinoma. There is a considerable degree of risk of complication, such as peritionitis, hemorrhage, or perforation of the gallbladder. As a consequence, this procedure should be considered if insulinoma is likely based on the hypoglycemia and hyperinsulinemia and yet ultrasound, CT scan, and arteriography have all been negative. Biochemical markers using α-hCG, β-hCG, or immunostaining technique have been found to be helpful in some patients. Differentiation of malignant lesions from benign by the histological criteria has been unreliable, because the morphological characteristics fail to correlate with the metastatic disease (100).

Treatment and Prognosis

To avoid irreversible neurological sequelae of persistent profound hypoglycemia, intense preoperative management is mandatory (101). Dietary management should be judicious, particularly ensuring a snack before bedtime. However, treatment is particularly difficult in infants and children due to the uncertainty in ensuring food intake at a timely manner and the variability of the pathology. Diffuse islet-cell involvement is more frequent than solitary adenomas, thus making complete surgical removal of the tumor difficult for the surgeons.

For preoperative patients as well as those with inoperable or undetectable tumor, pharmacological agents are indicated. Diazoxide, a benzothiadiazine derivative, reduces insulin secretion and increases the epinephrine release. When administered at a dosage of 100 to 800 mg daily, this maintains normoglycemia. Although side effects such as fluid retention and hypertrichosis are unacceptable, it is tolerated by most patients. Corticosteroid, used in conjunction with other agents, enhances the effectiveness of maintaining normoglycemia. A long-acting somatostatin analog, SMS 201–995, and calcium channel blockers, nifedipine, are also beneficial in correcting hypoglycemia (101,102). Surgical removal of the tumor is the mode of therapy in patients with insulinoma (103) and should be performed at the earliest possible time. Almost 90% of the tumors are benign, and carry a favorable diagnosis.

Glucagonoma

Clinical and diagnostic features of glucagonoma, in comparison with vipoma and somatostatinoma, are presented in Table 7. Apart from insulin, endocrine tumors of the pancreas can produce glucagon, pancreatic polypeptide, and somatostatin along with peptides that are not normally present therein, such

Table 7 Characteristic Features of Glucagonoma, Vipoma, and Somatostatinoma

Character	Glucagonoma	Vipoma	Somatostatinoma
Amino acid	29	28	14
Normal source	A-cells of pancreatic islet	Intestinal mucosa, central and peripheral nervous system	D-cells of pancreatic islet, hypo-thalamus-pituitary, intestinal mucosa
Physiological action	Raise blood glucose	Neurotransmitter; enhances intestinal secretion	Reduces intestinal secretion and motility
Characteristics	Rash, glossitis, stomatis	Persistent and profuse diarrhea mimics cholera	Diabetes, cholestasis, steatorrhea; may have hypoglycemia
Clinical features			
Malignancy (%)	75	60	60
Incidence in children	Not known, cases reported	Not known	Not known
Diagnosis	Elevated plasma level glucagons, insulin; CT scan; laparotomy	Elevated plasma level of VIP or PHM; hypokalemia; CT scan	Elevated serum level of somatostatin; CT scan to detect tumor mass

Abbreviations: CT, computed tomography; PHM, peptide histidine methionine.

as VIP, peptide histidine methionine, growth hormone-releasing factors, gastrin, and calcitonin. Due to cosecretion of these hormones by various tumors, clinical syndromes may overlap and appear to be nonspecific (104–106). It consequently becomes impractical to pursue the diagnosis in all patients who present with these symptoms. However, glucagonoma, vipoma, and somatostatinoma, despite being rare in children, are interesting because of the cause and effect relationship between the increased hormone levels and the distinct clinical syndromes (glucagons and hyperglycemia, vasoactice intestinal peptide and diarrhea, somatostatin and reduced motility of the gastrointestinal-biliary tract). For a practicing physician, the importance of being familiar with these syndromes is, therefore, obvious: distinct clinical expression caused by altered biochemical environment, increased availability of precise diagnostic tools, and, most of all, specific therapeutic implications.

The true incidence of glucagonoma is not really known. Postmortem studies in adult patients with neither clinical symptoms nor diabetes have disclosed the presence of glucagonoma (104). However, there are no such data available in children. Glucagon, a 29-amino-acid polypeptide, is mostly secreted by the α cells of the pancreatic islets. It stimulates the glycogenolytic process, resulting in elevation of blood glucose.

Clinical Features

A characteristic skin rash is the major manifestation in patients with glucagons-secreting islet-cell tumor (107,108). Commonly starting in the groin area as erythematous blotches, the lesions migrate to the buttocks, thighs, perineum, and distal extremities. The lesions are necrolytic, with raised and vesiculopustular appearance, and gradually become confluent. During the acute stage, these lesions are intensely painful and pruritic. After scaling, the lesions heal and become indurated and hyperpigmented. However, remission and relapse are typical of these lesions, which are due to the glycogenolytic action of glucagon. Glossitis, angular stomatitis, venous thrombosis, and occasional blackout spells occur in association with these lesions. The most common gastrointestinal symptoms are diarrhea and constipation, which is attributed to the altered motility of the intestine. Other findings include anemia and weight loss, which is primarily due to the anorexic and catabolic effect of glucagon. Specific biochemical findings include hypoproteinemia, hypoaminoacidemia, and hypocholesterolemia. Mild hyperglycemia, due to the glycogenolytic effect of glucagon, is also observed in some patients (108). Glucagonomas may also be associated with MEN 1, vipoma, and somastatinomas, and in such patients the clinical presentation will vary accordingly (109).

Diagnosis and Management

In the presence of suggestive clinical findings, the diagnosis is readily confirmed by the finding of elevated serum glucagon concentrations. Plasma insulin levels are also elevated, which explains the mildness of diabetes. A paradoxic rise of plasma glucagons during an oral glucose tolerance test or intravenous tolbutamide test provides additional support to the diagnosis of glucagonoma. However, the most valuable technique for tumor localization is selective arteriography; glucagonomas are highly vascular and produce prominent tumor blush.

Treatment involves surgical resection, which leads to dramatic improvement of the rash (110). Chemotherapy with dacarbazine (111) and strepzotocin (112) has been useful in case of nonresectable tumors. Somatostatin use alleviates the symptoms, because of the reduction of glucagon (113). Although zinc levels in the blood do not correlate with the presence or absence of the rash, oral zinc administration has been shown to improve the skin lesions (110). Zinc is contained in Zinc finger Gata-4 in the endocrine pancreas and activates glucagon gene expression (114). Adequate nutritional support and reversal of negative nitrogen balance stand at the

center of the medical and surgical management of these patients. Parenteral infusions of specific amino acids to correct the catabolic hypoaminoacidemia have led to disappearance of the skin lesions (115).

Vipoma

Since the first description of this condition in 1957 by Priest and Alexander (116), various investigators have reported its manifold clinical features (117–122). Various synonyms such as Verner–Morrison syndrome, watery diarrhea hypokalemia-achlorhydria syndrome, and pancreatic cholera syndrome have described the same condition (117). In 1973, Bloom and colleagues renamed this condition vipoma syndrome (118).

VIP is a 28-amino-acid peptide, distributed diffusely in the gastrointestinal submucosa as well as the central nervous system (123). It stimulates water and electrolyte secretion, leading to profuse water loss in the small intestine and colon. Sodium and potassium secretion into the lumen of the bowel also features prominently due to VIP's action. Relaxation of the smooth muscles of gastrointestinal and vascular system, reduction of the gastric acid output, and decrease in the motility of the gall bladder are also caused by the vipoma.

Clinical Features

Vipoma is an uncommon disorder in children. Mekhjian and O'Dorisio reported six patients (121), out of 29 diagnosed cases of this syndrome, who were below five years of age. Mean age of presentation is 47 years, and the incidence has been estimated at 1:10 million per year. There is a female preponderance.

Diarrhea, the major manifestation of the vipoma syndrome, is characterized as persistent, secretory, and large in volume, exceeding 700 mL/day. Stool is isotonic with plasma and mimics the description of cholera. Apart from the massive water loss, large amounts of potassium and bicarbonate are lost in the stool. Thus, dehydration, hypokalemia, and acidosis lead to significant morbidity and mortality.

Due to the inhibitory effect of VIP on pentagastrin-mediated acid secretion, gastric acid secretion is frequently decreased in these patients. Differentiation from Zollinger–Ellison syndrome is, therefore, based on diminished basal acid output. Although the serum phosphorus level is normal, hypercalcemia and hypomagnesemia occur in patients with vipoma syndrome. Hypercalcemia is explained on the basis of excessive bone resorption mediated by the VIP, although calcitonin-producing vipomas have been described (124). Hyperglycemia, due to the glucagon-like effect of the VIP, is also reported.

Diagnosis and Management

Correction of volume deficit, electrolyte abnormalities, hypercalcemia, and hypomagnesemia should be the priority before establishing the diagnosis of vipoma and localizing the tumor.

In the presence of characteristic clinical syndrome, the elevated plasma VIP concentration is diagnostic (125), although its significance is questionable in asymptomatic patients who present elevated levels (126). However, normal plasma VIP levels with increased level of peptide histidine methionine, another intestinal secretagogue similar in effect to the VIP, have been described in patients with this syndrome. An intestinal perfusion study is useful to document intestinal secretion and confirm the diagnosis of vipoma syndrome. Anatomical localization of the tumor and its metastasis is obtained by ultrasound and CT scan study (127–129). However, false-negative studies are reported, making exploratory laparotomy the most definitive diagnostic modality. Laparoscopic distal pancreatectomy has also been successful (128).

Surgical resection offers the best chance of cure and provides relief of symptoms (130). Radiofrequency ablation has also been a valuable alternative (131). In patients with nonresectable or metastatic disease, chemotherapy with streptozotocin may produce remission of symptoms and normalize the plasma VIP levels. Other drugs such as corticosteroids and lithium carbonates have been reported to control the symptoms of diarrhea. Treatment with octreotide, a somatostatin analog, may also be employed for the nonsurgical management of these patients.

Somatostatinoma

Clinical Features

This is also an unusual disorder in children. Most of the reported cases in literature involve adult patients (132,133). Somatostatin, a 14-amino-acid cyclic peptide, is present in the anterior pituitary, hypothalamus, thyroid follicle, D-cells of the pancreatic islet, and intestinal mucosa (134–137). As indicated by its name, it inhibits the pituitary, pancreatic, gastric, and biliary secretion. Thus, in patients with somatostatinoma and consequent increased serum somatostatin concentration, provocative gonadotropic hormone and thyroid-stimulating hormone response is inhibited, insulin and glucagon levels are diminished, and gastrointestinal and biliary secretion is decreased (137). Motility of the gastrointestinal and biliary tract is also reduced. Clinically, pancreatic somatostatinoma produces a triad of diabetes, cholelithiasis, and steatorrhea (138,139). Diabetes is usually mild; however, it may also lead to diabetic ketoacidosis (140). Cholelithiasis is associated with gallbladder stasis, and steatorrhea is due to insufficient exocrine function of the pancreas. However, cosecretion of the hormones can alter the picture, contributing to various nonspecific symptoms. For instance, diarrhea may be the prominent feature in case of calcitonin oversecretion. Due to the altered insulin glucagon balance, hypoglycemia has been reported in some patients with somatostatinoma (141).

Extra pancreatic somatostatinoma, usually located in the duodenal mucosa (142), is more likely to present with biliary obstruction than the above-mentioned constellation of the syndrome. Weight loss and anemia are also present in patients with long-standing disease. Somatostatinomas have also been associated with gastrointestinal stromal tumors and von Recklinghausen disease (143,144).

Diagnosis and Management

In presence of clinical syndrome, the serum somatostatin level is usually elevated in patients with somatostatinoma (145). Occasionally, there may also be high ghrelin levels and pancreatic ghrelinomas (146) Provocative endocrine studies using tolbutamide, arginine, or the glucose tolerance test may be required in doubtful cases to document lack of change in the serum levels of insulin and glucagon. Detection of tumor mass and metastasis requires radiological imaging study, usually with CT scan. Although total surgical resection offers the most favorable outcome, advanced or recurrent disease may require chemotherapy with streptozotocin and 5-fluorouracil. Therapy with 131I-MIBG has also been successful (147). Prognosis for treatment depends on the extent of the disease at the time of diagnosis.

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Disorders of Water Homeostasis

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INTRODUCTION

Maintenance of the tonicity of extracellular fluids within a very narrow range is crucial for proper cell function (1,2). Extracellular osmolality regulates cell shape as well as intracellular concentrations of ions and other osmolytes. Furthermore, proper extracellular ionic concentrations are necessary for the correct function of ion channels, action potentials, and other modes of intercellular communication. Extracellular fluid tonicity is regulated almost exclusively by the amount of water intake and excretion, whereas extracellular volume is regulated by the level of sodium chloride intake and excretion. In children and adults, normal blood tonicity is maintained over a 10-fold variation in water intake by a coordinated interaction among the thirst, vasopressin, and renal systems. Dysfunction in any of these systems can result in abnormal regulation of blood osmolality, which if not properly recognized and treated, may cause life-threatening dysfunction in neuronal and other cellular activities.

The posterior pituitary or neurohypophysis secretes the nonapeptide hormone vasopressin, which controls water homeostasis by its interaction with the renal V2-vasopressin receptor. This receptor regulates the activity of the water channel, aquaporin-2 (AQP2), in the distal nephron, which controls the reabsorption of water from the urine. Disorders of vasopressin secretion and its action in the kidney lead to clinically important derangements in water metabolism.

REGULATION OF FLUID BALANCE

Osmotic Sensor and Effector Pathways

Vasopressin Synthesis: Anatomy and Biochemistry

The sequence and synthesis of the nine-amino-acid-long biologically active vasopressin peptide was performed by Du Vigneaud et al. during the mid-1950s (3). Vasopressin was found to be closely related to oxytocin (OT), differing by only two amino acids. By replacement of l-arginine with d-arginine at position 8 of the vasopressin molecule, and amino-terminal deamidation, an analog with enhanced, prolonged antidiuretic-to-pressor activity ratio was found [desamino-d-arginine vasopressin (dDAVP)] (4). dDAVP is now routinely used in clinical practice (Fig. 1).

Vasopressin is initially synthesized as part of a larger precursor protein that also contains neurophysin. After biosynthesis, vasopressin and neurophysin are cleaved but remain associated within the cell prior to their secretion into the bloodstream. The function of neurophysin is not clear but may include stabilization of vasopressin against degradation during intracellular storage and its more efficient packaging or posttranslational processing by the proenzyme convertases within secretory granules (Fig. 2).

In all mammalian species thus far analyzed, the OT and vasopressin genes are adjacent in chromosomal location (chromosome 20 in the human) (5) and linked tail-to-tail in opposite transcriptional orientation. In the human, they are separated by 12 kb (5). This likely explains their origin from the ancient duplication of a common ancestral gene (6). This intergenic region contains the critical enhancer sites for cell-specific expression of vasopressin (VP) and OT in the magnocellular neurons (7,8).

Expression of the vasopressin and OT genes occurs in the hypothalamic paraventricular and supraoptic nuclei (9,10). The magnocellular components of each of these nuclei are the primary neuronal populations involved in water balance, with vasopressin synthesized in these areas carried via axonal transport to the posterior pituitary, its primary site of storage and release into the systemic circulation. The bilaterally paired hypothalamic paraventricular and supraoptic nuclei are separated from one another by relatively large distances (approximately one centimeter). Their axons course caudally, converge at the infundibulum, and terminate at different levels within the pituitary stalk and posterior pituitary gland. Vasopressin is also synthesized in the parvocellular

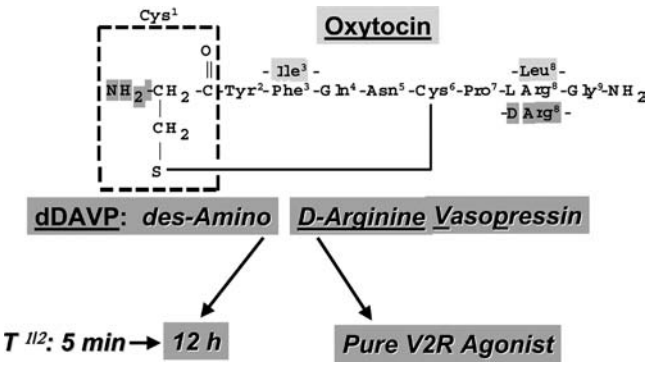

Figure 1 Structure of vasopressin (VP) and dDAVP. VP is a nine-amino-acid long peptide that is closely related to oxytocin. The disulfide bond between cystine molecules in positions 1 and 6 forms a six-amino chain within VP. Oxytocin (*light gray* shading) differs from VP by the replacement of phenylalanine with isoleucine in position 3 and of arginine with leucine in position 8. The synthetic analog dDAVP (*dark gray* shading) is created by the removal of the amino group at the amino terminus and by the replacement of L-arginine in position 8 with D-arginine. The first modification significantly increases the half-life from five minutes for VP to 12 hours for dDAVP. The second modification confers V2-receptor specificity to dDAVP. *Abbreviation*: dDAVP, desamino-D-arginine-VP.

neurons of the paraventricular nucleus, where it has a role in modulation of hypothalamic–pituitary–adrenal axis activity. In this site, vasopressin is co-localized in cells that synthesize corticotropin-releasing hormone (11,12), and both are secreted at the median eminence and carried via the portal–hypophyseal capillary system to the anterior pituitary where, together, they act as the major regulators of adrenocorticotropin synthesis and release (13). Vasopressin is also present in the hypothalamic suprachiasmatic nucleus, the circadian pacemaker of the body, where its function is unknown (Fig. 3).

Vasopressin Metabolism

Once in the circulation, vasopressin has a half-life of only 5 to 10 minutes because of its rapid degradation by a cysteine amino terminal peptidase, called vasopressinase. A synthetic analog of vasopressin, dDAVP (desmopressin, Fig. 1), is insensitive to amino terminal degradation and thus has a much longer half-life, 8 to 24 hours. During pregnancy, the placenta secretes increased amounts of this vasopressinase (14), resulting in a fourfold increase in the metabolic clearance rate of vasopressin (15). Normal women compensate with an increase in vasopressin secretion, but women with preexisting deficits in vasopressin secretion or action (16), or those with increased concentrations of placental vasopressinase associated with liver dysfunction (17) or multiple gestations (18), may develop diabetes insipidus in the last trimester, which resolves in the immediate postpartum period (19). As expected, this form of diabetes insipidus responds to treatment with dDAVP but not with vasopressin (20,21).

Physiological Actions of Vasopressin

Vasopressin Receptors

Vasopressin released from the posterior pituitary and median eminence affects the function of several tissue types by binding to members of a family of G-protein–coupled cell surface receptors, which subsequently transduce ligand binding into alterations of intracellular second messenger pathways (22). Biochemical and cell biological studies have defined at least three receptor types, designated V1, V2, and V3 (or V1b). The major sites of V1 receptor expression are on vascular smooth muscle (23) and hepatocytes (24–27) where receptor activation results in vasoconstriction (28,29) and glycogenolysis (30), respectively. The latter activity may be augmented by stimulation of glucagon secretion from the pancreas (30). The V1 receptor on platelets stimulates platelet aggregation (31). V1 receptor activation mobilizes intracellular calcium stores through phosphatidyl inositol hydrolysis (28,32). Despite its initial characterization as a powerful pressor agent, the concentration of vasopressin needed to significantly increase blood pressure is several-fold higher than that required for maximal antidiuresis (33), although substantial vasoconstriction in renal and splanchnic vasculature can occur at physiologic concentrations (34). The cloning of the V1 receptor (23–25) has greatly elucidated the relationship of the vasopressin (and OT) (35,36) receptors and, through sensitive in situ hybridization analysis, has further localized V1 expression to the liver and vasculature of the renal medulla as well as to many sites within the brain, including the hippocampus, amygdala, hypothalamus, and brainstem (26,27). The V3 (or V1b) receptor is present on corticotrophs in the anterior pituitary (37) and acts through the phosphatidylinositol pathway (38) to increase adrenocorticotrophic hormone secretion. Its binding profile for vasopressin analogs resembles more closely that of the V1 than the V2 receptor. The structure of this receptor has been determined in humans via cloning of its complementary DNA (38,39). Its structure is similar to that of the V1 and OT receptors and is expressed in the kidney as well as in the pituitary (Table 1).

Modulation of water balance occurs through the action of vasopressin upon V2 receptors located primarily in the renal collecting tubule along with other sites in the kidney, including the thick ascending limb of Henle's loop and periglomerular tubules (26,27,40). It is also present in vascular endothelial cells in some systemic vascular beds, where vasopressin stimulates vasodilation (41), possibly via activation of nitric oxide synthase (42). Vasopressin also stimulates von Willebrand's factor, factor VIIIa, and tissue plasminogen activator, via V2-mediated actions. Because of this, dDAVP is used to improve the prolonged

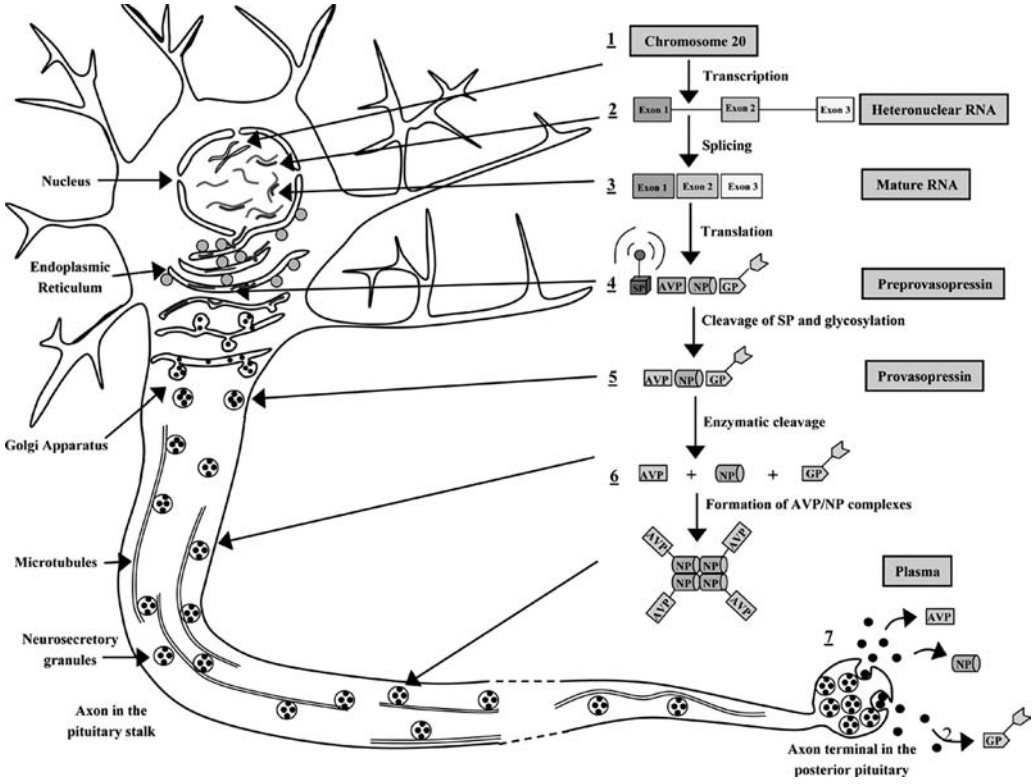

Figure 2 Steps in the synthesis and release of vasopressin.(1). *Nucleus*: Transcription of the vasopressin gene (chromosome 20) to heteronuclear ribonucleic acid (RNA) (2). *Nucleus*: Synthesis of the mature RNA by removal of the introns and the splicing of exons A, B and C and its subsequent passage into the cytoplasm. Attachment of the mRNA to ribosomes in the endoplasmic reticulum (ER) (3). *ER*: Translation of the exons to preprovasopressin. Exon A encodes the 19-amino-acid (AA), signal peptide (SP), the 9-AA vasopressin (AVP), and the amino terminal of the 93 to 95-AA neurophysin (NP), Exon B encodes the middle portion of NP and Exon C encodes the carboxyl terminal of NP and a 39 AA glycopeptide (GP) (4). *ER*: Glycosylation of the GP portion of the preprovasopressin and cleavage of the SP (5). *Golgi complex*: Entry of provasopressin into the Golgi complex and its packaging into neurosecretory granules. Attachment to and the subsequent transport of the granules along microtubules to the site of storage in the posterior pituitary gland (5). *Neurosecretory granules*: Enzymatic cleavage of provasopressin to vasopressin, NP, and GP during the transport within the acidic granules. Amidation of the vasopressin, formation of complexes consisting of one vasopressin molecule with either a dimer or a tetramer of NP (6). *Neurosecretory granules*: Fusion of the granule with the plasma membrane as a result of an action potential and the release of vasopressin, NP, and the GP into the extracellular space and plasma. *Source*: With permission from A. G. Robinson, M.D., UCLA.

bleeding times characteristic of uremia, Type I von Willebrand disease, and hemophilia (43). The V2 receptor consists of 370 amino acids encoding seven transmembrane domains characteristic of the G-protein–coupled receptors (40,44). These transmembrane domains share approximately 60% sequence identity with the V1 receptor but substantially less with other members of this family. Unlike the V1 and V3 receptors, the V2 receptor acts through adenylate cyclase to increase intracellular cyclic adenosine monophosphate (cAMP) concentration. The human V2-receptor gene is located on the long arm of the X chromosome (Xq28) (45,46) at the locus associated with congenital, X-linked vasopressin-resistant diabetes insipidus.

Renal Cascade of Vasopressin Function

Vasopressin-induced increases in intracellular cAMP, as mediated by the V2 receptor, trigger a complex pathway of events, resulting in increased permeability of the collecting duct to water and efficient water transit across an otherwise minimally permeable epithelium (Fig. 4) (47).

Activation of a cAMP-dependent protein kinase imparts remodeling of cytoskeletal microtubules and microfilaments that culminate in the insertion of aggregates of water channels into the apical membrane (48). Insertion of the water channels causes up to 100-fold increase in water permeability of the apical membrane, allowing water movement along its osmotic gradient into the hypertonic inner medullary interstitium from the tubule lumen and excretion of concentrated urine (Fig. 4). The molecular analysis of the water channels has revealed a family of related proteins, designated aquaporins, which differ in their sites of expression and pattern of regulation (49). Each protein consists of a single polypeptide chain with six membrane-spanning domains. Although functional as monomers, they are believed to form homotetramers in the plasma membrane (47). AQP2 is expressed

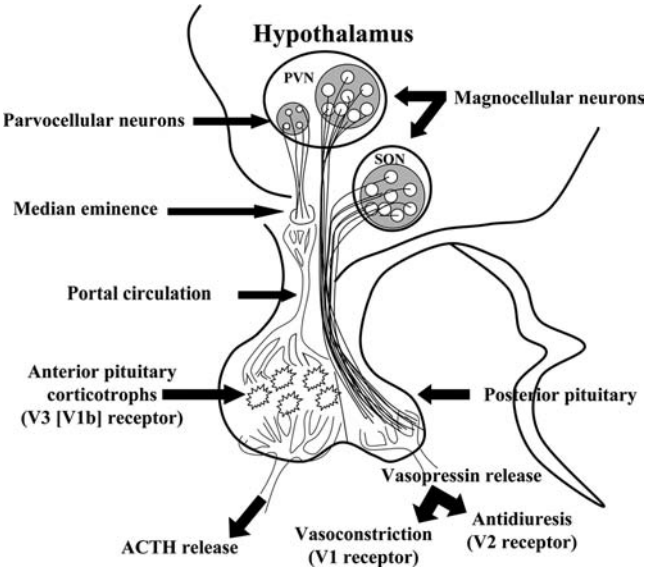

Figure 3 Anatomy of vasopressin (VP) producing neurons. The bilaterally paired paraventricular nucleus (PVN) and the supraoptic nucleus (SON) of the hypothalamus synthesize VP. The PVN contains magnocellular and parvocellular neurons while the SON contains only magnocellular neurons. The magnocellular neurons of the PVN and SON synthesize VP in their soma and carry it through their long axons to the posterior pituitary gland through the pituitary stalk to eventually secrete it from their terminals in the neurohypophysis. VP released from magnocellular neurons is primarily involved in water balance. The parvocellular neurons that synthesize VP along with corticotrophin-releasing hormone (CRH) have shorter axons that terminate in close proximity to the capillaries of the hypothalamic–hypophyseal portal system in the median eminence of the hypothalamus. VP secreted here is carried in the portal circulation directly to the anterior pituitary corticotrophs that secrete adrenocorticotrophic hormone (ACTH). VP from the parvocellular neurons, along with CRH, regulates ACTH production and release. The suprachiasmatic nucleus also produces VP, which is thought to regulate circadian rhythms (not shown).

mostly within the kidney (50), primarily within the collecting duct (51). It is also expressed in the vas deferens, at least in the rat, although it is not regulated by vasopressin in this location (52). Studies with immunoelectron microscopy have demonstrated large amounts of AQP2 in the apical plasma membrane and subapical vesicles of the collecting duct, consistent with the ''membrane shuttling'' model of water channel aggregate insertion into the apical membrane after vasopressin stimulation (53). In response to water restriction or dDAVP infusion in humans, the content of urinary AQP2 in both soluble and membrane-bound forms has been found to increase (54). In addition to AQP2, different aquaporins appear to be involved in other aspects of renal water handling. In contrast to the apical localization of AQP2, AQP3, and AQP4 are expressed on the basolateral membrane of the collecting duct epithelium. They appear to be involved in the flow of water and urea from the inside of the collecting duct cell into the extracellular renal medullary space.

Osmotic Regulation of Vasopressin Secretion and Thirst

The rate of secretion of vasopressin from the paraventricular and supraoptic nuclei is influenced by several physiologic variables, including plasma osmolality and intravascular volume, as well as nausea and a number of pharmacologic agents. The major osmotically active constituents of blood are sodium chloride and glucose (with insulin deficiency). Normal blood osmolality ranges between 280 and 290 mosmoles/kg H_20 (mOsm/kg).

The work of Verney (55) first demonstrated the relationship of increased vasopressin release in response to increasing plasma osmolality, as altered by infusion of sodium chloride or sucrose. At that time, the presence of intracranial sensors sensitive to changes in plasma osmolality was postulated. Multiple researchers have subsequently confirmed that plasma vasopressin concentration rises in response to increasing plasma tonicity, although the exact nature of the osmosensor has not been defined (56,57). Neurons of the supraoptic nucleus can respond directly to hypertonic stimuli with depolarization and vasopressin secretion (58), but the majority of evidence indicates that osmosensor and vasopressin-secreting neurons are anatomically distinct (59,60). The osmosensor is likely to reside outside the blood–brain barrier as implicated by differential vasopressin secretory response to similar changes in plasma osmolality, depending upon whether the change was induced by salt, sucrose, or urea (55,61). The organ vasculosum of the lamina terminalis (OVLT) and the subfornical organ areas of the preoptic hypothalamus outside the blood–brain barrier, are likely sites of osmosensing. Lesions of the OVLT, which is located in the anterior wall of the third ventricle, result in impaired arginine vasopressin (AVP) secretion and hypernatremia (59,60). Also, the site of

Table 1 Vasopressin Receptors, Their Intracellular Signaling, and Actions

Receptor	Target tissue	Intracellular messenger	Function
V1	Vascular smooth muscle, platelets, and hepatocytes	Protein kinase C, phosphotidyl-inositol	Vasoconstriction, hemostasis, and glycogenolysis
V2	Renal distal tubule and collecting duct	Protein kinase A, cyclic AMP	Aquaporin-2 mediated water reabsorption
V3 (V1b)	Anterior pituitary corticotrophs	Protein kinase C, phosphotidyl-inositol	ACTH release

Abbreviations: AMP; adenosine monophosphate; ACTH, adrenocorticotrophic hormone.

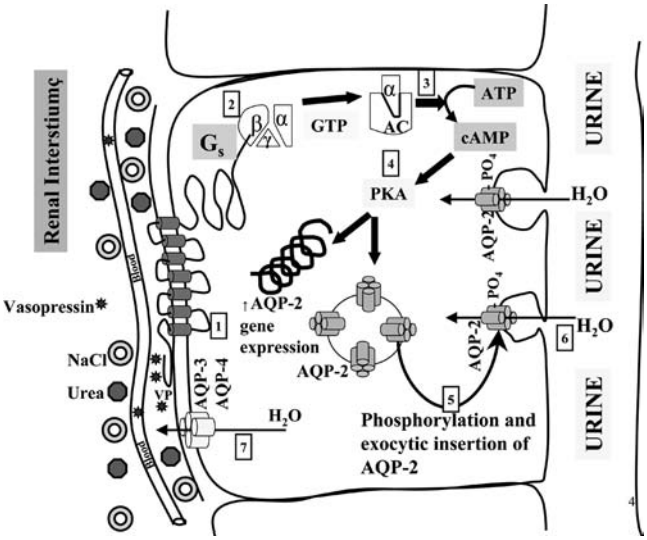

Figure 4 Action of vasopressin (VP) in the renal collecting duct epithelial cell (1). VP binds to the seven transmembrane G-protein–coupled V2 receptor on the surface of the epithelial cell (2). This activates the binding of GTP to the stuimulatory G_s protein causing the translocation of its α-subunit that activates adenylate cyclase (AC) (3). AC catalyzes the synthesis of cylic adenosine monophosphate (cAMP), which activates protein kinase A (PKA).(4) Activated PKA increases the expression of the gene for aquaporin-2 protein (AQP-2). It also catalyzes the phophorylation of serine 256 of the AQP-2 protein and its homotetramerization. Each tetramer forms a water channel.(5) The water channels in the vesicles move selectively toward and fuse with the luminal membrane. This exocytosis of the vesicle inserts the water channel into the membrane (6). Water moves freely into the epithelial cell from the lumen of the collecting duct, driven by the osmotic gradient (7). Intracellular water moves to the renal interstitium through the aquaporin 3 and 4 (AQP-3, AQP-4) channels that are preferentially localized to interstitial surface of the cell. This flow is driven by the osmotic gradient created mainly by urea and sodium chloride molecules. Hypercalcemia, hypokalemia, and treatment with demeclocycline or lithium interfere with these processes, possibly at the level of cAMP generation and AQP2 synthesis or action, thereby causing nephrogenic diabetes insipidus.

action of angiotensin II, infused intracerebrally or peripherally to produce vasopressin secretion and antidiuresis, resides within the OVLT (62–64). Using functional magnetic resonance imaging (MRI) techniques, systemic hypertonicity has been shown to activate neurons in the lamina terminalis and the anterior cingulate cortex in humans (65).

The pattern of secretion of vasopressin into blood has been characterized extensively in normal individuals and those with abnormalities in water homeostasis. Normally, at a serum osmolality of less than 280 mOsm/kg, the plasma vasopressin concentration is at or below 1 pg/mL, the lower limit of detection of most radioimmunoassays (56,57). Above 283 mOsm/kg, the normal threshold for vasopressin release, the plasma vasopressin concentration rises in proportion to plasma osmolality, up to a maximum concentration of about 20 pg/mL at a blood osmolality of approximately 320 mOsm/kg (Fig. 5). The osmosensor can detect as little as a 1% change in blood osmolality. Plasma concentrations in excess of 5 pg/mL are also found with nausea, hypotension, hypovolemia, and insulin-induced hypoglycemia, but further increments in urine concentration do not occur because peak antidiuretic effect is achieved at 5 pg/mL. The rate of rise of plasma vasopressin concentration, and thus the sensitivity of the osmosensor, exhibits substantial (as much as 10-fold) interindividual variation as plasma osmolality increases (67). The set-point for vasopressin secretion varies in a single individual in relation to changes in volume status and hormonal environment [e.g., pregnancy (68) or glucocorticoid status (69,70)]. After the seventh week of gestation, osmotic thresholds for both vasopressin release and thirst are reduced by approximately 10 mOsm/kg such that normal blood osmolality during pregnancy is approximately 273 mOsm/kg (serum sodium 135 mEq/L) (68,71).

The sensation of thirst, a more integrated cortical activity, is determined by other anatomically distinct hypothalamic neurons, with afferents involving the ventromedial nucleus (72). The activation of the thirst mechanism is also probably mediated by angiotensin II (73). Whether the osmosensor for thirst and vasopressin release are the same is not certain, although this is suggested by lesions in the anteroventral region of the third ventricle, which abolish both thirst sensation and vasopressin release (74). It makes physiologic sense that the threshold for thirst (approximately 293 mOsm/kg) is approximately

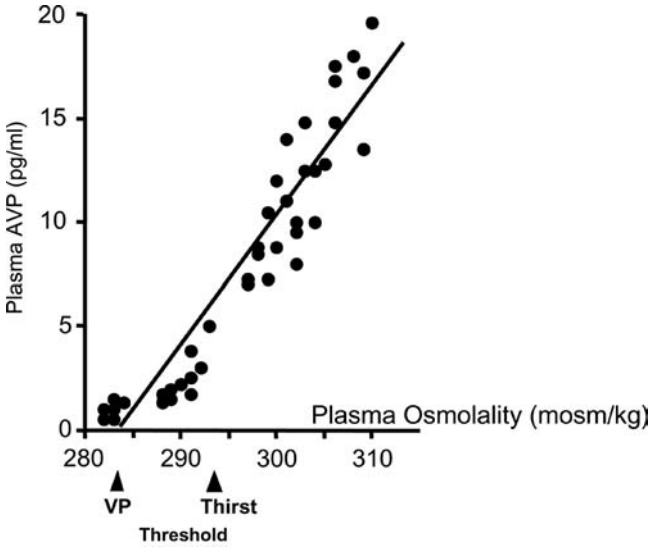

Figure 5 Osmotic regulation of vasopressin (VP) secretion and thirst. With water deprivation, plasma osmolality rises above the threshold for VP secretion. With continued hyperosmolality, VP secretion rises linearly. The threshold for thirst sensation is approximately 10 mOsm/kg greater than that for VP secretion. Solid arrowheads denote the thresholds for VP release and thirst sensation. *Source*: From Ref. 66, with permission.

10 mOsm/kg higher than that for vasopressin release (Fig. 5). Otherwise, during the development of hyperosmolality, the initial activation of thirst and water ingestion would result in polyuria without activation of vasopressin release, causing a persistent diuretic state. Immediately following water ingestion, prior to a change in blood osmolality or volume, vasopressin concentration falls and thirst ceases (75). The degree of suppression is directly related to the coldness (76) and volume (77) of the ingested fluid. This effect is probably mediated by chemoreceptors present in the oropharynx that guard against the rapid overdrinking of fluids after intense thirst during the time before the lowering of blood osmolality.

As noted above, water balance is regulated in two ways: vasopressin secretion stimulates water reabsorption by the kidney, thereby reducing future water loss, and thirst stimulates water ingestion, thereby restoring previous water loss. Ideally, these two systems work in parallel to efficiently regulate extracellular fluid tonicity (Fig. 6). However, each system by itself can maintain plasma osmolality in the near-normal range. For example, in the absence of vasopressin secretion but with free access to water, thirst drives water ingestion up to the 5 to 10 L/m^2 of urine output seen with vasopressin deficiency. Conversely, an intact vasopressin-secretory system can compensate for some degree of disordered thirst regulation. However, when both vasopressin secretion and thirst are compromised either by disease or iatrogenic means, there is great risk for the occurrence of life-threatening abnormalities in plasma osmolality.

Vasopressin is secreted in response to several nonosmotic stimuli. Physiologically, hypovolemia is the most important among them. Nausea, pain, motion sickness, and vasovagal reactions also stimulate vasopressin secretion. The very important concept of nonosmotic regulation of vasopressin by hypovolemia and hypotension is discussed further in this chapter under the section titled Volume Sensors and Effector Pathways (Fig. 7).

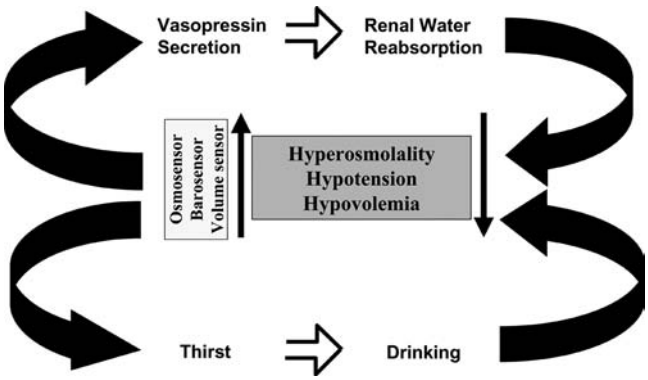

Figure 6 Regulation of vasopressin (VP) secretion and serum osmolality. Hyperosmolality, hypovolemia, or hypotension are sensed by osmosensors, volume sensors, or barosensors, respectively. These stimulate both VP secretion as well as thirst. VP, acting on the kidney, causes increased reabsorption of water (antidiuresis). Thirst causes increased water ingestion. The results of these dual negative feedback loops cause a reduction in hyperosmolality or hypotension/hypovolemia.

Volume Sensor and Effector Pathways

Renin–Angiotensin–Aldosterone System

In contrast to the vasopressin system, the classic or peripheral renin–angiotensin system primarily affects maintenance of intravascular volume as opposed to plasma tonicity. In addition to the well-established endocrine regulatory system, several local renin-angiotensin systems have emerged with both autocrine and paracrine effects in their tissue of synthesis, whose regulation is independent of the classic system. Finally, brain and pituitary angiotensin systems involved in blood pressure, autonomic function, and fluid balance have recently been characterized with extensive interaction with the vasopressin system.

Endocrine Renin–Angiotensin–Aldosterone System

Anatomy and Biochemistry. Renin, which is synthesized by the renal juxtaglomerular apparatus, is a proteolytic enzyme, which catalyzes the cleavage of angiotensinogen, synthesized by hepatocytes, into the decapeptide angiotensin I (78,79). Angiotensin I possesses no intrinsic vasoreactive or mineralocorticoid secretagogue activity but is efficiently cleaved by angiotensin-converting enzyme in the lungs as well as other peripheral sites to generate the octapeptide angiotensin II. Angiotensin II is further metabolized to the heptapeptide angiotensin III by removal of one amino-terminal amino acid. Angiotensin II possesses greater vasopressor activity and is present in approximately a fourfold greater amount than angiotensin III. Angiotensins II and III possess equivalent mineralocorticoid secretory activity on the adrenal glomerulosa cells.

Aldosterone, the primary and most potent endogenous mineralocorticoid released by the zona glomerulosa, acts on target tissues expressing the nuclear mineralocorticoid (or type I glucocorticoid) receptor to promote sodium absorption and potassium excretion. For control of intravascular volume, the primary target of action of aldosterone is the distal nephron. Here, aldosterone increases synthesis of apical membrane sodium channels, mitochondrial enzymes involved in adenosine triphosphate (ATP) production, and components of the Na^+-K^+ ATPase to cause increased sodium reabsorption and potassium excretion (80).

Regulation of Secretion. Decreased intravascular volume, as sensed by the renal juxtaglomerular apparatus, results in release of renin (78,81). Increased plasma renin activity then allows increased conversion of angiotensinogen to angiotensin I, which in turn is converted peripherally to angiotensins II and III. Increased angiotensin II

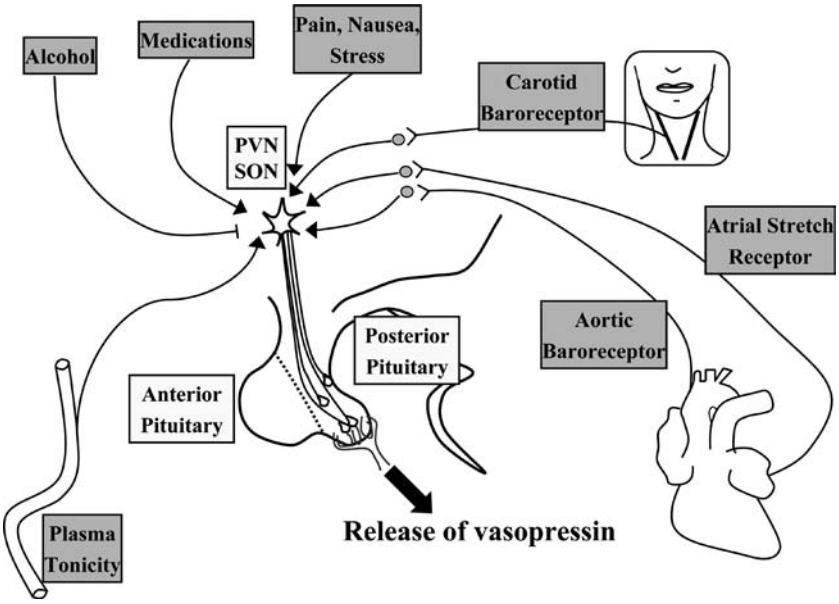

Figure 7 Regulation of vasopressin (VP) secretion. VP is controlled by osmotic and nonosmotic factors. Osmotic regulation occurs through osmosensors in the organum vasculosum of the lamina terminalis. An increase in plasma osmolality stimulates secretion of VP. Several nonosmotic factors regulate VP secretion. Blood-pressure–controlled regulation of VP is mediated by afferents from baroreceptors in the aorta and the carotid sinus via the cranial nerves IX and X with relays within the brainstem nuclei. Hypovolemia stimulates the secretion of VP via stretch receptors in the atria and pulmonary veins. Nausea is a potent stimulator of VP secretion. While pain, stress, and several medications stimulate the secretion of VP, alcohol inhibits its release. All these factors ultimately act upon the magnocellular vasopressinergic neurons of the paraventricular nucleus (PVN) and the supraoptic nuclei (SON) of the hypothalamus to regulate the synthesis and secretion of VP.

activity causes vasoconstriction and blood pressure elevation, whereas both angiotensins II and III stimulate aldosterone release from the zona glomerulosa, and subsequent salt and water retention and potassium excretion by the distal tubule of the kidney. Conversely, expanded intravascular volume causes decreased renin output and less sodium and water resorption in the kidney, serving to decrease intravascular volume and restore homeostasis.

Changes in vascular volume are not the only regulators of the renin–angiotensin–aldosterone system. Serum potassium concentration directly modulates aldosterone release by the adrenal glomerulosa by its effects on plasma membrane potential and activation of voltage-gated calcium channels (79,82). By membrane depolarization, increased serum potassium leads to increased aldosterone synthesis, which promotes renal potassium excretion, whereas low serum potassium reduces aldosterone synthesis and decreases urinary potassium losses. Pituitary adrenocorticotropin hormone and vasopressin act via their respective receptors on the glomerulosa cells to increase acute aldosterone secretion. These effects are of short duration because long-term chronic infusions do not chronically elevate aldosterone concentrations. Direct inhibitors of aldosterone secretion, and thus promoters of natriuresis, include atrial natriuretic peptide (ANP) (83,84), somatostatin (85–87), and dopamine (88,89).

Nonosmotic Regulation of Vasopressin Secretion

Separate from osmotic regulation, vasopressin has been shown to be secreted in response to alterations in intravascular volume. Afferent volume and baroreceptor pathways arising from the right and left atria and aortic arch (carotid sinus) are stimulated by increasing intravascular volume and stretch of vessel walls, and they send signals via the vagus and glossopharyngeal nerves, respectively, to the brainstem nucleus tractus solitarius (90,91). Noradrenergic fibers from the nucleus tractus solitarius synapse upon the hypothalamic paraventricular nucleus and supraoptic nucleus and, on stimulation, inhibit vasopressin secretion (92,93). Experimental verification of this pathway has included demonstration of increased vasopressin concentration following interruption of baroreceptor output to the brainstem and decreased plasma vasopressin concentration following mechanical stimulation of baroreceptors, an effect diminished by vagotomy (94,95).

The pattern of vasopressin secretion in response to volume as opposed to osmotic stimuli is markedly different (Fig. 8). While minor changes in plasma osmolality above 280 mOsm/kg evoke linear increases in plasma vasopressin, substantial alteration in intravascular volume is required for alteration in vasopressin output (96–98). No change in vasopressin secretion is seen until blood volume decreases by approximately 8%. With intravascular volume deficits exceeding 8%, vasopressin concentration rises exponentially. Furthermore, osmotic and hemodynamic stimuli can interact in a mutually synergistic fashion, so that the response to either stimulus may be enhanced by the concomitant presence of the other. When blood volume [or blood pressure (99–101)] decreases by approximately 25%, vasopressin concentrations are evident at 20- to 30-fold above normal and vastly exceeding those required for maximal antidiuresis. Surprisingly, the use of vasopressin

antagonists has suggested that the high concentration of vasopressin observed with hypotension does not contribute to the maintenance of blood pressure in humans (102).

Nausea, as evoked by apomorphine (103), motion sickness (104), and vasovagal reactions, is a very potent stimulus for vasopressin secretion. This effect is likely mediated by afferents from the area postrema of the brainstem and may result in vasopressin concentrations two to three orders of magnitude above basal levels. Nicotine is also a strong stimulus for vasopressin release (105). These pathways probably do not involve osmotic or hemodynamic sensor systems because blockade of the emetic stimulus with dopamine or opioid antagonists does not alter the vasopressin response to hypernatremia or hypovolemia.

Vasopressin secretion is inhibited by glucocorticoids; because of this, the loss of negative regulation of vasopressin secretion occurs in the setting of primary or secondary glucocorticoid insufficiency (106,107). The effects of cortisol loss, both enhancing hypothalamic vasopressin production and directly impairing free water excretion (108), are important considerations in the evaluation of the patient with hyponatremia, as is subsequently discussed.

The Natriuretic Peptide System

In addition to the classic vasopressin and renin–angiotensin–aldosterone systems, the recently defined natriuretic peptide families of ligands and their receptors add further potential for modulation of salt and water balance. The interaction of the natriuretic peptide system occurs both in the central nervous system via effects on vasopressin secretion and, peripherally, through its ability to both directly promote natriuresis in the kidney and indirectly inhibit adrenal aldosterone production.

Anatomy and Biochemistry

ANP was initially discovered as a component of cardiac atrial muscle that was able to induce natriuresis, a decrease in blood pressure, and rise in hematocrit when injected into rats (109,110).

The biologically active form of ANP consists of a 28-amino-acid peptide that includes a 17-amino-acid ring structure (111). The primary sequence of the

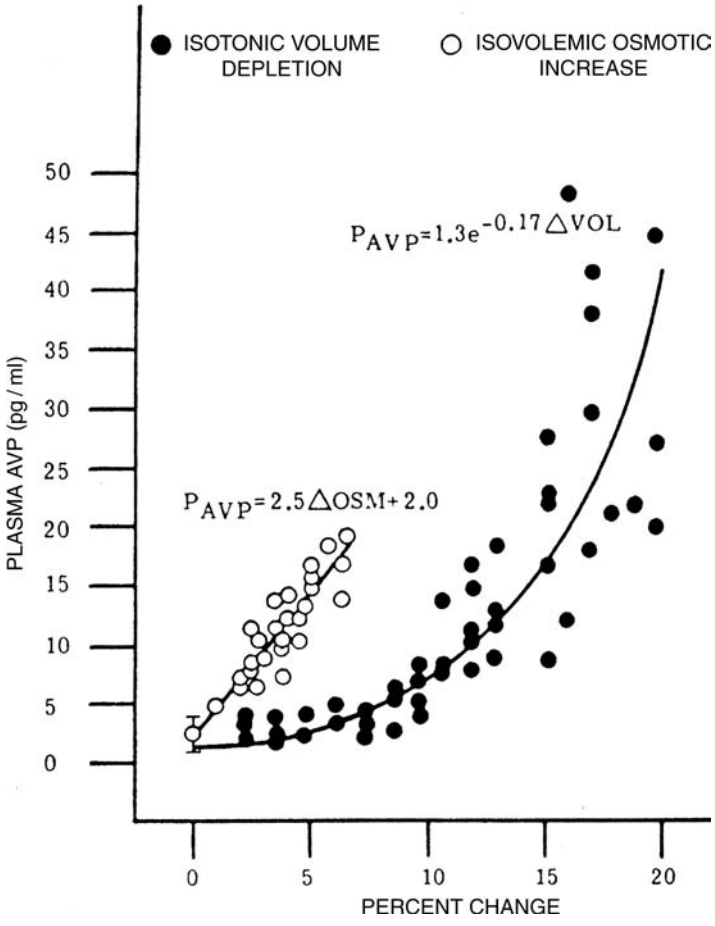

Figure 8 Relationships between osmotic and nonosmotic stimuli for vasopressin (VP) release. Response of plasma VP (AVP) concentration to the percent increase in blood osmolality (*open circles*) or to the decrease in blood volume (*closed circles*). *Source*: From Ref. 66, with permission.

peptide has been conserved among mammalian species and, in addition to synthesis in cardiac atrial tissue (112), has been detected in brain, spinal cord, pituitary, and adrenal gland (113–116).

Subsequent investigation defined a second peptide from porcine brain with structural homology to ANP (117). This peptide, designated brain natriuretic peptide (BNP), was later found to be secreted by the heart as well, in this case from both ventricular and atrial tissue (118–120).

A third member of this family, C-type natriuretic peptide (CNP), was also isolated from porcine brain (121). Little CNP can be detected in plasma and, in marked contrast to ANP and BNP, CNP does not increase in plasma in the setting of cardiac failure (122,123).

Regulation of Secretion and Function

Secretion of ANP by cardiac tissue occurs in response to increasing atrial transmural pressure from both left and right atria (122,124). Also, increased heart rate, especially increased atrial contractile frequency, results in increased ANP secretion. Ventricular production of ANP has also been demonstrated; it is increased in states of left-sided overload associated with ventricular hypertrophy (120).

The physiologic ramifications of increased ANP production are several. Infusion of ANP in the setting of normovolemia causes natriuresis, diuresis, and a small increase in divalent cation excretion (122,123,125). ANP, through the NPR-A receptor, primarily inhibits sodium reabsorption within the renal inner medullary collecting duct but also opposes the salt-retaining effects of angiotensin II at the level of the proximal tubule (125). ANP similarly inhibits the actions of vasopressin and aldosterone in the renal tubules (126–128).

ANP modulates mineralocorticoid production in a manner that results in the reduction of intravascular volume or pressure. Although direct reduction in plasma renin activity has been described with ANP infusion (129,130), the most dramatic response to ANP occurs at the level of the adrenal glomerulosa cell. ANP inhibits aldosterone production by inhibiting action of most aldosterone secretagogues, with the most pronounced reduction being angiotensin II activity (123–125).

BNP synthesis and secretion from cardiac ventricular tissue are augmented in congestive heart failure and, as for ANP, with hypertension, chronic renal, and chronic liver failure (119,122).

CENTRAL DIABETES INSIPIDUS

Causes of Central Diabetes Insipidus

Central (hypothalamic, neurogenic, or vasopressin-sensitive) diabetes insipidus can be caused by disorders of vasopressin gene structure; accidental or surgical trauma to vasopressin neurons; congenital anatomical hypothalamic or pituitary defects; neoplasms; infiltrative, autoimmune, and infectious diseases affecting vasopressin neurons or fiber tracts; and increased metabolism of vasopressin. In approximately 50% of children with central diabetes insipidus, the etiology is not apparent, even after long-term follow-up (Table 2) (131).

Genetic Causes

Familial, autosomal dominant central diabetes insipidus is manifest within the first half of the first decade of life (132). Vasopressin secretion, initially normal, gradually declines until diabetes insipidus of variable severity ensues. Patients respond well to vasopressin replacement therapy. The disease has a high degree of penetrance but may be of variable severity within a family (133) and may spontaneously improve in middle age (133,134). Vasopressin-containing neurons are absent from the magnocellular paraventricular neurons (135) but present in parvocellular regions (136). More than 50 mutations of the vasopressin gene (AVP) have been described that lead to the autosomal-dominant and -recessive forms of familial neurohypophyseal diabetes insipidus (details available at

Table 2 Causes of Central Diabetes Insipidus

Congenital
Septooptic dysplasia
Kabuki syndrome
Holoprosencephaly
Ectopia/hypogenesis of the pituitary
Other midline craniofacial defects
Familial
Autosomal dominant
Autosomal recessive (AR)
Wolfram syndrome (DIDMOAD) AR
Acquired
Trauma
Stalk transection
Septic shock (infarction)
Sheehan's syndrome (postpartum hemorrhage)
Hypoxic brain injury
Neoplasms
Craniopharyngioma
Germinoma
Pinealoma
Optic glioma
Pituitary adenoma
Metastatic tumors: leukemias
Infiltrative/autoimmune
Langerhans cell histiocytosis
Lymphocytic hypophysitis
Sarcoidosis
Drugs: ethanol, phenytoin, opiate antagonists, α-adrenergic agents
Infectious: (meningitis/encephalitis)
Cryptococcus neoformans
Listeria monocytogenes
Mycobacterium tuberculosis
Toxoplasmosis
Congenital cytomegalovirus infection
Aneurysm and cysts
Idiopathic
Pregnancy: increased vasopressinase

http://www.medicine.mcgill.ca/nephros) (137). Of the three families identified with the autosomal recessive form of the disease, two families had the phenotype of severe diabetes insipidus with onset before three months of age. Thus an early age of onset does not rule out the neurohypophyseal type of heritable diabetes insipidus (8).

Progressive loss of vasopressin-producing neurons in the supraoptic and paraventricular nuclei has been shown in murine knock-in models with a mutation associated with a more severe form of the disease. Retention of the precursor of vasopressin within the neuron with the induction of an endoplasmic reticulum (ER) chaperone protein (BiP) has been demonstrated in the same model (138).

Vasopressin deficiency is also found in the DIDMOAD syndrome, consisting of diabetes insipidus, diabetes mellitus, optic atrophy, and deafness (139,140). One (141), but not another (142), study has suggested that a mitochondrial defect is responsible for the disease. The gene for this syndrome complex, also known as Wolfram syndrome, was localized to human chromosome 4p16 by polymorphic linkage analysis (143) and has been identified (144).

Trauma

The axons of vasopressin-containing magnocellular neurons extend uninterrupted to the posterior pituitary over a distance of approximately 10 mm. Trauma to the base of the brain can cause swelling around or severance of these axons, resulting in either transient or permanent diabetes insipidus (145). Permanent diabetes insipidus can occur after seemingly minor trauma. Approximately one half of patients with fractures of the sella turcica will develop permanent diabetes insipidus (146), which may be delayed as long as one month following the trauma, during which time, neurons of severed axons may undergo retrograde degeneration (147). Septic shock (148) and postpartum hemorrhage associated with pituitary infarction (Sheehan syndrome) (149,150) may involve the posterior pituitary with varying degrees of diabetes insipidus. Diabetes insipidus is never associated with cranial irradiation of the hypothalamic–pituitary region, although this treatment can cause deficits in all of the hypothalamic releasing hormones carried by the portal–hypophyseal system to the anterior pituitary. Because vasopressin is carried directly to the posterior pituitary via magnocellular axonal transport, radiation may affect hypothalamic releasing hormone function by interruption of the portal–hypophyseal circulation.

Neurosurgical Intervention

One of the most common causes of central diabetes insipidus is the neurosurgical destruction of vasopressin neurons following pituitary–hypothalamic surgery. It is important to distinguish polyuria associated with the onset of acute postsurgical central diabetes insipidus from polyuria due to the normal diuresis of fluids given during surgery. In both cases, the urine may be very dilute and of high volume, exceeding 200 mL/m^2/hr. However, in the former case, serum osmolality will be high, whereas in the latter case it will be normal. A careful examination of the intraoperative record should also help distinguish between these two possibilities. Vasopressin axons traveling from the hypothalamus to the posterior pituitary terminate at various levels within the stalk and gland (Fig. 9). Because surgical interruption of these axons can result in retrograde degeneration of hypothalamic neurons, lesions closer to the hypothalamus will affect more neurons and cause greater permanent loss of hormone secretion. Of special interest is the triphasic pattern of vasopressin secretion often, but not always, seen following neursurgical

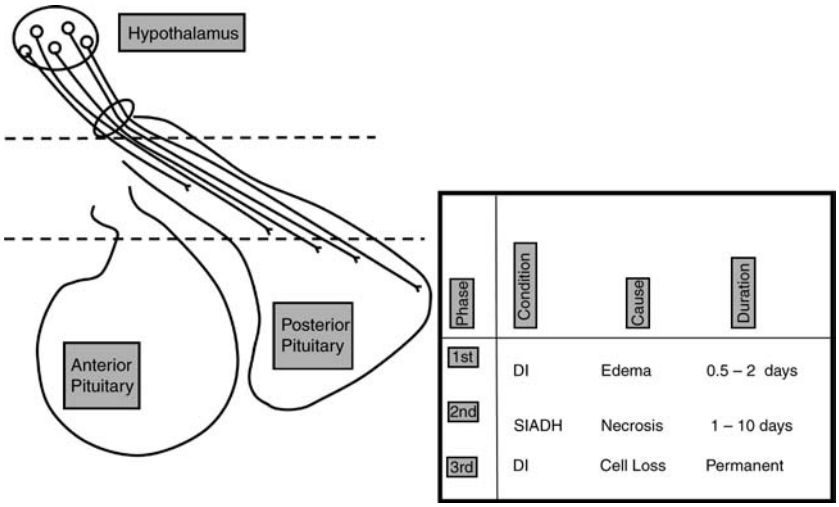

Figure 9 Diabetes insipidus following neurosurgery. Vasopressin (VP) neurons terminate at different levels of the posterior pituitary. Depending on the level of neurosurgical damage (*dashed lines*), different numbers of VP neurons will be permanently damaged. The three phases of the "triple-phase" response to neural damage, along with their causes and duration, are noted within the box. *Abbreviations*: DI, diabetes insipidus; SIADH, syndrome of inappropriate antidiuretic hormone.

procedures interfering with the supraoptic–hypophyseal tract (151). Following surgery, an initial phase of transient diabetes insipidus is observed, lasting one half to two days and possibly due to edema in the area interfering with normal vasopressin secretion (Fig. 9). If significant vasopressin cell destruction has occurred, this is often followed by a second phase of syndrome of inappropriate antidiuretic hormone secretion (SIADH) that may last up to 10 days, secondary to release of prestored vasopressin from damaged neurons. A third phase of permanent neurogenic diabetes insipidus may follow if more than 90% of vasopressin cells are destroyed. Usually, a marked degree of SIADH in the second phase portends significant permanent diabetes insipidus in the final phase of this response. In patients with coexisting vasopressin and cortisol deficits (e.g., in combined anterior and posterior hypopituitarism following neurosurgical treatment of craniopharyngioma), symptoms of diabetes insipidus may be masked because cortisol deficiency impairs renal free water clearance, as discussed subsequently. In such cases, institution of glucocorticoid therapy alone may precipitate polyuria, leading to the diagnosis of diabetes insipidus.

Congenital Anatomical Defects

Midline brain anatomic abnormalities such as septo-optic dysplasia with agenesis of the corpus callosum (152), the Kabuki syndrome (153), holoprosencephaly (154), and familial pituitary hypoplasia with absent stalk (155) may be associated with central diabetes insipidus. These patients need not have external evidence of craniofacial abnormalities (154). Central diabetes insipidus due to midline brain abnormalities is often accompanied by defects in thirst perception (152), suggesting that a common osmosensor may control both vasopressin release and thirst perception. Some patients with suspected defects in osmosensor function but with intact vasopressin neurons may have recumbent diabetes insipidus, with baroreceptor-mediated release of vasopressin while upright, and vasopressin-deficient polyuria while supine (Figs. 10 and 11) (156).

Neoplasms

Several important clinical implications follow from knowledge of the anatomy of the vasopressin system. Because hypothalamic vasopressin neurons are distributed over a large area within the hypothalamus, tumors that cause diabetes insipidus must either be very large or infiltrative or be strategically located at the point of convergence of the hypothalamo–neurohypophyseal axonal tract in the infundibulum. Germinomas and pinealomas typically arise near the base of the hypothalamus where vasopressin axons converge before their entry into the posterior pituitary and, for this reason, are among the most common primary brain tumors associated with diabetes insipidus. Germinomas causing the disease can be very small (158,159) and undetectable by MRI for several years following the onset of polyuria (160). For this reason, quantitative measurement of the b subunit of human chorionic gonadotropin, often secreted by germinomas and pinealomas, and regularly repeated MRI scans should be performed in children with idiopathic or unexplained diabetes insipidus. Empty sella syndrome, possibly due to unrecognized pituitary infarction, can be associated with diabetes insipidus in children (161). Craniopharyngiomas and optic gliomas can also cause central diabetes insipidus when very large, although this is more often a postoperative complication of the treatment for these tumors. Hematologic malignancies can cause diabetes insipidus. In some cases such as with acute myelocytic leukemia, the cause is infiltration of the pituitary stalk and sella (162–164)However, more than 30 patients with monosomy or deletion of chromosome 7 associated with acute blast transformation of myelodysplastic syndrome presented with central diabetes insipidus (165–168) without evidence of infiltration of the posterior pituitary by neoplastic cells, leaving the cause of the diabetes insipidus unresolved (Fig. 12).

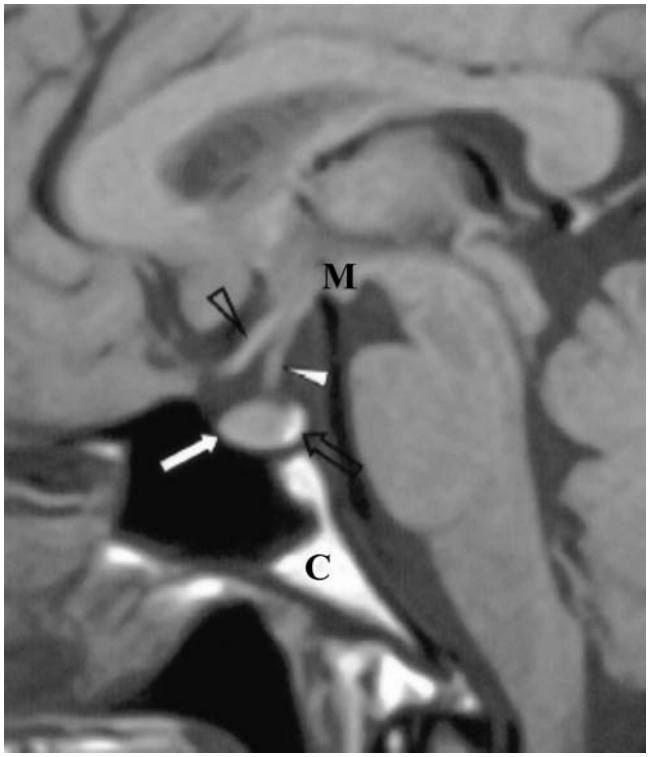

Figure 10 Magnetic resonance image (MRI) appearance of the normal pituitary gland. T1-weighted MRI shows the sagittal view of the normal anatomy of the sella turcica and juxtasellar region. The anterior lobe (*thick white arrow*), posterior lobe (*open arrow*), pituitary stalk (*thick white arrowhead*), optic chiasm (*open arrowhead*), mamillary body (M), and clivus (C) are indicated. *Source*: From Ref. 157.

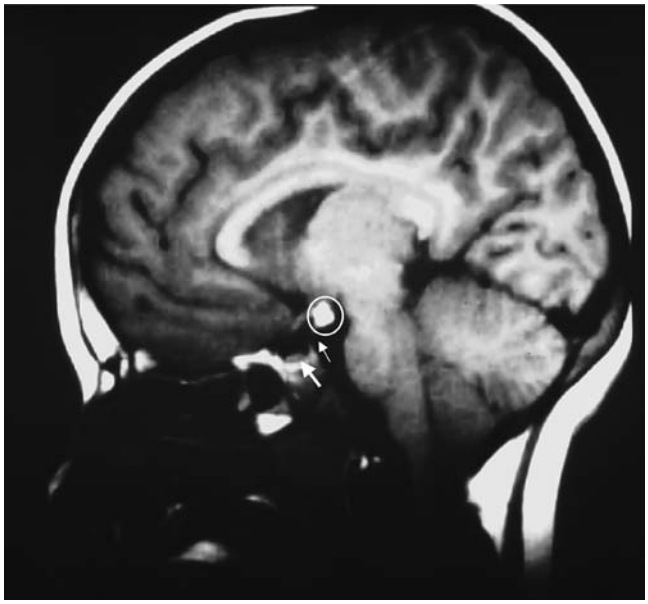

Figure 11 Ectopic posterior pituitary bright spot. T1-weighted magnetic resonance image of the sagittal section of the brain with gadolinium contrast showing an ectopic posterior pituitary bright spot (within the *white circle*) that is placed higher than the normal bright spot. Note the absence of the bright spot in the empty sella turcica (*white arrow*).

Infiltrative, Autoimmune, and Infectious Diseases

Langerhans cell histiocytosis and lymphocytic hypophysitis are the most common types of infiltrative disorders causing central diabetes insipidus. Approximately 10% of patients with histiocytosis will have diabetes insipidus. These patients tend to have more serious multisystem disease for longer periods of time than those without diabetes insipidus (169,170), and anterior pituitary deficits often accompany posterior pituitary deficiency (171). MRI characteristically shows thickening of the pituitary stalk (172). One report suggests that in patients with Langerhans cell histiocytosis, radiation treatment to the pituitary region within 14 days of onset of symptoms of diabetes insipidus may result in return of vasopressin function in more than one-third of affected patients (173).

Lymphocytic infundibuloneurohypophysitis may account for over one half of patients with ''idiopathic'' central diabetes insipidus (174). This entity may be associated with other autoimmune diseases (175). Image analysis discloses an enlarged pituitary and thickened stalk (174,176), and biopsy of the posterior pituitary reveals lymphocytic infiltration of the gland, stalk, and magnocellular hypothalamic nuclei (177). A necrotizing form of this entity has been described which also causes anterior pituitary failure and responds to steroid treatment (178). Diabetes insipidus can also be associated with pulmonary granulomatous diseases (179) including sarcoidosis (180). Whether antibody-mediated destruction of vasopressin cells occurs is controversial. Over one half of patients with central diabetes insipidus of a nontraumatic cause have antibodies directed against vasopressin-containing cells (181), and patients with other autoimmune diseases have such antibodies without evidence of diabetes insipidus (182). Many patients with central diabetes insipidus also have antivasopressin peptide antibodies, although their appearance usually follows institution of vasopressin treatment (183). It is very possible that antibodies directed against vasopressin-containing cells or vasopressin are not pathogenetic but, instead, are markers of prior neuronal cell destruction.

Infections involving the base of the brain such as meningococcal (184), cryptococcal, listeria (185), and toxoplasmosis (186) meningitis, congenital cytomegalovirus infection (187), and nonspecific inflammatory disease of the brain (188), can cause central diabetes insipidus. The disease is often transient, suggesting that it is due to inflammation rather than destruction of vasopressin-containing neurons. Central diabetes insipidus has also been described in association with congenital toxoplasmosis (189) and in a patient with an acquired cytomegalovirus infection of the hypothalamic area (190).

Brain Death

Central diabetes insipidus can appear in the setting of hypoxic brain death (191). Although its presence has been suggested as a marker for brain death in children (192), in some studies, only a minority of patients with brain death manifest the disorder (193), and up to 15% of patients with cerebral insults and diabetes insipidus ultimately recover brain function (194). Polyuria in the setting of brain death can be accompanied by high concentrations of plasma vasopressin (195), suggesting that some cases mistaken for diabetes insipidus are actually due to other causes such as cerebral salt-wasting with polyuria, as discussed subsequently.

Increased Metabolism of Vasopressin

The metabolic clearance rate of vasopressin increases fourfold during pregnancy due to the elaboration of a vasopressinase by the placenta (15). If the mother cannot respond with a concomitant increase in vasopressin action because of preexisting subclinical central or nephrogenic diabetes insipidus (16), overt, transient disease will appear, usually early in the third trimester, and resolve within one week of delivery (19,196). Even without prior defects in vasopressin function, an extreme elevation in vasopressinase concentrations in primigravidas, with either preeclampsia, liver dysfunction, or multiple gestation (17,18,20,197–199), may result in development of the syndrome.

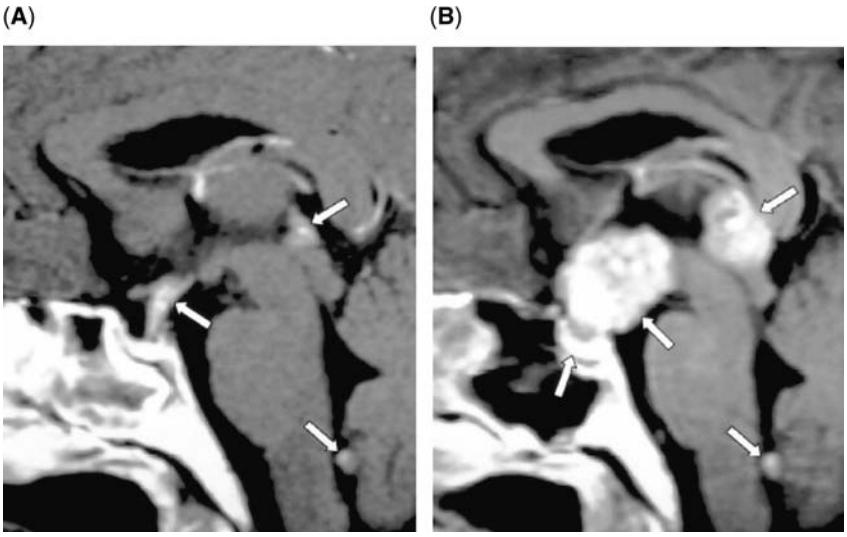

Figure 12 Magnetic resonance (MR) images of the pituitary stalk with a germinoma. (A) Initial contrast-enhanced sagittal T1-weighted MR image depicts mild thickening and homogeneous enhancement of the pituitary stalk. In addition, small nodular enhancing lesions are seen in the pineal gland and outlet of the fourth ventricle (*arrows*). (B) Contrast-enhanced sagittal T1-weighted MR image obtained one year later without treatment shows a marked increase in the size and inhomogeneous enhancement of those lesions and also of the pituitary gland (*arrows*). The focal enhancing lesion seen within the fourth ventricle appears to be cerebrospinal seeding. Germinoma was pathologically proven. *Source*: From Ref. 157.

Drugs

The most common agent associated with inhibition of vasopressin release and impaired urine-concentrating ability is ethanol (200). Because inhibition of vasopressin release by ethanol can be overcome in the setting of concurrent hypovolemia, clinically important diabetes insipidus due to ethanol ingestion is uncommon (201). Phenytoin, opiate antagonists, halothane, and α-adrenergic agents have also been associated with impaired vasopressin release (202,203).

Treatment of Central Diabetes Insipidus

Fluid Therapy

Patients with otherwise untreated diabetes insipidus crave cold fluids, especially water. With complete central diabetes insipidus, maximum urine-concentrating ability is approximately 200 mOsm/kg. Because 2.5 L of urine would be required to excrete an average daily solute load of 500 mOsm/m^2, and under normal conditions one loses 0.5 L/m^2/day via nonrenal (insensible) routes, fluid intake must be at least 3 L/m^2/day to maintain normal plasma tonicity. With an intact thirst mechanism and free access to oral fluids, a person with complete diabetes insipidus can maintain plasma osmolality and sodium in the high normal range, although at great inconvenience. Furthermore, long-standing intake of these volumes of fluid in children can lead to hydroureter (204) and even hyperfluorosis in communities that provide fluoridated water (Table 3) (205).

There are two situations in which central diabetes insipidus is sometimes best treated solely with high levels of fluid intake, without vasopressin. Vasopressin therapy coupled with excessive fluid intake (usually greater than 1 L/m^2/day as discussed subsequently) can result in unwanted hyponatremia. Because neonates and young infants receive all of their nutrition in liquid form, the obligatory high oral fluid requirements for this age (3 L/m^2/day), combined with vasopressin treatment, are likely to lead to this dangerous complication (206). Such neonates may be better managed with fluid therapy alone. Although children managed with such a regimen may be chronically thirsty, parents may have difficulty keeping up with the voluminous fluid intake and urine output, and poor growth may occur if adequate calories are not provided along with water (207), these problems are more easily addressed than is life-threatening hyponatremia. In difficult cases, thiazide and/or amiloride diuretics may be added to facilitate renal proximal tubular sodium and water reabsorption (208) and thereby decrease oral-fluid requirements. In older children, the use of the short-acting agent AVP (Pitressin) will decrease fluid needs while minimizing the possible occurrence of hyponatremia (see section Vasopressin and Vasopressin Analogs) (Table 4).

Table 3 Calculating Fluid Requirements

Obligatory renal excretion of solutes ingested and produced by the body: 500 mOsm/m^2
Minimum urine osmolality under full suppression of vasopressin production: 50 mOsm/kg H_2O
Urine osmolality in complete central diabetes insipidus: 200 mOsm/kg H_2O
Maximum urine osmolality upon stimulation by vasopressin: 1000 mOsm/kg H_2O
Insensible fluid losses under normal conditions (respiration and perspiration): 500 mL/m^2
Formula for calculating fluid requirement: [(500 mOsm/m^2 × body surface area in m^2) ÷ urine osmolality with or without vasopressin] + insensible losses
Formula for body surface area (m^2): $\sqrt{[\text{weight(kg)} \times \text{height(cm)} \div 3600]}$

Table 4 Fluid Management in Infants with Central Diabetes Insipidus

Caloric requirement: 1200–1500 kcal/m^2/day
At least 350 kcal/day (body surface area $\approx$ 0.3–0.4 m^2)
Breast milk = 75 kcal/100 mL
350 kcal/day = 470 mL/day
Normal maximum fluid intake: 4 L/m^2/day
1.2 L/day
Maximum fluid intake on desamino-D-arginine vasopressin: 1 L/m^2/day
300 mL/day = 225 kcal in milk

In the acute postoperative management of central diabetes insipidus occurring after neurosurgery in young children, vasopressin therapy may be successfully employed (209,210), but caution must be exerted with its use. While under the full antidiuretic effect of vasopressin, a patient will have a urine osmolality of approximately 1000 mOsm/kg and become hyponatremic if she/he receives an excessive amount of fluids, depending on the solute load and nonrenal water losses. With a solute excretion of 500 mOsm/m^2/day, normal renal function, and nonrenal fluid losses of 500 mL/m^2/day, fluid intake of greater than 1 L/m^2/day will result in hyponatremia. In addition, vasopressin therapy will mask the emergence of the SIADH phase of the triple-phase neurohypophyseal response to neurosurgical injury (as has been discussed). For these reasons, it may be best to manage acute postoperative diabetes insipidus in young children with fluids alone, avoiding the use of vasopressin (211). This method consists of matching input and output hourly, using between 1 to 3 L/m^2/day (40–120 mL/m^2/hr). If intravenous therapy is used, a basal 40 mL/m^2/hr should be given as 5% dextrose (D5) in one-fourth normal saline (normal saline = 0.9% sodium chloride) and the remainder, depending on the urine output, as D5 in water. Potassium chloride (40 mEq/L) may be added if oral intake is to be delayed for several days. No additional fluid should be administered for hourly urine volumes under 40 mL/m^2/hr. For hourly urine volumes above 40 mL/m^2/hr, the additional volume should be replaced with D5 up to a total maximum of 120 mL/m^2/hr. For example, in a child with a surface area of 1 m^2 (approximately 30 kg), the basal infusion rate would be 40 mL/hr of D5 in one-fourth normal saline. For an hourly urine output of 60 mL, an additional 20 mL/hr D5 would be given for a total infusion rate of 60 mL/hr. For urine outputs above 120 mL/hr, the total infusion rate would be 120 mL/hr. In the presence of diabetes insipidus, this will result in serum sodium in the 150 mEq/L range and a mildly volume-contracted state, which will allow one to assess both thirst sensation as well as the return of normal vasopressin function or the emergence of SIADH. Patients may become mildly hyperglycemic with this regimen, particularly if they are also receiving postoperative glucocorticoids. However, because it does not use vasopressin, this fluid-management protocol prevents any chance of hyponatremia.

Vasopressin and Vasopressin Analogs

Intravenous therapy with synthetic aqueous vasopressin (Pitressin) is useful in the management of central diabetes insipidus of acute onset (209,210). If continuous vasopressin is administered, fluid intake must be limited to 1 L/m^2/day (assuming normal solute intake and nonrenal water losses as described). The potency of synthetic vasopressin is still measured using a bioassay and is expressed in bioactive units, with one milliunit (mU) equivalent to approximately 2.5 ng of vasopressin. For intravenous vasopressin therapy, 1.5 mU/kg/hr results in a blood vasopressin concentration of approximately 10 pg/mL (212), twice that needed for full antidiuretic activity (213). Vasopressin's effect is maximal within two hours of the start of infusion (213), and one must beware of it sticking to intravenous bottles and tubing. Occasionally following hypothalamic (but not transsphenoidal) surgery, higher initial concentrations of vasopressin are required to treat acute diabetes insipidus, which may be attributable to the release of a substance related to vasopressin from the damaged hypothalamo–neurohypophyseal system that acts as an antagonist to normal vasopressin activity (214). Much higher rates of vasopressin infusion, resulting in plasma concentrations above 1000 pg/mL, should be avoided, as they may cause cutaneous necrosis (215), rhabdomyolysis (215,216), and cardiac rhythm disturbances (217). Patients treated with vasopressin for postneurosurgical diabetes insipidus should be switched from intravenous to oral fluid intake at the earliest opportunity because thirst sensation, if intact, will help regulate blood osmolality, as discussed. Intravenous dDAVP (Desmopressin) should not be used in the acute management of postoperative central diabetes insipidus, for it offers no advantage over vasopressin, and its long half-life (8–12 hours) compared with that of vasopressin (5–10 minutes) is a distinct disadvantage, as it may increase the chance of causing water intoxication (218). In fact, the use of intravenous dDAVP, 0.3 mg/kg, to shorten the bleeding time in a variety of bleeding disorders (as has been discussed), has been associated with water intoxication (219), particularly in young children who have high obligate oral-fluid needs (Table 5).

A special problem arises when a patient with established central diabetes insipidus must receive a high volume of fluid for therapeutic reasons, e.g., accompanying cancer chemotherapy. Such patients can either be managed by discontinuing antidiuretic therapy and increasing fluid intake to 3 to 5 L/m^2/day (rendering the patient moderately hypernatremic), or by using a low dose of intravenous vasopressin (0.1 mU/kg/hr, approximately one-eighth the full antidiuretic dose), with which the partial antidiuretic effect allows the administration of higher amounts of fluid without causing hyponatremia (220).

In the outpatient setting, treatment of central diabetes insipidus in older children should begin with

Table 5 Medications for the Management of Central Diabetes Insipidus

Desamino-D-arginine vasopressin (dDAVP)(desmopressin acetate)
Oral: 100, 200 μg tablets
Dose: 100–400 μg, every 12 hr
10–20 times the intranasal dose of dDAVP
Onset of action: ~15–30 min
Duration: 8–12 hr
May become tachyphylactic
Intranasal: rhinal tube (10 μg/0.1 mL, 0.025 mL/squirt), nasal spray (10 μg/0.1 mL, 0.1 mL/spray)
Dose: ~10–20 μg/dose, every 12 hr
Onset of action: 5–15 min
Duration: 8–12 hr
Advantage: can be used when vomiting
Disadvantages: some discomfort, variable absorption with rhinitis
Vasopressin (pitressin): 20 units/mL (0.5, 1.0, and 10 mL): conversion: 1 unit = 2.5 μg
Intramuscular/subcutaneous route
Dose 6.25–25 μg, every 6–12 hr, as needed
Onset of action: min
Duration: 2–8 hr (in oil vehicle)
Intravenous route
Dose: 0.1–1.5 milliunits/kg/hr
Onset of action: min
Half-life: 5–10 min

Table 6 Exercise in Calculating Fluid Requirements

What is the maximum amount of water a 10-yr-old with a body surface area (BSA) of 1 m^2 can drink without becoming hyponatremic?
Renal losses: 500 mOsm/m^2/day ÷ 50 mOsm/L = 10 L/m^2/day
Insensible losses = 0.5 L/m^2/day
Total maximum daily fluid intake = 10.5 L/m^2/day
Adjust for solute (salt) excretion and nonrenal losses [fever, gastrointestinal (GI) losses]
What is the minimum amount of water that a 10-yr-old with a BSA of 1 m^2 can drink in 24 hr before his/her sodium concentration begins to rise?
Minimum renal loss: 500 mOsm/m^2/day ÷ 1000 mOsm/L = 0.5 L/m^2/day
Insensible losses = 0.5 L/m^2/day
Total minimum daily fluid requirement = 1 L/m^2/day
Adjust for solute (salt) ingestion and nonrenal losses (fever, GI losses)
What are the minimum (to prevent hypernatremia) and maximum (to prevent hyponatremia) amounts of water the same child can drink if he/she is being treated with desamino-D-arginine vasopressin (or has syndrome of inappropriate antidiuretic hormone)?
Same as above = 1 L/m^2/day for both minimum and maximum (because urine osmolality fixed at 1000 mOsm/L)
Adjust for solute (salt) excretion and nonrenal losses (fever, GI losses)
If he/she has complete central diabetes insipidus, what are the maximal and minimal fluid requirements?
Maximum fluid loss = same as above (with no arginine vasopressin action)
Minimum = assume urine osmolality = 200 mOsm/kg H_2O
Renal losses: 500 mOsm/m2/d ÷ 200 mOsm/L = 2.5 L/m^2/day
Insensible losses = 0.5 L/m^2/day
Total minimum daily fluid loss = 3 L/m^2/day
Adjust for solute (salt) excretion and nonrenal losses (fever, GI losses)

oral dDAVP in doses of 25 to 300 mcg every 8 to 12 hours. Oral dDAVP tablets have come into widespread use. Although when given orally, dDAVP is at least 20-fold less potent than when given via the intranasal route, it is highly effective and safe in children (221–223). Alternatively, intranasal dDAVP (10 mcg/0.1 mL), 0.025 mL (2.5 mcg) is given by rhinal tube at bedtime and the dose increased to the lowest amount that gives an antidiuretic effect. If the dose is effective but has too short a duration, it should be increased further, or a second morning dose should be added. Patients should escape from the antidiuretic effect for at least one hour before the next dose to ensure that any excessive water will be excreted. Otherwise, water intoxication may occur. dDAVP is also available as a nasal spray in the same concentration with each spray delivering 10 mcg (0.1 mL). This is the standard preparation used for treatment of primary enuresis.

In addition to polyuria and polydipsia, decreased bone mineral density has been reported in patients with central diabetes insipidus (224). The decreased bone density was not corrected by vasopressin analog treatment alone, suggesting that institution of bisphosphonate or other therapies designed to prevent bone loss may be of long-term benefit in the treatment of diabetes insipidus (Table 6).

Children with Primary Enuresis

Although normal children have a nocturnal rise in plasma vasopressin associated with an increase in urine osmolality and a decrease in urine volume, those with primary enuresis have a blunted or absent rise in vasopressin and excrete a higher urine volume of lower tonicity (225,226). This has suggested that enuretic children have a primary deficiency in vasopressin secretion, although the same outcome could be caused solely by excessive water intake in these children. The use of the V2 agonist dDAVP is highly effective in abolishing bed-wetting episodes, although relapse is high once therapy is stopped (227–229). Fluid intake must be limited while a child is exposed to the antidiuretic action of dDAVP to guard against water intoxication.

NEPHROGENIC DIABETES INSIPIDUS

Causes of Nephrogenic Diabetes Insipidus

Nephrogenic (vasopressin-resistant) diabetes insipidus can be due to genetic or acquired causes. Genetic causes are less common but more severe than acquired forms of the disease, although genetic etiologies are more common in children than in adults (Table 7).

Genetic Causes

Congenital X-linked Nephrogenic Diabetes Insipidus: V2-receptor Mutations

The vasopressin type 2 receptor (V2R) belongs to the rhodopsin subfamily of the guanine-nucleotide (G) protein coupled receptors. The gene for the V2R is found in the chromosome region Xq28, and it contains 3 exons and 2 small introns. Congenital X-linked

Table 7 Causes of Nephrogenic Diabetes Insipidus

Congenital
X-linked: V2 receptor defect
Autosomal dominant: aquaporin-2 defect
Autosomal recessive: aquaporin-2 defect
Acquired
Metabolic
Hypercalcemia
Hypokalemia
Renal diseases
Polycystic kidney disease
Medullary cystic kidney
Sickle cell nephropathy (disease/trait)
Chronic pyelonephritis
Acute tubular necrosis
Obstructive uropathy
Primary polydipsia: washout of the gradient
Drugs
Lithium
Demeclocycline
Amphotericin B
Foscarnet
Cisplatin
Rifampin
Methicillin
Ifosfamide

nephrogenic diabetes insipidus is caused by inactivating mutations of the vasopressin V2 receptor. Due to its mode of transmission, it is a disease of males, although rarely females may be affected, presumably due to extreme Lyonization during X chromosome inactivation (230). In keeping with a germline, as opposed to somatic, mutation in the V2 receptor, these patients are deficient in all systemic V2-receptor–mediated actions (231,232) and have intact V1-receptor–mediated responses (233,234). As expected, the V2-receptor defect is proximal to the activation of renal adenylate cyclase (235,236). Unlike the function of other G-protein–coupled seven transmembrane receptors such as the parathyroid hormone and thyroid-stimulating hormone receptors, that of the V2 receptor is unaffected in patients with pseudohypoparathyroidism who have inactivating mutations in the alpha subunit of G_s (237).

Because of vasopressin resistance in congenital nephrogenic diabetes insipidus, the kidney elaborates large volumes of hypotonic urine with osmolality ranging between 50 and 100 mOsm/kg. Manifestations of the disease are usually present within the first several weeks of life (238) but may only become apparent after weaning from the breast. The predominant symptoms are polyuria and polydypsia. Thirst may be more difficult to satisfy than in central diabetes insipidus. Many infants initially present with fever, vomiting, and dehydration, often leading to an evaluation for infection. Growth failure in the untreated child may be secondary to the ingestion of large amounts of water, which the child may prefer over milk and other higher-caloric substances (239). Mental retardation of variable severity as a result of repeated episodes of dehydration was described frequently in early reports (240). However, the majority of patients reported in recent studies have a normal intelligence, very likely due to an early diagnosis and appropriate treatment (241). Intracerebral calcification of the frontal lobes and basal ganglia is not uncommon in children with X-linked nephrogenic diabetes insipidus (242–245). Because this appears early and is not seen in children with central diabetes insipidus of equivalent severity, cerebral calcification is probably unrelated to the level of dehydration or therapeutic intervention. It is possible that elevated vasopressin concentrations acting via intact V1 or V3 receptors, contribute to some of the unique manifestations of X-linked nephrogenic diabetes insipidus such as cerebral calcification, intense thirst, vomiting, and growth failure. Older children may present with enuresis or nocturia. They may learn to reduce food intake (and therefore solute load) to decrease polyuria, which may contribute to growth failure. After long-standing ingestion and excretion of large volumes of water, patients may develop nonobstructive hydronephrosis, hydroureter, megabladder, and impairment of bladder function (204,246,247). Chronic renal failure from bilateral hydronephrosis has also been noted in affected individuals (248).

Although one founder (arriving in North America from Scotland in 1761 on the ship *Hopewell*) was initially postulated to be the ancestor of most North American subjects with congenital, X-linked nephrogenic diabetes insipidus (249), as many as 183 putative disease-causing mutations in the gene for the V2 receptor have been identified in 287 families with nephrogenic diabetes insipidus, with some appearing to have arisen independently more than once (250–263). Among these, there are 89 missense, 18 nonsense, 45 frame-shift deletion or insertion, 7 in-frame deletion or insertion, 4 splice site, and 19 large deletion mutations, which are distributed fairly evenly throughout the receptor protein (8,137). Mutations may affect either vasopressin binding, cAMP generation, or possibly transcriptional regulation (264–268). Patients with different mutations will likely be found to exhibit phenotypic heterogeneity, including in severity of disease and response to treatment. Genetic heterogeneity may underlie the variable response of patients with X-linked diabetes insipidus to dDAVP treatment. In a family with a known mutation, prenatal or early postnatal DNA screening can unambiguously identify affected males, allowing the institution of appropriate therapy (269). Interestingly, different mutations affecting the amino acid arginine in position 137 of the V2R have been shown to have opposite phenotypes. This is part of a highly conserved region of the receptor protein that appears to be critical for its function. Nephrogenic DI is seen with the R137H mutation, which leads to a loss of function of the V2 receptor, while nephrogenic SIADH is seen with the R137L and R137C mutations, due to a gain of function of the V2 receptor (270).

Congenital, Autosomal Nephrogenic Diabetes Insipidus: Aquaporin-2 Mutations

After the initial description of X-linked nephrogenic diabetes insipidus (271), several patients were reported with similar clinical findings except for autosomal recessive transmission of the disease (272) or normal V2-receptor function outside of the kidney (273). The human AQP2 gene is located in the chromosome region 12q13. It contains four exons and three introns. With the cloning of the complementary DNA for the renal water channel, AQP2, many patients with autosomal recessive nephrogenic diabetes insipidus have been reported who have different mutations in this gene (268). Thirty-five putative disease-causing mutations in the AQ-2 gene have been identified to date. Of these, 25 are missense, 2 nonsense, 6 frame-shift deletion or insertion, and 2 splice site mutations (8,137). They are scattered throughout the molecule, including within four of the five transmembrane domains, two of three extracellular domains, and two of four intracellular domains. Recently, an autosomal dominant mode of inheritance for nephrogenic diabetes insipidus has been described, associated with mutations in AQP2. One of these dominant mutations results in mixed tetramers of the wild type and mutant alleles being retained in the Golgi apparatus (274). AQP2 mutations impair the ability of the luminal membrane to undergo an increase in water permeability following signaling through the V2 receptor. Such mutations may exist in patients previously described who had a normal rise in urinary cAMP in response to vasopressin without a concomitant increase in urine osmolality (235). AQP2 protein is excreted in the urine in both soluble and membrane-bound forms. AQP2 excretion is low in untreated central and nephrogenic diabetes insipidus but, following dDAVP administration, increases markedly in the former, but not in the latter, disease. For this reason, its measurement in urine has been suggested as an aid in the differential diagnosis of diabetes insipidus (54).

Acquired Causes

Acquired causes of nephrogenic diabetes insipidus are more common and less severe than genetic causes. Nephrogenic diabetes insipidus may be caused by drugs such as lithium (275–277) and demeclocycline (278,279), both of which are thought to interfere with vasopressin-stimulated cAMP generation or action. Approximately 50% of patients receiving lithium have impaired urinary concentrating ability, although only 10% to 20% of them develop symptomatic nephrogenic diabetes insipidus, which is almost always accompanied by a reduction in the glomerular filtration rate (GFR) (280,281). The risk increases with duration of therapy. Lithium impairs the ability of vasopressin to stimulate adenylate cyclase (282), resulting in a 90% fall in AQP2 messenger RNA expression in renal collecting duct (283), which may be the basis for its causing nephrogenic diabetes insipidus.

Demeclocycline treatment causes nephrogenic diabetes insipidus by inhibiting transepithelial water transport (284). For this reason, it is useful in the treatment of dilutional hyponatremia associated with inappropriate secretion of vasopressin, as will be discussed. Other causes of nephrogenic diabetes insipidus include hypercalcemia (285,286), hypokalemia, and therapy with foscarnet (used in treatment of cytomegalovirus infection in immunosuppressed patients) (287,288), clozapine (288), amphotericin (289,290), methicillin (291), and rifampin (292). Whether any of these agents cause nephrogenic diabetes insipidus by interfering with the expression or insertion into apical collecting duct membranes of AQP2 water channels is not yet known. Local intracellular increases in calcium concentration have been shown to play a key role in triggering the fusion of the aquaporin vesicles to the target membrane, and vasopressin is thought to release calcium from its intracellular stores (293). A calcium receptor has been identified on aquaporin vesicles, and an increase in the urinary calcium concentration has been shown to decrease the expression and trafficking of AQP2 (293). A systematic review of the literature (294), which examined over 2000 case reports, cohort studies, and cross-sectional studies, identified 9 definite causes of reversible nephrogenic diabetes insipidus based on Koch's postulates. They included treatment with amphotericin B, demeclocycline, large dose of dexamethasone (295), dopamine (296), hypercalcemia, ifosfamide (297), lithium, and ofloxacin (298). Ureteral obstruction (299), chronic renal failure, polycystic kidney disease, medullary cystic disease, Sjogren's syndrome (300), and sickle cell disease can also impair renal concentrating ability. Osmotic diuresis, due to glucosuria in diabetes mellitus or to sodium excretion with diuretic therapy, will interfere with renal water conservation. Primary polydypsia can result in secondary nephrogenic diabetes insipidus because the chronic excretion of dilute urine lowers the osmolality of the hypertonic renal interstitium, thus decreasing renal concentrating ability. Finally, decreased protein or sodium intake, also, can lead to diminished tonicity of the renal medullary interstitium and nephrogenic diabetes insipidus.

Treatment of Nephrogenic Diabetes Insipidus

Early diagnosis and treatment of affected infants can reduce the risk of future development of physical and mental retardation resulting from recurrent episodes of dehydration and hypernatremia (8). The treatment of acquired nephrogenic diabetes insipidus focuses on elimination, if possible, of the underlying disorder, such as offending drugs, hypercalcemia, hypokalemia, or ureteral obstruction. Congenital nephrogenic diabetes insipidus is often difficult to treat. The main goals should be to ensure the intake

of adequate calories for growth and to avoid severe dehydration. Foods with the highest ratio of caloric content to osmotic load shoud be ingested to maximize growth and minimize the urine volume required to excrete urine solute. However, even with the early institution of therapy, growth and mental retardation are not uncommon (301). While advising patients to urinate frequently and to double-void would help prevent the large dilations of the urinary tract, impairment of bladder function, and the rare possibility of chronic renal failure, annual renal and abdominal ultrasound studies would assist early identification of these complications (8).

Thiazide diuretics in combination with amiloride or indomethacin are the most useful pharmacologic agents in the treatment of nephrogenic diabetes insipidus. Thiazides promote sodium excretion by interfering with sodium reabsorption in the distal tubule of the nephron and altering inner medullary osmolality; the former promotes increased proximal tubule reabsorption of sodium, and the latter leads to increased free water reabsorption from the collecting duct (208,302). Indomethacin 2 mg/kg/day, further enhances proximal tubular sodium and water reabsorption (208,303,304), although this effect is not mediated by inhibition of cyclooxygenase (305). The combination of thiazide and amiloride diuretics is the most commonly used regimen for the treatment of congenital, X-linked nephrogenic diabetes insipidus because amiloride counteracts thiazide-induced hypokalemia (238), avoids the nephrotoxicity associated with indomethacin therapy, and is well tolerated even in infants (306). In addition, amiloride decreases the uptake of lithium by renal epithelial cells and, for this additional reason, has been proposed in combination with thiazide as treatment for lithium-induced nephrogenic diabetes insipidus (307). High-dose dDAVP therapy, in combination with indomethacin, has been reported to be helpful in treating some subjects with nephrogenic diabetes insipidus (308). This treatment may prove to be useful in patients with genetic defects in the V2 receptor that reduce the binding affinity for vasopressin. Paradoxically, V2-receptor antagonists may also be useful in this disease (discussed subsequently).

The majority of the mutations in the genes for the V2R and the AQP-2 proteins result in the formation of aberrant proteins that are misfolded during the processing within the endoplasmic reticulum (ER). This leads to retention of the protein within the ER without the normal trafficking to its target membrane due to any one of the following reasons: rapid degradation of the misfolded protein within the cell, misrouting of the protein, or destruction of the cell from toxicity of the accumulated aberrant protein. Recently there have been reports in the literature of the successful use of membrane permeable V2R antagonists as pharmacological chaperones to help the aberrant V2R proteins pass through the ER and reach their target membranes (309). Because these proteins may retain some degree of functionality despite the mutation, they have been shown to partly restore function to the cell once they reach their target membranes (8). The selective nonpeptide V2R antagonists SR121463A and VPA-985 increased cell-surface expression of eight mutant V2Rs that cause nephrogenic diabetes insipidus, by promoting their proper folding and maturation, and thus rescued their function within tissue cultures (310). Another V2R antagonist partly treated the nephrogenic diabetes insipidus in a clinical study involving five adult men who had three different mutations of the V2R gene (8). These findings have opened a new avenue for the future therapy of hereditary nephrogenic diabetes insipidus as well as other conditions arising from errors in the normal processing and trafficking of cellular proteins.

ADIPSIC HYPERNATREMIA

Introduction

Patients with adipsic hypernatremia do not feel the urge to drink fluids even in the presence of hyperosmolar dehydration due to a disruption in the regulation of thirst by the osmosensor. The condition is characterized by chronic or recurrent hypernatremic dehydration, usually accompanied by a deficient response of vasopressin to osmotic stimulation. The hypernatremia develops over time and is uncovered or accelerated by events such as gastroenteritis, fever, or excessive sweating which increase renal or nonrenal fluid losses. Clinical manifestations of dehydration such as tachycardia, decreased skin turgor, orthostatic hypotension, muscle weakness, and those of hypernatremia such as lethargy, weakness, irritability, nausea, vomiting, seizures, coma, and even death, can occur. Laboratory manifestations include hypernatremia, azotemia, hypokalemia, and alkalosis. Complications such as acute renal failure, deep vein thrombosis (311), and rhabdomyolysis (312) have been observed in this group of patients.

Pathophysiology

Hyperosmolality stimulates both thirst and vasopressin release. Lesions in the anterior hypothalamus can affect the thirst mechanism causing adipsia. Hypernatremia with adipsia can occur even in the absence of associated diabetes insipidus because the free water intake may be inadequate to match the obligatory renal and nonrenal losses. On the other hand, the lack of vasopressin, despite causing significant polyuria, rarely causes significant hypernatremia when the osmosensor-thirst mechanism is intact (313). Disordered thirst mechanism is seen in about 10% of patients with diabetes insipidus. The vasopressin response to osmotic stimulation is often partially or completely deficient in this condition. However, the vasopressin response to nonosmotic stimuli like hypotension may be intact (314). Rarely, the osmoregulation of vasopressin is intact (315–317). A recent

study showed that secondary hyperaldosteronism was not the cause of the hypokalemia and alkalosis occurring in this condition. Chronic renal losses of potassium are hypothesized to lead to hypokalemia and alkalosis (318).

Causes

Adipsic hypernatremia can potentially occur with a malformation of or any injury to the osmosensor for thirst. Malformations of the brain such as holoprosencephaly (319), hypoplasia of the corpus callosum (320), vascular lesions such as anterior communicating artery aneurysms or their ligation (321–323), and neoplasms such as craniopharyngioma (324,325), suprasellar germinoma (326), and pinealoma (327,328) have been associated with adipsia. Other associations include granulomatous diseases such as histiocytosis and sarcoidosis and miscellaneous conditions like hydrocephalus (329,330), arachnoidal cysts (331), idiopathic hypothalamic dysfunction with precocious puberty (332), AIDS, and cytomegalovirus encephalitis (333) and pseudotumor cerebri with an empty sella turcica (334).

Management

In the presence of normal osmoregulation of AVP release, patients with adipsic hypernatremia are managed with controlled intake of daily fluids along with close monitoring of body weight, urine output, clinical symptoms, and serum sodium levels. While the patients should consume a minimum amount of fluids calculated from the renal and nonrenal losses, this amount may vary with events that increase these losses. The intact vasopressin response to osmotic stimulus should accommodate for variations in the fluid balance by regulating renal free-water excretion. However, caution must be exercised in order to avoid fluid overloading even in patients without significant polyuria because some of these individuals may be unable to fully suppress AVP release when faced with lower serum osmolalities (313).

Fluid management of adipsic hypernatremia is more difficult when it is associated with diabetes insipidus. There is the additional danger of hypoosmolar overhydration with fluid intake in the presence of dDAVP. A practical method of managing these patients is to keep them antidiuresed on a fixed dose of ddAVP and then adjusting the controlled fluid intake based on changes in body weight, urine output, and the presence or absence of events associated with increased renal or nonrenal fluid losses. A target body weight with the patient in a euvolemic state is identified. The intake of fluids for a particular day, then, could be adjusted to keep the daily weight close to this target weight (314). Frequent revision of the target weight would be required for a growing child. Education of the family is vital in the management of this condition. Frequent monitoring of serum sodium levels using portable home analyzers may be helpful in the management of children with this problem (335).

DIAGNOSTIC APPROACH TO AND THE DIFFERENTIAL DIAGNOSIS OF POLYURIA

The salient features of neurogenic diabetes insipidus are polyuria and polydipsia. In children, it must first be determined whether pathological polyuria or polydypsia (exceeding $1.5\,L/m^2/day$) is present by asking the following questions: Is there a psychosocial reason for either polyuria or polydipsia? Can either be quantitated? Has either polyuria or polydypsia interfered with normal activities? Is nocturia or enuresis present? If so, does the patient also drink following nocturnal awakening? Does the history (including longitudinal growth data) or physical examination suggest other deficient or excessive endocrine secretion or an intracranial neoplasm? If pathological polyuria or polydypsia is present, the following should be obtained in the outpatient setting: serum osmolality; serum concentrations of sodium, potassium, glucose, calcium, and blood urea nitrogen (BUN); and urinalysis, including measurement of urine osmolality, specific gravity, and glucose concentration. A serum osmolality greater than 300 mOsm/kg, with urine osmolality less than 300 mOsm/kg, establishes the diagnosis of diabetes insipidus. If serum osmolality is less than 270 mOsm/kg, or urine osmolality is greater than 600 mOsm/kg, the diagnosis of diabetes insipidus is unlikely. If on initial screening, the patient has a serum osmolality less than 300 mOsm/kg, but the intake/output record at home suggests significant polyuria and polydipsia, which cannot be attributed to primary polydipsia (i.e., the serum osmolality is greater than 270 mOsm/kg), the patient should undergo a water deprivation test to establish a diagnosis of diabetes insipidus and to differentiate central from nephrogenic causes (Table 8) (Fig. 13).

After a maximally tolerated overnight fast (based on the outpatient history), the child is admitted to the outpatient testing center in the early morning of a day when an 8- to 10-hour test can be carried out and is deprived of water (336,337). The physical signs and biochemical parameters shown in the accompanying protocol are measured (Fig. 13). If at any time

Table 8 Diagnostic Approach to Polyuria/Polydipsia

True polyuria/polydipsia: $> 1.5\,L/m^2/day$
Body surface area $(m^2) = \sqrt{[\text{weight(kg)} \times \text{height(cm)} \div 3600]}$
Measure: serum osmolality, sodium, glucose, calcium, and potassium
Measure: urine osmolality, specific gravity, and glucose
If serum Osm $>$ 300 mOsm/kg, with urine Osm $<$ 300 mOsm/kg: the diagnosis is diabetes insipidus
If urine Osm $>$ 600 mOsm/kg or serum Osm $<$ 270 mOsm/kg: unlikely to be diabetes insipidus
If serum Osm 270–300 mOsm/kg and I/O record shows true polyuria/polydipsia: perform the water deprivation test

Water Deprivation Test

ENDOCRINE FUNCTION TEST MR

DIAGNOSIS: Rule out Diabetes Insipidus

TEST: Water Deprivation

Present Health _____Good _____Fair _____Poor

Diet for previous two days (attach diet history): _____Good _____Fair _____Poor

Period of Fast _____Hours Recent Medications _____

Body Weight _____Kg

No	Hour	Interval Minutes	BS mg/dL	Serum Na	Serum OSM	Serum BUN	Serum VP*	Body weight	Vital signs	Urine Na	Urine OSM	Urine S.G.	Urine vol/hr
		-30	Place IV hep lock										
		0		X	X	X	X	X	X	X	X	X	X
		60		X	X			X	X	X	X	X	X
		120		X	X			X	X	X	X	X	X
		180		X	X			X	X	X	X	X	X
		240		X	X		X	X	X	X	X	X	X
		300		X	X			X	X	X	X	X	X
		360		X	X			X	X	X	X	X	X
		420		X	X			X	X	X	X	X	X
		480		X	X		X	X	X	X	X	X	X

*If patient has DI, last VP sample at last time point before VP admininstration (see below)

AT ANY TIME DURING TEST:

If serum osm <300 (Na<145), urine osm <600, continue test unless vital signs disclose hypovolemia

If urine osm >1000, or >600 and stable (<30 mosm change for 2 time points), stop test = NORMAL

If serum osm >300 and urine osm<600=DIABETES INSIPIDUS. Give Pitressin, 1U/m^2 SQ and measure:

TIME AFTER PITRESSIN ADMINISTRATION:

		0									X	X	X
		30									X	X	X
		60									X	X	X

COMMENTS: *VP = vasopressin (ADH)

Figure 13 Protocol for evaluation of diabetes insipidus using water deprivation. *Abbreviations*: IV, intravenous; OSM, osmolality; S.G., urinary specific gravity; SQ, subcutaneous.

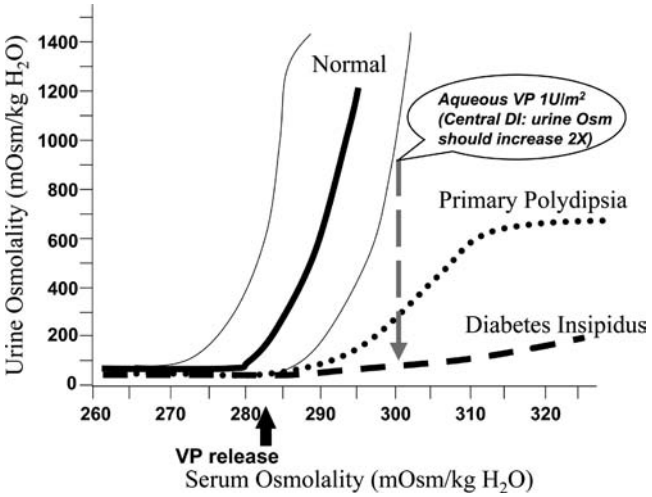

Figure 14 Relationship between serum and urine osmolalities during a water deprivation test. With water deprivation, serum osmolality rises above the threshold for VP secretion, causing antidiuresis and concentration of the urine. In normal persons, when serum osmolality exceeds 300 mOsm/kg H_2O, urine osmolality exceeds 600 mOsm/kg H_2O (*solid thick* and *thin lines*, mean and 95% confidence intervals for normal children). In patients with diabetes insipidus (*dashed line*), urine osmolality does not exceed 200 to 300 mOsm/kg H_2O, even with marked serum hyperosmolality. In patients with primary polydipsia (*dotted line*), urine osmolality may not exceed 600 mOsm/kg H_2O, due to partial nephrogenic diabetes insipidus caused by dilution of the osmotic gradient in the renal interstitium (see text). Solid arrowhead denotes the threshold for VP release. Once diagnosis of diabetes insipidus is made, administration of aqueous VP, 1 U/m^2, results in a rise in urine osmolality in patients with central, but not nephrogenic, diabetes insipidus. *Abbreviations*: DI, diabetes insipidus; VP, vasopressin.

during the test, the urine osmolality exceeds 1000 mOsm/kg or 600 mOsm/kg and is stable over one hour, the patient does not have diabetes insipidus (Fig. 14).

If at any time, the serum osmolality exceeds 300 mOsm/kg and the urine osmolality is less than 600 mOsm/kg, the patient has diabetes insipidus. If the serum osmolality is less than 300 mOsm/kg and the urine osmolality is less than 600 mOsm/kg, the test should be continued unless vital signs disclose hypovolemia. A common error is to stop a test too soon, based on the amount of body weight lost, before either urine osmolality has plateaued above 600 mOsm/kg or a serum osmolality above 300 mOsm/kg has been achieved. Unless the serum osmolality rises above the threshold for vasopressin release, a lack of vasopressin action (as inferred by a nonconcentrated urine) cannot be deemed pathological. If the diagnosis of diabetes insipidus is made, aqueous vasopressin, (Pitressin; 1 U/m^2), should be given subcutaneously. If the patient has central diabetes insipidus, urine volume should fall and osmolality should at least double during the next hour, compared with the value prior to vasopressin therapy. If there is less than a two-fold rise in urine osmolality following vasopressin administration, the patient probably has nephrogenic diabetes insipidus. dDAVP should not be used for this test, as it has been associated with water intoxication in small children in this setting (218). Patients with longstanding primary polydypsia may have mild nephrogenic diabetes insipidus because of dilution of their renal medullary interstitium. This should not be confused with primary nephrogenic diabetes insipidus, because patients with primary polydypsia should have a tendency toward hyponatremia, rather than hypernatremia, in the basal state. Patients with a family history of X-linked nephrogenic diabetes insipidus can be evaluated for the disorder in the prenatal or perinatal period by DNA sequence analysis, as will be discussed, thus allowing therapy to be initiated without delay (269).

The water deprivation test should be sufficient in most patients to establish the diagnosis of diabetes insipidus and to differentiate central from nephrogenic causes. Plasma vasopressin concentration may be obtained during the procedure, although it is rarely needed for diagnostic purposes in children (338). It is particularly helpful in differentiating between partial, central, and nephrogenic diabetes insipidus, in that plasma vasopressin is low in the former and high in the latter situation (339). If urine osmolality concentrates normally, but only after serum osmolality is well above 300 mOsm/kg, the patient may have an altered threshold for vasopressin release, also termed a reset osmostat. This may occur following head trauma, neurosurgery, or with brain tumors (340). MRI is not very helpful in distinguishing central from nephrogenic diabetes insipidus (341). Normally, the posterior pituitary is seen as an area of enhanced brightness (Fig. 10) in T1-weighted images following administration of gadolinium (342). The posterior pituitary ''bright spot'' is diminished or absent in both forms of diabetes insipidus, presumably because of decreased vasopressin synthesis in central, and increased vasopressin release in nephrogenic disease (342–344). In primary polydypsia, the bright spot is normal, probably because vasopressin accumulates in the posterior pituitary during chronic water ingestion (342), whereas it is decreased in SIADH, presumably because of increased vasopressin secretion (341). Recently, dynamic, fast-frame, magnetic resonance image analysis has allowed estimation of blood flow to the posterior pituitary (345). With this technique, both central and nephrogenic diabetes insipidus are associated with delayed enhancement in the area of the neurohypophysis (346).

In the inpatient, postneurosurgical setting, central diabetes insipidus is likely if hyperosmolality (serum osmolality $>$ 300 mOsm/kg) is associated with urine osmolality less than serum osmolality. One must beware of intraoperative fluid expansion with subsequent hypoosmolar polyuria masquerading as diabetes insipidus.

HYPERNATREMIA

Other Causes of Hypernatremia

Hypernatremia can occur from either excessive intake of sodium or from excessive loss of free water relative to sodium. Excessive intake of sodium causes hypernatremia transiently. In the presence of intact thirst mechanism, the ability to drink water, and intact renal function, it is followed by water intake leading to volume expansion and natriuresis, thus normalizing the serum sodium levels. Similarly, excessive loss of free water is usually compensated by increased intake driven by the intact thirst mechanism. Inability to drink water freely as seen in infants and neurologically impaired children can thus lead to hypernatremia from either excessive ingestion of sodium or from excessive free water loss. Infants in particular also have a limited ability to excrete the osmotic solute load from their kidneys and hence can have persistent hypernatremia from accidental or deliberate inclusion of excessive amounts of salt in their food.

An algorithm for hypernatremia in most children with normal thirst mechanism and a normal ability to drink water is thus limited to the identification of a source for the free water loss. The presence of polyuria with dilute urine confirms renal loss of free water. The diagnostic approach for polyuria has been discussed previously in this chapter. Free water may be lost relatively in excess to sodium loss through nonrenal routes such as the skin as in burns and excessive sweating, the respiratory tract, or the gastrointestinal tract as occurs with diarrhea or through fistulae.

Management of Hypernatremia

The management of hypernatremia should involve identification of the underlying cause and the duration of the hypernatremia. Treatment is directed at the underlying cause where one can be identified. An estimation of the free water deficit is made using the formula:

$$\text{Free } H_2O \text{ deficit(L)} = [(\text{Serum sodium level} - 140)/140] \times \text{Weight(kg)} \times 0.6$$

This free water deficit is best corrected with oral or nasogastric free water over a period of 24 to 48 hours. Ongoing losses should be replaced simultaneously while closely monitoring the clinical status and the serum electrolytes. In children who are unable to receive free water through the enteral route, a parenteral solution containing either D5, or 0.45% to 0.225% saline can be used.

The brain cells adapt to hypernatremia over a period of days by increasing the intracellular osmolyte concentration in order to prevent intracellular dehydration and cell shrinkage. They do so by increasing the levels of electrolytes such as sodium, potassium, amino acids like taurine and glutamine, and other osmotically active substances such as myoinositol, and betaine (347). While this process is protective during chronic hypernatremia, rapid lowering of the serum sodium can cause swelling of the brain cells. The brain, being enclosed within the cranial vault that is nonexpandable after the closure of the fontanelles, can herniate infratentorially. Seizures, coma, and even death can occur with rapid lowering of chronically elevated serum sodium levels. Hence, it is widely accepted that the serum sodium level should be corrected at least over a period of 48 hours.

HYPONATREMIA

The evaluation of hyponatremia requires the exclusion of states of hyperproteinemia, hyperlipidemia, and hyperglycemia that may falsely lower the measurement of serum sodium by flame-emission spectrophotometry. More modern analytical methods measure actual sodium concentration rather than content and are thus immune to artifactual hyponatremia.

Hyponatremia (serum sodium < 130 mEq/L) in children is usually associated with severe systemic disorders. It is most often due to intravascular volume depletion or excessive salt loss, as will be discussed, and is also encountered with hypotonic fluid overload, especially in infants. Inappropriate excessive vasopressin is one of the least common causes of hyponatremia in children, except following vasopressin administration for treatment of diabetes insipidus.

In evaluating the cause of hyponatremia, one should first determine whether the patient is dehydrated and hypovolemic. This is usually evident from the physical examination (decreased weight, skin turgor, central venous pressure) and laboratory data (high BUN, renin, aldosterone, uric acid). With a decrease in the glomerular filtration rate, proximal tubular reabsorption of sodium and water will be high, leading to a urinary sodium less than 10 mEq/L. Patients with decreased ''effective'' intravascular volume due to congestive heart failure, cirrhosis, nephrotic syndrome, or lung disease will present with similar laboratory data but will also have obvious signs of their underlying disease, which often includes peripheral edema. Patients with primary salt loss will also appear volume depleted. If the salt loss is from the kidney (e.g., diuretic therapy or polycystic kidney disease), then the urine sodium will be elevated, as may be the urine volume. Salt loss from other regions (e.g., the gut in gastroenteritis or the skin in cystic fibrosis) will cause urine sodium to be low, as in other forms of systemic dehydration. Cerebral salt-wasting is encountered with central nervous system insults and results in high serum ANP concentrations, leading to high urine sodium and urine excretion.

SIADH exists when a primary elevation in vasopressin secretion is the cause of hyponatremia. It is

characterized by hyponatremia, an inappropriately increased urinary concentration of sodium (>100 mOsm/kg), normal or slightly elevated plasma volume, and normal-to-high urine sodium (because of volume-induced suppression of aldosterone and elevation of ANP). Serum uric acid is low in patients with SIADH, whereas it is high in those with hyponatremia due to systemic dehydration or other causes of decreased intravascular volume (348). Measurement of plasma vasopressin is not very useful because it is elevated in all causes of hyponatremia except for primary hypersecretion of ANP (349). Because cortisol and thyroid deficiency cause hyponatremia by several mechanisms discussed subsequently, they should be considered in all hyponatremic patients. Drug-induced hyponatremia should be considered in patients on potentially offending medications, as discussed below. In children with SIADH who do not have an obvious cause, a careful search for a tumor (thymoma, glioma, bronchial carcinoid) causing the disease should be considered. Clinically, patients present with nonspecific symptoms of hyponatremia: anorexia, lethargy, weakness, and in severe cases, obtundity and convulsions. Signs of diminished intravascular volume, edema, hypothyroidism, adrenal insufficiency, and renal disease are absent by definition.

Hyponatremia with Normal Regulation of Vasopressin

Hyponatremia with Appropriately Decreased Secretion of Vasopressin

Increased Water Ingestion (Primary Polydipsia)

Introduction. In primary polydipsia, it is the excessive intake of water that drives the polyuria. In a hypoosmolar state with vasopressin secretion normally suppressed, the kidney can excrete urine with an osmolality as low as 50 mOsm/kg. Under these conditions, a daily solute load of 500 mOsm/m^2 could be excreted in 10 L/m^2 of urine per day. Neonates cannot dilute their urine to this degree and are prone to develop water intoxication at levels of water ingestion above 4 L/m^2/day (approximately 60 mL/hr in a newborn). This may happen when concentrated infant formula is diluted with excess water, either by accident or in a misguided attempt to make it last longer (350). A primary increase in thirst without apparent cause, leading to hyponatremia has been reported in infants as young as five weeks of age (207). In older children, with a normal kidney and the ability to suppress vasopressin secretion, hyponatremia does not occur unless water intake exceeds 10 L/m^2/day, a feat that is almost impossible to accomplish. Longstanding ingestion of large volumes of water will decrease the hypertonicity within the renal medullary interstitium, which will impair water reabsorption and guard against water intoxication (351). However, hyponatremia will occur at lower rates of water ingestion when renal water clearance is impaired, either because of inappropriately elevated vasopressin secretion (as has been discussed) or for other reasons (as will be discussed).

Despite the presence of polyuria and polydipsia, this entity should not be confused with diabetes insipidus. Differentiating primary polydipsia from diabetes insipidus is important because treating primary polydipsia with dDAVP will abolish the protective diuresis, leading to potentially fatal fluid overload and profound hyponatremia. In primary polydipsia, although the urinary osmolality may be low, the serum osmolality and serum sodium levels are not elevated. Relative excess of free water being the underlying problem, the body almost never overcorrects it to cause hypernatremia as seen in diabetes insipidus. Primary polydipsia may be classified as thirst-driven (dipsogenic) or non–thirst-driven as seen in the setting of psychiatric illnesses (psychogenic).

Psychogenic Polydipsia. This condition is associated with schizophrenia and other psychiatric disorders. It may be a form of compulsive behavior, a means of stress reduction, or secondary to the ''positive'' symptoms of schizophrenia. ''Inner-cleansing'' appears to be a common reason for the polydipsia in patients with disordered thought (352). The prevalence of polydipsia in psychiatric inpatients has been reported to be between 6% and 17% (353). Treatment options include controlled fluid intake, behavioral strategies, and pharmacotherapy. Patients do not complain of thirst when their fluid intake is restricted to the normal amount. Case reports have described therapy with medications such as clonidine and enalapril (354), clozapine (355), propranolol (356), naloxone (357), lithium (358), demeclocycline (359), risperidone, and olanzapine (360). A Cochrane database review (361) of randomized controlled trials in the literature which studied the pharmacotherapy of psychosis associated polydipsia concluded that there was a lack of proper evidence to support the use of any of these medications. Therapy with ddAVP is not advised because the patient can have profound hyponatremia with impairment of the renal clearance of free water in the presence of unregulated fluid intake.

Dipsogenic Polydipsia. Unlike in psychogenic polydipsia, the excessive water intake is driven by an altered thirst mechanism. Although this is seen in association with hypothalamic disease, quite often it is idiopathic. Normally, the threshold for the osmotic stimulation of thirst is approximately 10 mOsm/kg higher than for osmotic stimulation of AVP secretion. An individual's set plasma osmolality lies between these two thresholds (313). The rare patient in whom the osmotic thresholds for thirst and vasopressin release are reversed, illustrates the importance of the normal relationship between these two responses to osmotic stimulation (362). If thirst is activated below the

threshold for vasopressin release, water intake and resulting hypoosmolality will occur, suppressing vasopressin secretion, thus leading to persistent polydipsia and polyuria. Such individuals will drink plenty of fluids even as they are unable to concentrate their urine appropriately. However, when their plasma osmolality is allowed to rise to the threshold for vasopressin release by means of water deprivation, they are able to secrete vasopressin appropriately. The renal response to the vasopressin, however, may be blunted by the loss of the intrarenal concentration gradient due to the chronic polyuria. As long as daily fluid intake is less than $10\,L/m^2$, hyponatremia will not occur. Treatment of such a patient with dDAVP may lower serum osmolality below the threshold for thirst, suppressing water ingestion and the consequent polyuria.

Decreased Renal Free Water Clearance

Adrenal insufficiency, either primary or secondary in nature, has long been known to result in compromised free water excretion (70,108). The mechanisms by which glucocorticoids and mineralocorticoids modulate water diuresis have been the subject of substantial investigation. Some studies have demonstrated increased plasma vasopressin activity in the context of glucocorticoid insufficiency (363,364), consistent with more recent molecular biological evidence that glucocorticoids inhibit transcription of the vasopressin gene (365). However, other investigators have failed to detect vasopressin in plasma of patients with adrenal insufficiency and abnormal water clearance (366). Consistent with vasopressin-independent actions of adrenal steroids on water metabolism, Brattleboro rats with hypothalamic diabetes insipidus manifest impaired excretion of a water load after adrenalectomy (108). In adrenalectomized Brattleboro rats, glucocorticoid administration restored urine flow rate but did not restore maximal urinary diluting capacity. Conversely, mineralocorticoid administration restored maximal urinary diluting capacity but not flow rate. Thus, both mineralocorticoids and glucocorticoids are required for normal free water clearance. In part, these vasopressin-independent actions of mineralocorticoids and glucocorticoids have been attributed to the increased glomerular filtration rate arising from reexpansion of extracellular fluid volume (reduced due to salt-wasting) and improved cardiovascular tone, respectively (69,367,368). By restoring the glomerular filtration rate, more free water is delivered to the distal tubule for excretion. Additionally, volume repletion reduces the nonosmotic stimuli for vasopressin release of volume depletion and hypotension. Glucocorticoid deficiency causes upregulation of AQP2 expression in rodent kidney (369). Recently, nitric oxide has been found to stimulate cyclic guanosine monophosphate–dependent membrane insertion of AQP2 into renal epithelial cells (370). As glucocorticoid has been shown to inhibit endothelial nitric oxide (371), it is possible that under conditions of glucocorticoid deficiency, high levels of nitric oxide synthase result in elevated levels of endothelial nitric oxide in the renal vasculature, which in the distal renal tubule stimulate increased, vasopressin-independent, AQP2 activity and decreased free water clearance (370).

Thyroid hormone is also required for normal free water clearance, and its deficiency likewise results in decreased renal water clearance and hyponatremia. While some studies suggest that vasopressin mediates the hyponatremia of hypothyroidism because ethanol increases free water excretion in hypothyroid patients, this effect has not been found in other reports (372). Additionally, in severe hypothyroidism, hypovolemia is not present, and hyponatremia is accompanied by appropriate suppression of vasopressin (373). Similar to the consequences of isolated glucocorticoid deficiency described above, hypothyroidism impairs free water clearance more than maximal urine-diluting capacity (374). This decrease in free water clearance may result from diminished glomerular filtration rate and delivery of free water to the diluting segment of distal nephron as suggested by both animal (375) and human studies (376).

Given the often-subtle clinical findings associated with adrenal and thyroid deficiency, all patients with hyponatremia should be suspected of these disease states and have appropriate diagnostic tests performed, if indicated. Moreover, patients with coexisting adrenal failure and diabetes insipidus may have no symptoms of the latter until glucocorticoid therapy unmasks the need for vasopressin replacement (377,378). Similarly, resolution of diabetes insipidus in chronically polyuric and polydipsic patients may suggest inadequate glucocorticoid supplementation or noncompliance with glucocorticoid replacement.

Some drugs may cause hyponatremia by inhibiting renal water excretion without stimulating secretion of vasopressin, an action that could be called ''nephrogenic'' *SIADH*. In addition to augmenting vasopressin release, both carbamazepine (379,380) and chloropropamide (381,382) increase the cellular response to vasopressin. Acetaminophen also increases the response of the kidney to vasopressin (381); however, this has not been found to cause hyponatremia. High-dose cyclophosphamide treatment (15 to 20 mg/kg intravenous bolus) is often associated with hyponatremia, particularly when it is followed by a forced water diuresis to prevent hemorrhagic cystitis (383–385). Plasma vasopressin concentrations are normal, suggesting a direct effect of the drug to increase water resorption (386). Similarly, vinblastine, independent of augmentation of plasma vasopressin concentration or vasopressin action (387), and cisplatinum (388,389) cause hyponatremia. These drugs may damage the collecting duct tubular cells, which are normally highly impermeable to water, or may enhance AQP2 water channel activity and thereby increase water reabsorption down its osmotic gradient into the hypertonic renal interstitium.

Treatment

Hyponatremia because of cortisol or thyroid hormone deficiency reverses promptly following institution of hormone replacement. Because hyponatremia is often chronic, too rapid a rise in serum sodium should be avoided if possible, as has been discussed. When drugs that impair free water excretion must be used, water intake should be limited as if the patient has SIADH, to $1\,L/m^2/24\,hr.$, using the regimen discussed.

Hyponatremia with Appropriately Increased Secretion of Vasopressin

Increased vasopressin secretion causing hyponatremia may either be an appropriate response or an inappropriate response to a pathological state. Inappropriate secretion of vasopressin, also called SIADH, is the much less common of the two entities (390,391). Whatever the cause, hyponatremia is a worrisome sign often associated with increased morbidity and mortality (392).

Causes of Hyponatremia with Appropriately Increased Secretion of Vasopressin

Systemic Dehydration. As discussed above, systemic dehydration (water in excess of salt depletion) initially results in hypernatremia, hyperosmolality, and activation of vasopressin secretion. In addition, the associated fall in the renal glomerular filtration rate results in an increase in proximal tubular sodium and water reabsorption, with a concomitant decrease in distal tubular water excretion. This limits the ability to form dilute urine, and along with the associated stimulation of the renin–angiotensin–aldosterone system and suppression of ANP secretion, results in the excretion of urine that is very low in sodium. As dehydration progresses, hypovolemia and/or hypotension become major stimuli for vasopressin release, much more potent than hyperosmolality (as has been discussed). This effect, by attempting to preserve volume, decreases free water clearance further and may lead to water retention and hyponatremia, especially if water replacement in excess of salt is given. In many cases, hyponatremia because of intravascular volume depletion is evident from physical and laboratory signs such as decreased skin turgor, low central venous pressure, hemoconcentration, and elevated BUN. However, the diagnosis may be subtle. For example, patients with meningitis may present with hyponatremia for which water restriction has been advocated in the belief that it is due to SIADH. However, several studies have found that volume depletion, rather than SIADH, is often the cause of the hyponatremia (393,394), and that it resolves more readily when supplemental, rather than restricted, fluid and solute are administered (395). In patients with hyponatremia following head trauma, volume depletion rather than SIADH is the cause in approximately one half (396). The same has been found in the treatment of pediatric gastroenteritis, where hyponatremia is due to salt loss and secondary elevation of vasopressin (397), and isotonic fluid is better than hypotonic fluid treatment (398).

Primary Loss of Sodium Chloride. Salt can be lost from the kidney in patients with congenital polycystic kidney disease, acute interstitial nephritis, and chronic renal failure. Mineralocorticoid deficiency, pseudohypoaldosteronism (sometimes seen in children with urinary tract obstruction or infection), diuretic use, and gastrointestinal disease (usually gastroenteritis with diarrhea and/or vomiting) can also result in excess loss of sodium chloride. Hyponatremia can also result from salt loss in sweat in cystic fibrosis, although obstructive lung disease with elevation of plasma vasopressin probably plays a more prominent role, as has been discussed. With the onset of salt loss, any tendency toward hyponatremia will initially be countered by suppression of vasopressin and increased water excretion. However, with continuing salt loss, hypovolemia and/or hypotension ensues, causing nonosmotic stimulation of vasopressin. This, plus increased thirst, which leads to ingestion of hypotonic fluids with low solute content, result in hyponatremia. Weight loss is usually evident, as is the source of sodium wasting. If it is the kidney, it is accompanied by a rate of urine output and a urine sodium content greater than those associated with most other causes of hyponatremia except a primary increase in ANP secretion.

Decreased Effective Plasma Volume. Congestive heart failure, cirrhosis, nephrotic syndrome, positive pressure mechanical ventilation (399), severe burns (400), and lung disease [bronchopulmonary dysplasia (401–403) (in neonates), cystic fibrosis with obstruction (404,405), and severe asthma (406,407)] are all characterized by a decrease in ''effective'' intravascular volume (372,408). This occurs either because of impaired cardiac output, an inability to keep fluid within the vascular space, or impaired blood flow into the heart, respectively. As with systemic dehydration, in an attempt to preserve intravascular volume, water and salt excretion by the kidney are reduced, and decreased barosensor stimulation results in a compensatory, appropriate increase in vasopressin secretion, leading to an antidiuretic state and hyponatremia (409). Because of the associated stimulation of the renin–angiotensin–aldosterone system, these patients also have an increase in the total body content of sodium chloride and may have peripheral edema, which distinguishes them from those with systemic dehydration. In patients with impaired cardiac output and elevated atrial volume (e.g., congestive heart failure or lung disease), ANP concentrations are elevated, which contributes to hyponatremia by promoting natriuresis (as will be discussed).

Treatment

Patients with systemic dehydration and hypovolemia should be rehydrated with salt-containing fluids such as normal saline or lactated Ringer's solution. Because of activation of the renin–angiotensin–aldosterone system, the administered sodium will be avidly conserved, and a water diuresis will quickly ensue as volume is restored and vasopressin concentrations fall (410). Under these conditions, caution must be taken to prevent too rapid a correction of hyponatremia that may itself result in brain damage, as will be discussed.

Hyponatremia due to a decrease in effective plasma volume caused by cardiac, hepatic, renal, or pulmonary dysfunction is more difficult to reverse. The most effective therapy is the least easily achieved: treatment of the underlying systemic disorder. Patients weaned from positive pressure ventilation undergo a prompt water diuresis and resolution of hyponatremia, as cardiac output is restored, and vasopressin concentrations fall. The only other effective route is to limit water intake to that required for the renal excretion of the obligate daily solute load of approximately 500 mOsm/m^2 and to replenish insensible losses. In a partial antidiuretic state with a urine osmolality of 750 mOsm/kg and insensible losses of 500 mL/m^2, oral intake would have to be limited to approximately 1200 mL/m^2/day. Because of concomitant hyperaldosteronism, the dietary restriction of sodium chloride needed to control peripheral edema in patients with heart failure may reduce the daily solute load and further limit the amount of water that can be ingested without exacerbating hyponatremia. However, hyponatremia in these settings is often slow to develop, rarely causes symptoms, and usually does not need treatment. If the serum sodium falls below 125 mEq/L, water restriction to 1 L/m^2/day is usually effective in preventing a further decline. Because water retention in these disorders is a compensatory response to decreased intravascular volume, an attempt to reverse it with drugs such as demeclocycline or specific V2-receptor antagonists (which induce nephrogenic diabetes insipidus, as discussed above) could result in worsening hypovolemia with potentially dire consequences (411).

In general, patients with hyponatremia due to salt loss (including those with cerebral salt-wasting syndrome) require ongoing supplementation with sodium chloride and fluids. Initially, intravenous replacement of urine volume with fluid containing sodium chloride150 to 450 mEq/L depending on the degree of salt loss may be necessary; oral salt supplementation may be required subsequently (349). This treatment contrasts with that of SIADH where water restriction without sodium supplementation is the mainstay.

Precautions in the Emergency Treatment of Hyponatremia

Severe hyponatremia requires rapid treatment but not rapid correction. Most children with hyponatremia develop the disorder gradually, are asymptomatic, and should be treated with water restriction alone. However, the development of acute hyponatremia, or a serum sodium concentration below 120 mEq/L, may be associated with lethargy, psychosis, coma, or generalized seizures, especially in younger children. Acute hyponatremia causes cell swelling due to the entry of water into cells (Fig. 15), which can lead to neuronal dysfunction from alterations in the ionic

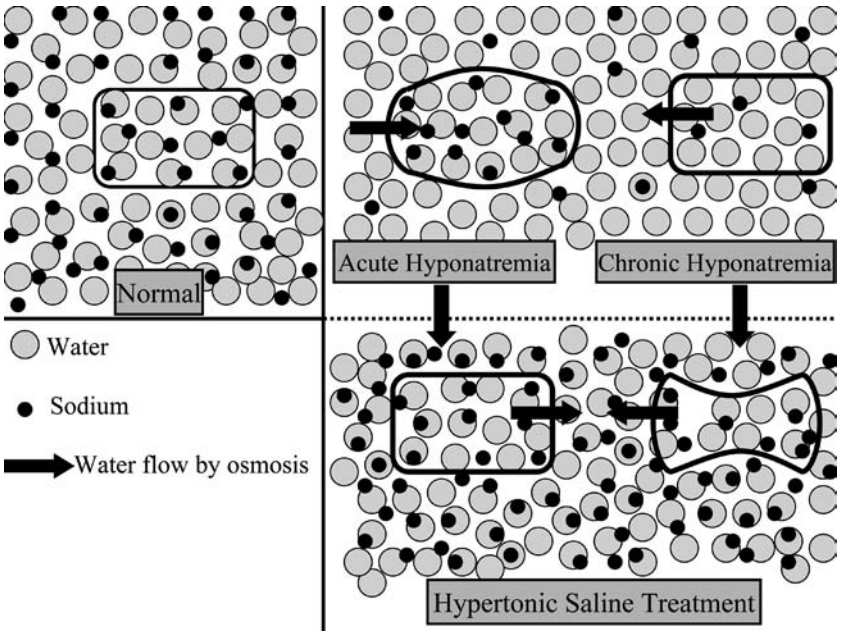

Figure 15 Changes in organic osmolytes with hyponatremia and following its correction. Under normal conditions, osmotic balance exists between extracellular and intracellular compartments. With acute hyponatremia, water enters cells, causing cell swelling. After approximately 24 hours of continued hyponatremia, intracellular organic osmolytes decrease, restoring cell volume toward normal. Hypertonic saline treatment of acute hyponatremia results in restoration of normal cell volume, whereas the same treatment of chronic hyponatremia results in cell shrinkage. *Note*: *Large circles*, water; *closed smaller circles*, solute; *arrow*, direction of water flow.

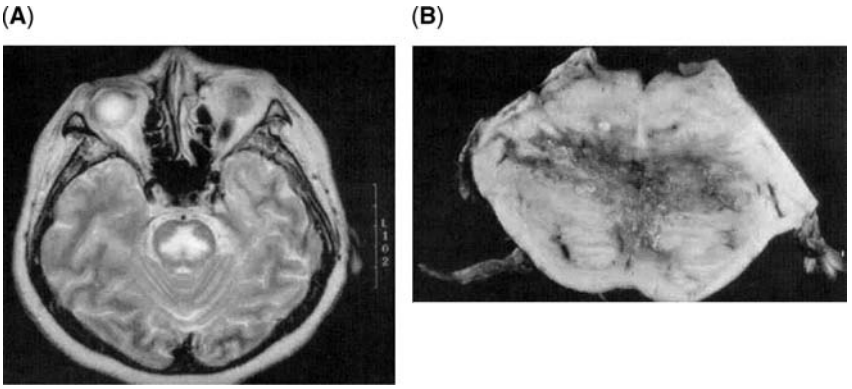

Figure 16 Magnetic resonance imaging and autopsy images of central pontine myelinolysis. (A) T2-weighted magnetic resonance imaging scan showing a symmetrical area of increased signal in the center of the pons, consistent with central pontine myelinolysis. (B) Section of pons with central pontine myelinolysis. *Source*: From Ref. 416.

environment or to cerebral herniation because of the encasement of the brain in the cranium. If present for more than 24 hours, cell swelling triggers a compensatory decrease in intracellular organic osmolytes, resulting in the partial restoration of normal cell volume in chronic hyponatremia (412). The proper emergency treatment of cerebral dysfunction depends on whether the hyponatremia is acute or chronic (1,413). In all cases, water restriction should be instituted. If hyponatremia is acute, and therefore probably not associated with a decrease in intracellular organic osmolyte concentration, rapid correction with hypertonic 3% sodium chloride administered intravenously may be indicated. As a general guide, this solution given in the amount of 12 mL/kg body weight will result in a rise in serum sodium of approximately 10 mEq/L. If hyponatremia is chronic, hypertonic saline treatment must be undertaken with caution because it may result in both cell shrinkage (Fig. 14) and the associated syndrome of central pontine myelinolysis (414). As previously stated in this chapter, brain cells extrude electrolytes and osmotically active organic compounds to equilibrate the intracellular osmolality with that of the extracellular environment in a bid to avoid a change in the cell volume (347). Rapid elevation of the serum osmolality can thus lead to osmotic flow of water from the relatively hypoosmolar brain cell to the extracellular compartment causing cell shrinkage. Central pontine myelinolysis affects the central portion of the basal pons as well as other brain regions (Fig. 16) and is characterized by axonal demyelination with sparing of neurons. It becomes evident within 24 to 48 hours following a too-rapid correction of hyponatremia, has a characteristic appearance by computed tomography and MRI, and often causes irreversible brain damage (414,416,417). If hypertonic saline treatment is undertaken, the serum sodium should be raised only high enough to cause an improvement in mental status, and in no case faster than 0.5 mEq/L/hr or 12 mEq/L/day (413,414,416,417). In the case of systemic dehydration, the rise in serum sodium may occur very rapidly using this regimen. The associated hyperaldosteronism will cause avid retention of the administered sodium, leading to rapid restoration of volume and suppression of vasopressin secretion, and resulting in a brisk water diuresis and a rise in serum sodium (410).

Acute treatment of hyponatremia is more difficult in patients with decreased effective plasma volume. This is both because the underlying disorder makes it difficult to maintain the administered fluid within the intravascular space, and because an associated increase in ANP promotes natriuresis and loss of the administered salt. Furthermore, patients with cardiac disease who are administered hypertonic saline may require concomitant treatment with a diuretic such as furosemide to prevent worsening of heart failure, which will also increase natriuresis.

Hyponatremia with Abnormal Regulation of Vasopressin

Hyponatremia with Inappropriate Increased Secretion of Vasopressin (Syndrome of Inappropriate Antidiuretic Hormone)

Causes of Syndrome of Inappropriate Antidiuretic Hormone Secretion

SIADH is uncommon in children (390,391,418). It can occur with encephalitis, brain tumor (419), head trauma (396,420), or psychiatric disease (421), in the postictal period after generalized seizures (422); after prolonged nausea (423,424), pneumonia (425,426), or AIDS (Table 9) (427). Many drugs have been associated with impaired free water clearance as indicated

Table 9 Diagnostic Criteria for Syndrome of Inappropriate Antidiuretic Hormone

Hyponatremia ($<$ 135 mmol/L) with serum hypoosmolality ($<$ 275 mOsm/kg)
Absence of clinical evidence of hypovolemia
Inappropriately raised urinary concentration ($>$ 100 mOsm/kg)
Increased urinary sodium loss in the presence of a normal intake of salt and water
Absence of thyroid, adrenal, or renal insufficiency and diuretic use

Source: From Ref. 429.

Table 10 Causes of Syndrome of Inappropriate Antidiuretic Hormone

Trauma/subdural hematoma/subarachnoid hemorrhage
Meningitis/encephalitis/brain abscess
Brain tumors
Pulmonary infections: viral, bacterial, fungal, and mycobacterial pneumonias, empyema
Neonatal hypoxia
Tumors: leukemia, mediastinal: thymoma, bronchogenic carcinoma
Prolonged nausea
Prolonged seizures
Acquired immunodeficiency syndrome
Postoperative (triple-phase response)
Drugs
Angiotensin converting enzyme inhibitors: lisinopril
Anticonvulsants: carbamazepine, oxcarbazine, valproic acid
Antineoplastics: cisplatinum, vincristine, vinblastine
Antiparkinsonian: amantadine, trihexyphenidyl
Antipsychotics: haloperidol, thioridazine
Antipyretics: acetaminophen
Hypolipidemics: clofibrate
Oral hypoglycemics: chlorpropamide, tolbutamide
Selective serotonin reuptake inhibitors: fluoxetine, sertraline
Tricyclic antidepressants

Source: Adapted from Refs. 381–383, 388, 430–436.

in Table 10. Impaired free water clearance can result from alteration in vasopressin release, increased vasopressin effect at the same plasma vasopressin concentration, or vasopressin-independent changes in distal collecting tubule water permeability. Common drugs that have been shown to increase antidiuretic hormone secretion and result in hyponatremia include carabamazepine (380), chlorpropamide (436), vinblastine (387), vincristine (437), and tricyclic antidepressants (438,439). Newer sulfonylurea agents, including glyburide, are not associated with SIADH (440). Although it has been believed to be the cause of hyponatremia associated with viral meningitis, volume depletion is more commonly the etiology (393,395). In contrast, the majority of children with tuberculous meningitis have hyponatremia and SIADH, which predict more severe disease and poor outcome (441–443). SIADH is the cause of the hyponatremic second phase of triple-phase response seen after hypothalamic–pituitary surgery, as has been discussed. Hyponatremia with elevated vasopressin secretion is found in up to 35% of patients one week after transsphenoidal pituitary surgery (444,445). The mechanism is most likely retrograde neuronal degeneration with cell death and vasopressin release. Secondary adrenal insufficiency causing stimulation of vasopressin release (106) may also play a role, because hyponatremia most commonly follows the removal of adrenocorticotropin hormone-secreting corticotroph adenomas (445). In the vast majority of children with SIADH, the cause is the excessive administration of vasopressin, whether to treat central diabetes insipidus (206,218) or, less commonly, bleeding disorders (219) (as has been discussed previously) or, most uncommonly, following dDAVP therapy for enuresis.

Variable aldosterone levels and dissociation between the plasma renin activity and the serum aldosterone levels have been described in this condition (446). There is both an acute and a chronic elevation of the atrial natriuretic factor with the water retention of SIADH that is, at least in part, contributory to the natriuresis (447). A normal saline (0.9% saline) bolus in a patient with SIADH will thus lead to lowering of the serum sodium level. Due to the mild volume expansion and the increased activity of the ANP, the sodium content of the bolus is excreted in the urine while, at the same time, there is relative retention of the free water content of the bolus due to the presence of vasopressin. This response to a normal saline (0.9%) bolus may at times help the clinician in differentiating SIADH from cerebral salt-wasting where there is usually an elevation of the serum sodium levels after such a bolus.

Treatment of Syndrome of Inappropriate Antidiuretic Hormone Secretion

The key principle of treatment of chronic SIADH is to lower the amount of free water relative to the amount of osmotic solutes within the body. This can be accomplished by either restricting free water intake, blocking the action of the vasopressin, or by increasing the intake of osmotically active solutes relative to the intake of free water. The renal action of vasopressin can be blocked using either a selective V2-receptor antagonist or medications like demeclocycline and lithium that interfere with the intracellular actions of vasopressin.

Chronic SIADH is best treated by chronic oral fluid restriction. Under full vasopressin antidiuretic effect (urine osmolality of 1000 mOsm/kg), a normal daily obligate renal solute load of 500 mOsm/m^2 would be excreted in 500 mL/m^2 water. This, plus a daily nonrenal water loss of 500 mL/m^2, would require that oral fluid intake be limited to 1000 mL/m^2/day to avoid hyponatremia, as has been discussed more fully. In young children, this degree of fluid restriction may not provide adequate calories for growth. In this situation, the creation of nephrogenic diabetes insipidus using demeclocycline therapy may be indicated to allow sufficient fluid intake for normal growth (448). Demeclocycline is superior to lithium for this purpose (449). However, its use is not recommended in children under eight years of age and pregnant women, because tetracyclines are extensively incorporated into bones and tooth enamel. Specific V2-receptor antagonists are also being developed for this purpose (450). Acute treatment of hyponatremia due to SIADH is only indicated if cerebral dysfunction is present. In that case, treatment is dictated by the duration of hyponatremia and the extent of cerebral dysfunction, as has been discussed (Table 11).

Sodium chloride given orally or parenterally and oral urea are two ways in which the osmotic solutes can be increased relative to the free water.

Table 11 Treatment of Chronic Syndrome of Inappropriate Antidiuretic Hormone

Fluid restriction: poor compliance, inadequate calories in growing children
Furosemide with oral or intravenous sodium loading: poor compliance with oral salt therapy, nephrocalcinosis, hypokalemia, and metabolic alkalosis with furosemide
Oral urea therapy: at low doses reduces natriuresis and at higher doses causes osmotic diuresis; 30% solution, 0.1–2.0 g/kg/day in 4 divided doses. Unpalatable
Nonpeptide V2-receptor antagonists: not studied in children
Demeclocycline: staining of teeth, impairment of bone growth
Lithium: hypothyroidism and arrhythmias, narrow therapeutic index

Most of the ingested sodium chloride is excreted because of the elevated ANP level and also because of the mildly volume expanded state. Free water is lost along with it by osmotic diuresis. This flow of sodium in and out of the body can be accelerated by giving sodium orally or intravenously along with Furosemide therapy (451). Close monitoring of the serum electrolytes is required during such therapy. It is also important that if the sodium chloride is being given as a solution, the osmolality of such a solution should be well above the urinary osmolality in SIADH in order to have a net loss of free water from the body. The unpalatability of sodium chloride tablets or solutions may be a factor affecting compatablity with chronic therapy. Furosemide is associated with hypokalemia, alkalosis, and nephrocalcinosis when used over a long period of time.

Studies performed on patients with chronic renal failure have shown urea in the blood to be relatively safe even in high levels (452–455). Urea plays a vital role in the concentrating mechanism of the kidney. Treatment of patients with SIADH with low doses of urea leads to a decreased loss of sodium in the urine while higher doses cause loss of free water in the urine by osmosis (456). Oral urea therapy has been used successfully and without any significant side effects at doses between 30 and 60 g/day in adults with chronic SIADH for as long as five years (457,458). In these studies, treatment with urea eliminated the need for strict fluid restriction and yet increased the serum sodium levels significantly. Recently, it has been used successfully in a group of infants and children with chronic SIADH (459).

Hyponatremia with Inappropriate Decreased Secretion of Vasopressin Because of Increased Secretion of Atrial Natriuretic Peptide (Cerebral Salt-Wasting Syndrome)

Although ANP does not usually play a primary role in the pathogenesis of disorders of water metabolism, it may have an important secondary role (401,460–462). Patients with SIADH have elevated ANP concentrations, probably due to hypervolemia, which may contribute to the elevated natriuresis of SIADH and which decreases as water intake is restricted (460). Likewise, the suppressed ANP concentrations found in central diabetes insipidus, probably due to the associated hypovolemia, rise after dDAVP therapy (460). However, hyponatremia in some patients, primarily those with central nervous system disorders including brain tumor, head trauma, hydrocephalus, neurosurgery, cerebral vascular accidents, and brain death, may be due to the primary hypersecretion of atrial natriuretic peptide (349,463–465). This syndrome, called cerebral salt-wasting, is defined by hyponatremia accompanied by elevated urinary sodium excretion (often more than 150 mEq/L), excessive urine output, hypovolemia, suppressed vasopressin, and elevated ANP concentrations (>20 pmol/L) (349). Thus, it is distinguished from SIADH in which normal or decreased urine output, euvolemia, only modestly elevated urine sodium concentration, and elevated vasopressin concentration occur. Direct measurement of intravascular volume status with a central venous line is often helpful. The distinction is important because the therapies of the two disorders are markedly different. Treatment of patients with cerebral salt-wasting consists of restoring intravascular volume with sodium chloride and water, as with the treatment of other causes of systemic dehydration. The underlying cause of the disorder, which is usually due to acute brain injury, should also be treated if possible (Table 12).

Other Causes of True and Factitious Hyponatremia

True hyponatremia is associated with hyperglycemia, which causes the influx of water into the intravascular space. For every increment in blood glucose above 100 mg/dL, serum sodium will decrease by 1.6 mEq/L.

Table 12 Clinical Criteria to Differentiate SIADH from Cerebral Salt Wasting

Criterion	SIADH	Cerebral salt wasting
Body weight	Increased	Decreased
Plasma volume	Increased	Decreased
Serum sodium and body weight	Move in opposite directions	Move in the same direction
Urine osmolality	Higher than the plasma osmolality	Isotonic with the plasma
Urine sodium	High (usually >80 mmol/L)	High (usually >150 mmol/L)
Hematocrit/BUN/creatinine /albumin	Decreased	Increased
Serum uric acid	Decreased	Variable

Abbreviation: SIADH, syndrome of inappropriate antidiuretic hormone.

Glucose is not ordinarily an osmotically active agent and does not stimulate vasopressin release, probably because it is able to equilibrate freely across plasma membranes. However, in the presence of insulin deficiency and hyperglycemia, glucose acts as an osmotic agent, presumably because its normal intracellular access to osmosensor sites is prevented (466). Under these circumstances, an osmotic gradient exists, and this stimulates vasopressin release. In diabetic ketoacidosis, this, together with the hypovolemia caused by the osmotic diuresis secondary to glycosuria, results in marked stimulation of vasopressin secretion (467–470). Rapid correction of hyponatremia may follow soon after the institution of fluid and insulin therapy. Whether this contributes to the pathogenesis of cerebral edema, occasionally seen following treatment of diabetic ketoacidosis is not known. Elevated concentrations of triglycerides may cause factitious hyponatremia, as can obtaining a blood sample downstream from an intravenous infusion of hypotonic fluid.

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Endocrine Disorders After Cancer Therapy

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INTRODUCTION

Advances in the treatment of malignant diseases have resulted in a dramatic fall in mortality rates for most of them, which means that an increasing number of survivors may have to cope with the late effects of cancer treatment. The protocols used include surgery, tumor-targeted radiotherapy, chemotherapy, total body irradiation, and/or intensive chemotherapy followed by bone marrow transplantation. Appropriate therapeutic decisions can be taken to avoid or minimize severe complications such as dwarfism, abnormal pubertal development, and infertility. Although the primary goal is still to cure the malignant disease, knowledge of the side effects needs to be considered in the choice of any new therapeutic protocol.

According to the British National Registry of Childhood Tumors, acute leukemias, mostly lymphoblastic leukemia occurring in early childhood, account for one-third of all registrations. Lymphomas, most frequently non-Hodgkin's type, account for a further 10% with a higher incidence in late childhood. Brain and spinal tumors make up 25% of all tumors. Retinoblastoma, which is often bilateral and familial, is a major condition requiring cranial irradiation in infants. Most of these tumors require treatment with high doses of radiation, which severely damage the hypothalamus and pituitary gland. The remaining childhood cancers include gonadal, bone and soft tissue sarcomas, and embryonal tumors such as Wilms' tumors and neuroblastoma (1).

Because most children with malignant diseases are treated according to nationally or internationally driven protocols in pediatric oncology centers, it has now become clear that the most constructive evaluation and follow-up of cancer survivors might be undertaken by a joint endocrinology and oncology clinic. Most treatment protocols combine chemotherapy and irradiation, but chemotherapy is becoming an important and sometimes exclusive form of treatment in many conditions. It is therefore important to consider the detailed structure of a given treatment for each child, focusing on the location of the radiation fields causing direct damage to endocrine glands or to the skeleton and on the use of cytotoxic chemotherapy that could be responsible for direct damage to the gonads.

In general, the time at which the late endocrine effects may occur is quite variable (2). Table 1 summarizes the endocrine abnormalities occurring after external cranial irradiation, according to radiation doses delivered to the hypothalamic–pituitary region.

HYPOTHALAMIC–PITUITARY AXIS AND GROWTH HORMONE DEFICIENCY

The severity and frequency of pituitary defects vary according to the initial disease and its specific therapeutic regimens, but basically the radiation dose effectively delivered to the hypothalamic–pituitary region defines the risk factor. It depends on the total dose, the number of fractions, and the duration of treatment: a given dose delivered in a shorter time period is more likely to cause growth hormone (GH) deficiency than one delivered over a long period (3,4). Hormonal deficits are also time dependent, due to delayed effects of irradiation. It necessitates prolonged follow-up with repeated evaluation.

The first case of induced hypopituitarism after cranial radiation for a tumor distant from the hypothalamic–pituitary region was reported in 1966 (5). GH deficiency is at present the most common pituitary defect after radiation (Table 1). The hypothalamus is more radiosensitive than the pituitary gland, so that GH deficiency is probably caused by a dysfunction of GH-releasing hormone [luteinizing hormone–releasing hormone (LHRH)] and somatostatin control. This may explain the differences observed between spontaneous and pharmacologically stimulated GH secretion, as well as the persistence of normal GH responses to the growth hormone–releasing hormone stimulation test (6–8). If growth is retarded despite normal GH peak responses, it has been suggested to measure the spontaneous GH secretion during nocturnal profile. If the later is subnormal, one may consider a partial GH deficiency. This condition has been described as GH neurosecretory dysfunction, and may essentially occur after low-dose irradiation (Vol. 2; Chap. 3) (9). It is now largely accepted that assessment of GH secretion requires

Table 1 Endocrine Abnormalities After External Cranial Irradiation in Patients Evaluated at Least Four Years After Irradiation[a]

		Frequency of cases with endocrine abnormality (%)				
		GH deficiency				
Cause	Cases (*n*)	Complete	Partial	Thyroid[b]	ACTH	LHRH[c]
Leukemia, 24 Gy	86	30	22	2	0	3
Face and neck tumors, 25-45 Gy	56	46	22	35	7	16
Medulloblastoma, 25-45 Gy	59	52	24	47	8	20
Optic glioma, 45-55 Gy	39	77	23	46	3	40

[a]Expressed as the percentage of affected cases in each patient group. GH deficiency; complete, after stimulation, GH peak < 5 ng/mL; partial, 5-8ng/mL.
[b]Includes elevated plasma TSH after direct thyroid irradiation.
[c]LH/FSH deficiency or precocious puberty. Evaluated in patients reaching pubertal age. Does not include primary gonadal failure.
Abbreviations: ACTH, adrenocorticotropic hormone; LH, luteinizing hormone; GH, growth hormone; TSH, thyroid-stimulating hormone; LHRH, LH-releasing hormone; FSH, follicle-stimulating hormone.
Source: From Ref. 3.

a pharmacologic GH stimulation test (10). Plasma IGF-I measurement is a convenient screening method: in patients irradiated with doses higher than 3000 cGy, IGF-1 values correlated with spontaneous GH secretion (11). However, normal IGF-I concentrations may be observed in patients with partial GH deficiency after low-dose cranial irradiation (12) and the level of IGF-I is more difficult to interpret because many of these patients are moderately obese (13). Repeated GH testing may be necessary in some cases before GH deficiency is diagnosed, particularly in patients treated with low-dose radiation. In any case, GH responses and IGF-I values must be interpreted according to the pubertal status of the patient (3).

GH deficiency occurs in about 75% of the children treated with cranial irradiation doses between 3000 and 4500 cGy. All children irradiated with higher doses are affected and eventually develop panhypopituitarism. The greater the radiation dose, the earlier it is that the GH deficiency develops, with intervals ranging from one year in patients irradiated with 4000 cGy for cranial tumors to more than four years in leukemic patients receiving lower radiation doses (2). The age at time of cranial irradiation is another important risk factor, younger children being more vulnerable (14). It is therefore recommended that cranial irradiation be delayed whenever possible until the child is over three years old. Children treated for acute leukemia with radiation doses of 2400 cGy or less do not always develop GH deficiency, and the deficiency may be restricted to the pubertal period (15). Complete GH deficiency is not reversible. However in a recent study (16), only 64% of the children irradiated for brain tumors who developed complete GH deficiency fulfilled the criteria of severe GH deficiency at adulthood when retested at final height attainment. This may be partly because of difficulties in quantitatively assessing GH secretion at different ages. The long-term outcome of partial GH deficiency, as observed in patients treated with low-dose cranial irradiation, is not firmly established. In the group of patients with severe GH deficiency, gonadotropins, adrenocorticotropic hormone (ACTH) , and thyroid-stimulating hormone (TSH) defects can occur years later. After total body irradiation, preparing for bone marrow transplantation, the risk of GH deficiency depends on the radiation protocol (17–20). A single dose of 1000 cGy, less commonly used, generally impairs GH secretion. Doses superior to 700 cGy, even fractionated, induce a 50% risk of GH deficiency. Generally there seems to be no correlation between GH secretion and growth, at least during the first few years following irradiation (17). This is not surprising, because many other etiologic factors may play a role.

THYROID

Central Hypothyroidism

The reported incidence of central hypothyroidism after cranial irradiation varies with the diagnostic criteria used, because most patients do not develop overt clinical hypothyroidism. Low free T4 (FT4) with normal or low TSH is observed in only a small percentage of patients, in the high-risk group (2). Only 6% of brain tumors survivors have evidence of hypothyroidism after a mean interval of 12 years (21). In another study, because of the lack of overt clinical presentation, the incidence of TSH dysregulation was assessed by combining three criteria: basal FT4, TSH response to TRH, and nocturnal TSH surge. Using such sensitive biological criteria, it was shown that central hypothyroidism would have remained undiagnosed by baseline thyroid function tests alone. After a mean of six years follow-up, 36% had evidence of central hypothyroidism (22). Mixed central and primary hypothyroidism is expected to occur if the thyroid was also irradiated. TSH deficiency occurred even before GH deficiency, although undiagnosed by the conventional insensitive tests (Table 1) (22). The incidence of central hypothyroidism is related to the total radiation dose. Chemotherapy with busulfan and cyclophosphamide may exacerbate the effects of irradiation. Transient hypothyroidism has also been reported during induction chemotherapy with L-asparaginase (23). The issue of latent central hypothyroidism is complex as one may consider that it should be treated with L-thyroxin to optimize

Table 2 Current Chemotherapy Agents Cytotoxic for the Testis and Ovary

Chemotherapy	Disease
Alkylating agents	
Cyclophosphamide	Lymphoblastic leukemia
	Non-Hodgkin's lymphoma
	Various tumors
Ifosfamide	Soft tissue tumors
	Ewing sarcoma
	Nephroblastoma
Melphalan	Bone marrow transplantation
Busulfan	Bone marrow transplantation
	Chronic myeloid leukemia
Dacarbazine (DTIC)	Soft tissue tumors
Carmustine (BCNU)	Hodgkin's disease
	Brain tumors
Lomustine (CCNU)	Hodgkin's disease
Semustine (methyl-CCNU)	Brain tumors
Other agents	
Cytarabine (cytosine arabinoside)	Lymphoblastic leukemia
Vincristine	Various tumors
Vinblastine (VLB)	Hodgkin's disease
Procarbazine	Hodgkin's disease
Cisplatin (cis-DDP)	Various tumors

spontaneous or human growth hormone (hGH)-treated growth as well as intellectual and school performance of affected children. More studies are needed to evaluate long-term thyroid replacement therapy in cancer survivors.

Primary Thyroid Damage

Primary hypothyroidism due to direct thyroid irradiation is diagnosed by TSH elevation. It is essentially found in patients irradiated for Hodgkin's disease or after craniospinal irradiation. Hyperfractionation radiation therapy lowers its incidence in the long term (24). Interestingly, primary hypothyroidism may also occur as a consequence of scattered irradiation of the neck from both cranial and spinal fields (21).

The risk of developing benign nodules or thyroid cancer in later adult life is increased after radiation of the neck (Vol. 2; Chap. 3). A low-dose thyroid radiation exposure (up to 500 cGy) is a known cause of neoplasm, thus both head and neck therapeutic irradiation must be considered as a risk factor. Thyroid nodules may also occur after radiation doses of 1000 to 4200 cGy. Although most data are derived from patients treated for Hodgkin's disease (25), children given spinal or total body irradiation should also be followed up through adulthood. Ultrasonography of thyroid is more sensitive than physical palpation; however, it is so sensitive and nodules are so prevalent in the normal population that great caution is needed in interpreting the results. Serum thyroglobulin correlates with the number of nodules (26). Surgery is a difficult decision to take. The percentage of biopsied nodules containing malignant cells (mostly papillary carcinoma) varies from 14% to 40% depending on the method of detection (27). Therefore it is important to inform all patients at risk of the need for prolonged follow-up.

GONADAL AND REPRODUCTIVE FUNCTION

Risk factors for gonadal toxicity include local irradiation, type and dosage of chemotherapy, age, pubertal status, and sex of the patient (Table 2).

There are important differences between male and female gametogenesis and its relationship to gonadal endocrine function. The subfertility risk varies according to the initial malignant disease, hence its most appropriate therapy (28) (Table 3).

In Girls

There is a fixed number of primordial follicles at five months of gestation and a progressive loss occurs with age. However if ovocytes are damaged or destroyed by anticancer therapy, accelerated reduction in their number results in the absence of puberty; at a later age there may be temporary or permanent cessation of ovulation with shortening of the reproductive period.

High-dose cranial irradiation induces gonadotropins deficiency and must be treated by sex steroid replacement therapy at age of puberty, by exogenous LHRH or recombinant gonadotropins for fertility purpose.

Chemotherapy-related damage to the ovary depends on the drug, its dosage, and age of the patient because gonadotoxic effects increase with age. They are minimal in prepubertal girls who have a greater reserve of germ cells and primary follicles. Alkylating agents, essentially cyclophosphamide at total dose higher than 20 g or 500 mg/kg (29) and high-dose busulfan, permanently suppress ovarian function. Combination chemotherapy regimens have generally less effect on ovarian function in adolescent girls than in adult females. In prepubertal girls, if treated for leukemia, a favorable outcome was reported (30–32). In Hodgkin's disease, mechlorethamine, vincristine, procarbazine, and prednisone (MOPP)

Table 3 Subfertility Risk After Current Treatments for Cancers and Leukemia in Childhood and Adolescence

Low subfertility risk ($<20\%$)
Acute lymphobiastic leukemia
Wilm's tumor
Retinoblastoma
Brain tumors with surgery and low radiation dose
Soft tissue sarcoma stage 1
Germ cell tumors without irradiation
Medium subfertility risk
Hodgkin's disease
Non-Hodgkin's lymphoma
Brain tumor with cranial/craniospinal irradiation
Neuroblastoma
Ewing's sarcoma
Acute myeloblastic leukemia
Soft tissue sarcoma stage 2 or 3
High subfertility risk ($>80\%$)
Total body irradiation (TBI)
Gonadal irradiation
Alkylating agents for TBI or Hodgkin's disease
Metastatic carcinomas

Source: From Ref. 28.

and MVVP regimens, which induce a high incidence of transient or permanent amenorrhea in adults, have little effect on ovarian function in girls provided they did not receive additional pelvic irradiation (33,34). In contrast, a favorable outcome was reported after ABVD regimen (35).

Ovarian function may be severely impaired by direct irradiation with fractionated doses over 700 cGy, with complete destruction of the ovocytes at 2000 cGy (36) as used in patients treated for abdominal, pelvic, or genital tumors and Hodgkin's disease. Primary ovarian dysfunction may also occur in patients who received spinal irradiation with scattered irradiation to the ovaries (37). Ovarian transposition before abdominal irradiation has been shown to protect patients from ovarian failure if performed before puberty (38).

Total body irradiation (TBI) given for bone marrow transplantation induces a high risk of ovarian failure. Permanent and total damage to the ovary occurs as well in girls given high doses of busulfan, often associated with cyclophosphamide (39,40). In contrast chemotherapy regimens excluding busulfan have a favorable outcome. Young age at TBI and a fractionated irradiation are associated with increased ovarian recovery. Whatever the disease the time required for possible recovery, may vary from 7 (41) to 10 years (42). Transient increase in plasma gonadotropins, and ultimate recovery were observed in 35% of the girls treated with TBI for leukemia (43). Therefore when estrogen replacement therapy is given, it should be stopped periodically to detect any ovarian recovery.

Future fertility is the main issue in all these patients. The prospects for normal fertility are rather favorable in girls treated with nonintensive chemotherapy for leukemia (44,45) or with nitrosoureas for brain and spinal tumors. The correlation between plasma gonadotropin levels and fertility in amenorrheic girls is poor, as persistently patients with elevated plasma gonadotropins may become pregnant. Presently there is no evidence, in spite of favorable animal studies, that gonadal activity suppression during anticancer therapy decreases the risk of gonadal toxicity. The assessment of future fertility requires the knowledge of the degree of ovarian damage. In adult survivors with spontaneous menstrual cycles, ovarian volume and total number of antral follicules are correlated with circulating inhibin B and antimullerian-hormone levels (46–48).

In a recent study, a global fertility deficit of 23% was observed in females treated during childhood with alkylating agents and abdominal-pelvic irradiation with large variations according to treatment regimens (49). Radiation is the most deleterious factor. Radiation also damages the uterus and its vascularization, which may result in sterility. This occurs with a higher frequency in girls given TBI before puberty, or in a high frequency of miscarriages or premature births (40,44,50). These lesions are more severe when girls were treated in early childhood (51,52). There is no increased frequency of congenital malformations. Because of uterine atrophy as shown by reduced uterine length and endometrial thickness at ultrasound examination, some women are also unlikely to benefit from in vitro fertilization with donor ovocytes (53). In addition, estrogen replacement therapy may be inadequate to generate normal uterine growth (54). If fertility is maintained, there is no increased frequency of congenital malformations.

Fertility preservation (28) remains to be proven in prepubertal girls, in view of recently reported success in adult women (55). In the latter case, frozen ovarian tissue allowed autologous transplantation. Some groups advocate removing and freezing one ovary, when girls are to be given treatments that are known to be associated with a full risk of ovarian atrophy. However there is at present no precise outcome for such procedure. Transplantation remains the most promising option for the future, provided it is performed within acceptable ethical guidelines.

Clinical evaluation of ovarian activity is easy in cases with complete gonadal failure. It is less precise in secondary amenorrhea with elevated follicle-stimulating hormone (FSH) and LH plasma concentrations. The later may be the only sign of ovarian dysfunction observed during posttreatment follow-up. Estrogen and progesterone replacement therapy is necessary to fully feminize these patients and allow a normal pubertal growth. It is suggested to periodically interrupt this replacement therapy in order to revaluate the ovarian function. Breast atrophy may result from scattered irradiation after whole abdominal or flank irradiation performed before puberty. It may require cosmetic surgery (51).

In Boys

The germinal epithelium is more sensitive to irradiation than Leydig cells (56). In prepubertal boys, local radiation doses between 300 and 1000 cGy, as delivered during abdominal or low spinal irradiation (craniospinal irradiation may result in a scattered dose to the gonad) , induce transient or permanent rise in serum FSH at time of puberty with later oligospermia or azoospermia and reduced testicular volume (57). Germ cell dysfunction occurs after TBI, single or fractionated, with a high risk of azoospermia (43). Recovery of germ cell function is rare, even more so after fractionated irradiation (58). When irradiated during puberty similar data were reported, and recovery of gametogenesis was not assured, though may occur years later. Chemotherapy is essentially aggressive to germ cells. It includes alkylating agents, procarbazine, vinblastine, cytarabine, and cisplatin (Table 3). Except for the very aggressive, intensive cytotoxic chemotherapy with high doses of cyclophosphamide or busulfan, there are remarkable individual variations that may not be predictable and

long-term follow-up is necessary. The elevation of serum FSH is consistent with germinal damage (59) and circulating inhibin B is a potential marker in childhood and adolescence. Each regimen used in the management of a given cancer should be evaluated for its potential harm to the gonads. For instance, the effects of cyclophosphamide on sperm density are proportional to the dose given before adulthood (60). A total dose of 200 mg/kg or more is followed by evidence of germ cell damage, probably more if treated after the onset of puberty and the prognosis of fertility are poor in most of them (61). Combination chemotherapy, such as MOPP or MVPP to treat Hodgkin's disease, is generally more gonadotoxic than individual agents. Therefore new regimens, which have low gonadal toxicity, are being developed (62). For fertility preservation, the value of semen cryopreservation in adolescent patients has been shown, yet it is often of poor quality at that age (28).

The Leydig cell function, in general, is preserved. Testosterone secretion and pubertal development are normal following local irradiation with doses inferior to 2000 cGy or TBI although elevated serum LH with normal testosterone values may be observed in some cases. Only males who received high-dose testicular irradiation after leukemia relapse experience testicular failure and require androgen replacement therapy (63).

OTHER ENDOCRINE AND METABOLIC COMPLICATIONS

Adrenocorticotropic Hormone Deficiency

ACTH deficiency and secondary adrenal insufficiency with clinical symptoms is a rare complication that occurs after high doses of cranial radiation given for brain tumors. Evaluation should continue beyond 10 years after irradiation. It is absent after low radiation doses as used for leukemia therapy. In children, it may cause hypoglycemia especially if combined with severe GH deficiency. It may occur as early as the first two years after radiotherapy (64). Its diagnosis has been made by combined evaluation of early morning plasma cortisol and ACTH, or more precisely by a metyrapone test (64), insulin tolerance test (but many groups are reluctant to perform this test), glucagon stimulation test, or short synacthen test (65,66). Hydrocortisone replacement therapy is required (Vol. 2; Chap. 8).

Prolactin Secretion

Hyperprolactinemia (usually below 100 ng/mL) after high-dose cranial irradiation develops during adolescence or adulthood without any clinical expression. It is an additional indicator of hypothalamic damage (64).

Obesity

In its severe form, it is a late effect in brain tumor survivors, due to hypothalamic damage, essentially after radiation doses of 5000 cGy or higher at a young age. Obesity is assumed to be the consequence of VMH dysfunction resulting in hyperphagia and/or an excessive insulin secretion (Vol. 1; Chap. 1) (67). It also occurs at a lesser extend, mostly in girls, in ALL survivors. However a survey on self-reported body mass index after treatment for brain tumors showed that it was not different from sibling controls, except for females treated at a younger age (68). In addition, a metabolic syndrome including elevated systolic blood pressure, increased waist–hip ratio, and an abnormal lipid profile (Vol. 1; Chap. 8) have been reported in brain tumors survivors, with more severity in untreated GH deficiency (69). An opposite situation has been observed in Hodgkin survivors with frequent underweight and adverse health (70).

Bone Mineralization

Several factors lead to suboptimal acquisition and mineralization of bone mass in survivors of childhood cancer. They include radiation, chemotherapy, nutritional deficiency, limited physical activity, and several endocrine factors such as GH deficiency, sex steroid deficiency, and corticosteroid therapy. Bone mineral density should be evaluated as part of each protocol. For instance, in survivors of acute lymphoblastic leukemia treated with chemotherapy alone, a spontaneous recovery from decreased total bone mineral density was observed within a few years (71). In GH-deficient patients, adequate and prolonged GH treatment may be necessary to allow progression to the peak bone mass (72).

Importantly cranial irradiation is never accompanied by posterior pituitary dysfunction. Calcium homeostasis remains normal although primary hyperparathyroidism had been reported to rarely occur after low-dose neck irradiation. Finally, there is no reported evidence of primary endocrine pancreas or adrenal dysfunction after abdominal irradiation.

GROWTH

Growth depends on GH secretion and the timing of puberty but also on a number of factors unrelated to pituitary deficiency, such as chemotherapy, associated acute and chronic disease effects, and exposure of cartilage plates to irradiation, as seen in children with spinal or total body irradiation. Irradiation impairs growth through alterations at its various targets (Table 4).

After High-Dose Cranial Irradiation

Radiation doses in excess of 3000 cGy reduce final height in most children. The height loss is progressive, reaching about 1 standard deviation (SD) before puberty and 2 SD at final height. Growth retardation develops more rapidly, within two years, in patients given 4500 cGy or more. Bone age is delayed, and

Table 4 Critical Targets for Growth According to Radiation Protocols

	Cranial	Craniospinal	Total body
Growth hormone[a]	++	++	+/−
Gonadal steroids[b]	+	+	+[c]
Thyroid hormones[c]	+	++	++
ACTH	+	+	
Skeleton		++	+

[a]Hypothaiamic and pituitary deficiency.
[b]Primary and/or secondary defects according to radiation protocols.
[c]Essentially in girls.
Abbreviation: ACTH, adrenocorticotropic hormone.

typical features of GH deficiency may appear in prepubertal patients (2).

After Low-Dose Cranial Irradiation

Variable patterns of growth have been reported. Typically, there is a moderate height reduction during the acute phase of the disease and the associated induction chemotherapy. This is followed by a subnormal or normal growth rate until puberty. An additional height loss of 1 SD may occur during puberty (73). A few patients have shown normal growth after irradiation, despite a GH deficiency (73). The overall mean loss in adult height in patients treated with cranial doses of 1800 to 2400 cGy varies from 0.9 to 1.4 SD (74–76). Final short stature is more likely to occur after intensive induction chemotherapy in children irradiated at a younger age if puberty began earlier and in patients with familial short stature. Because GH deficiency remains the prime candidate as a cause of growth retardation, all children should be tested for GH secretion before and at onset of puberty if they demonstrate a significant decrease in linear growth. This issue is even more critical in patients, most frequently in girls, presenting with sexual precocity. These irradiated patients are prone to obesity in the long-term with increase circulating leptin levels independent of GH status (77).

After Spinal or Total Body Irradiation

Some protocols include extensive skeletal irradiation, and these patients are exposed to more severe and early growth retardation. The first group of patients are those given spinal irradiation (generally 2400 cGy) in addition to cranial irradiation for medulloblastoma. They may lose up to 2 SD of height within two years following irradiation and have a mean final height loss of 2 to 3 SD, with a short upper segment largely attributed to the lack of spinal growth (78,79). When the lower dose of 1800 cGy is given to the spine, growth is improved (80). Children irradiated before age six years are more severely affected. Some degree of reduced spinal growth and disproportion also occurs after whole abdomen or, more rarely, after flank irradiation, as performed for abdominal malignancies, such as Wilms' tumors (81). Total body irradiation as conditioning for bone marrow transplantation is another therapy that leads to growth retardation unrelated to GH deficiency. It is increasingly used as the ultimate therapy in leukemia and in some nonmalignant diseases (82,83). The outcome of growth in these patients depends on the radiation dose and its fractionation (17). Immediate growth retardation was observed in patients given a single 1000 cGy dose. The more recent protocols with fractionated doses of 800 to 1000 cGy have little impact on short-term growth. Final height has been reported to decrease by 1 SD with diminished sitting height. Similar results, with a more compromised growth at puberty in boys, were recently reported (84). GH deficiency was found in 30% of these patients, with some improvement of GH production over the years (85). Difference in treatment protocols may explain some discrepancy among reported data. Other factors such as prolonged corticosteroid therapy, renal failure, and chronic graft-versus-host disease may also contribute to growth retardation. The frequency and severity of GH deficiency depends on the radiation protocols, and GH may not play a major role in the growth disturbance of these patients (17–19,86). In adult long-term survivors, serum IGF-I values were only partly correlated with GH secretion. Normal IGF-I was found in contrast with evidence of GH deficiency (11,12) and could be explained by increased adiposity.

Primary thyroid insufficiency occurs in most patients given total body irradiation, elevated plasma TSH appearing within two years, but less than 10% of them have overt hypothyroidism, which must be treated. Most of the boys and girls irradiated before puberty develop primary gonadal failure with elevated LH and FSH levels. Delayed or absence of sex steroid secretion then contributes to growth retardation at the age of puberty and requires replacement therapy.

After Chemotherapy

The effect of chemotherapy on growth is difficult to assess because many factors, such as differences between protocols, infection, poor nutrition, and the disease itself, may play a role. These patients do not develop GH deficiency after treatment by chemotherapy alone, but the moderate, early growth retardation, as reported in children irradiated for leukemia (87) or cranial tumors (79), may be related to the induction chemotherapy and caused by a transient insensitivity to GH. However, the final height of patients treated for leukemia with chemotherapy alone was normal (76). Interestingly, there may even be catch-up growth in immunodeficient growth-retarded children after preparative chemotherapy for bone marrow transplantation (17). Because some data still suggest that chemotherapy has a moderately detrimental effect on growth (75), a follow-up of all patients' remains necessary. Yet growth is unlikely to be a critical issue in nonirradiated patients.

PUBERTY AND GONADOTROPIN SECRETION

Surprisingly, children who have received cranial irradiation may present with early or true precocious puberty (88,89). This is in contrast with the delayed puberty usually accompanying idiopathic GH deficiency. Early puberty occurred essentially in girls after cranial irradiation for leukemia, and the children who had been irradiated when very young tended to have the earliest puberty (90,91). This is an important consideration because of the risk of excessive bone maturation and early epiphyseal closure. It also tends to narrow the window of opportunity to treat with hGH before secretion of sex steroids. Puberty not only occurs earlier but it is also shortened with early menarche (30). The final height loss may even be more severe if full-blown precocious puberty is associated with untreated GH deficiency: in patients with optic glioma presenting with precocious puberty at the time of irradiation, the persistence of a normal growth rate within one or two years after cranial radiation may be misinterpreted, and excessive progression of bone age will lead to early cessation of growth. GH testing is then necessary one year after irradiation to unmask any associated GH deficiency and allow GH therapy to begin (92).

In contrast, gonadotropin deficiency may develop within a few years after high-dose cranial radiation for tumors, as indicated by arrested puberty, primary amenorrhea in girls, and absence of luteinizing hormone (LH) and FSH response to an LHRH stimulation test (93). Some girls suffer only from menstrual irregularities, and their impact on fertility has not been documented. Gonadotropin deficiency is usually associated with GH deficiency and moderate hyperprolactinemia, a combination indicating multiple hypothalamic–pituitary deficiencies.

FOLLOW-UP AND MANAGEMENT

Long-term assessment of oncology patients is required for growth, puberty, fertility, and other late endocrine complications. Adult endocrinologists should now focus on GH deficiency–related symptoms, obesity, bone mineral density decrease, intellectual cognitive functions, and sexual life of the survivors. Regular checkups are necessary to decide upon replacement treatments.

Growth Hormone Replacement Therapy

After cranial irradiation for brain and facial tumors, because GH deficiency is generally severe, recombinant GH given at the usual dose of 0.2 to 0.3 mg/kg/wk), produces a significant improvement in growth rate (80). In earlier reports, the long-term results and final heights achieved have been rather disappointing (94–96). Several factors may have had negative effects when compared with patients with idiopathic GH deficiency: failure to catch up

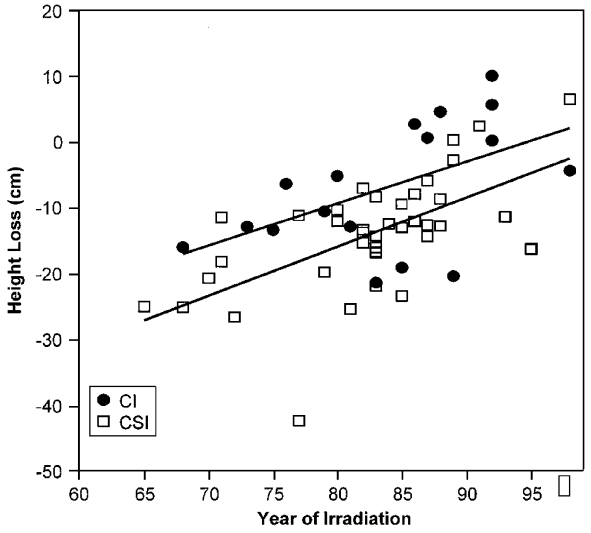

Figure 1 Improvement in final height in the last 25 years in children with brain tumors treated for radiation-induced GH deficiency. Patients with craniospinal irradiation had the best improvement in final height. *Abbreviations*: CI, cranial irradiation; CSI, craniospinal irradiation. *Source*: From Ref. 16.

with normal height may have been related to low hGH dosage, delayed treatment, and/or early puberty, which accelerated the skeletal maturation faster than the increase in growth rate (16). It is therefore essential to commence hGH therapy as soon as GH deficiency and growth retardation are documented, but preferably not less than two years after the primary treatment except for severe cases.

This issue is more complex in patients treated for leukemia with low doses of cranial irradiation. In this group, GH therapy should be started before puberty only if the height loss exceeds 1 SD. Some of them maintain normal growth rates for several years until puberty despite partial GH deficiency. But at time of onset of puberty if there is a familial short stature and confirmed GH deficiency, GH treatment will help avoiding a significant adult height loss. Again, early or precocious puberty is an additional risk factor of short stature. LHRH analog therapy should then be considered in association with the hGH treatment. In this group of patients, most authors restrict the use of hGH and/or LHRH analog therapy to the GH-deficient patients that are at most risk of final adult short stature, because of familial short stature or cranial irradiation at a younger age. Whatever the cranial radiation dose, those who have received additional spinal irradiation have a poor response to hGH treatment (96,97). The same suboptimal growth response occurs after high-dose total body irradiation.

In patients with cranial irradiation, there is now sufficient evidence that growth can be improved by hGH therapy (Fig. 1). Combination with LHRH analog allows these patients to reach height in the normal range although frequently lower than calculated target

height (98,99). Reduced final height remains a critical problem in patients with craniospinal or TBI.

One major concern is the potential oncogenic effect of hGH treatment. However, there is at present no evidence that hGH promotes leukemia or tumor relapse (100,101). A high rate of secondary malignancies has been reported in children treated before age of three with alkylating agents and etoposide with or without irradiation (102). There is a question mark on this issue in patients treated for leukemia (101).

Continuation of GH therapy after final height is reached has been suggested after reevaluation of GH secretion (16). A significant improvement of quality of life was observed after short-term GH treatment (103). These adults are often obese, with poor physical performance. However the need for prolonged GH therapy in adulthood may be questionable in patients who do not show profound GH deficiency (Vol. 2; Chaps. 4 and 5). More studies are needed to evaluate its metabolic benefit on body composition, muscular mass, and bone mineralization.

Other Issues During Follow-Up

Presently, GH replacement therapy with conventional doses is well defined and other issues should be given priority on the long term and according to the oncology therapy. Skeletal dysplasia may be secondary to irradiation and appropriate replacement sex steroid therapy is necessary to minimize loss of bone mineral density. Thyroid nodules occur after many years. Gonadal failure requires replacement therapy. Risk of fertility can be anticipated but long-term evaluations are still necessary in both sexes and fertility preservation has become a main issue. On all issues, parents and patients should be given appropriate information and helped in order to face potential problems after cure of the cancer, and accept a prolonged medical follow-up (104).

INTELLECTUAL AND NEUROPSYCHOLOGICAL RISKS

The risk of intellectual dysfunction has been recognized for a long time and a comprehensive analysis was provided by Duffner and Cohen (105) in the early 1990s. With the protocols followed at that time, precise conclusions were presented. The most important variable was age at time of diagnosis and treatment: children younger than three years have a significantly worse intelligence quotient (Iq) than patients treated at older ages. This led to postponement of cranial irradiation and to treat with postoperative chemotherapy in an attempt to delay radiation until the children could better tolerate its effects. A second risk factor is large volume irradiation, with significant changes in IQ scores after whole brain irradiation. The dose of radiation that produces neuropsychological sequelae is not precisely known.

More recent studies have shown that only-chemotherapy protocols among leukemic children had modest late effects on nonverbal cognitive skills, particularly girls (106). However sequelae were reported when leukemic children had cranial irradiation (24 or 18 cGy) with language and education disabilities (107). Similarly an impaired academic achievement was reported as well after central nervous system chemotherapy prophylaxis in these patients. More recent studies have shown that only-chemotherapy protocols among leukemic children had modest late effects on nonverbal cognitive skills, particularly among girls.

Children with medulloblastoma treated with irradiation at high doses most frequently show severe neuropsychological deficit, requiring specific support (108). Over time, deficits in nonverbal and information processing skills may deteriorate (109).

Survivors of pediatric bone marrow transplantation are being more precisely evaluated and a recent study concluded that with or without TBI, bone marrow transplantation entails minimal neurocognitive sequelae in patients who are six years of age or older at time of therapy (110). Altogether, because there is a significant incidence of learning disabilities after cranial irradiation, early and appropriate monitoring of cognitive skills may improve the neurocognitive future of these patients.

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Radiation Terrorism

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INTRODUCTION

Unlike the well-known incidents involving biological and chemical terrorism, there has not been a domestic act of terrorism that involved the use of radiation-emitting devices or explosives. The potential for terrorism using radiation is very real. Such an incident would create fear among civilian populations and their governments, as well as have the potential to cause significant loss of life. It is only a matter of time before a radioactive device will be used as an act of terrorism. Therefore, a review of the subject is appropriate and timely. There have been several large-scale disasters causing radiation exposure since the atomic bomb detonations in Hiroshima and Nagasaki, Japan six decades ago (1). These exposures were primarily from nuclear power plants and fallout from nuclear bomb testing (2). There are several excellent review articles on the subject of radiation exposure, the health consequences, and medical management that provide considerable information in great depth (1–12). This chapter will focus on the effects of radiation on the vulnerabilities of the endocrine system and metabolism, and discuss important coexisting effects on other organ systems as appropriate.

The acquisition of potent forms of radiation for use in terrorism presents a unique challenge, for the transport of radioactive materials is hazardous to humans unless the materials are secured properly to prevent or minimize radiation exposure. Customarily, large, heavy containers are needed for storage and transport, making a clandestine transport of these materials difficult. However, traditional explosives could be used on radioactive-containing structures, such as nuclear power plants, to cause the release of large amounts of radioactivity. A more likely scenario is the use of a ''dirty bomb,'' more appropriately termed a radiation dispersal device. This is a radiation weapon, which combines radioactive material with conventional explosives. While the dirty bomb is designed to disperse radioactive material over a large area, the conventional explosive is more likely to produce an immediate lethal effect than the radioactive material.

Radiation disasters through war and accidents have provided data on the risk of the health consequences of radiation exposure, and provide some information on the dangers of high doses of radiation exposure, especially to children (1). Most of the current information is derived from studies following the detonation of atomic bombs in Nagasaki and Hiroshima, Japan, in 1945 (11), and the accidental nuclear power plant disaster at Chernobyl, Ukraine that affected large populations of all ages principally in Belarus, Northern Ukraine, and Western Russia (12,13).

IONIZING RADIATION

Definitions

Unstable atoms emit energy in the form of *ionizing radiation* and, therefore, become stable (1). The high-frequency particles and electromagnetic energy released causes various adverse biological effects, depending upon the type and dose of the energy released (4). Adverse biological effects include the production of free radicals, damage to DNA, production of new macromolecules, and disruption of chemical bonds (1,4).

Radionuclides, such as isotopes of carbon (e.g., deuterium and uranium) that emit ionizing radiation, may exist in the environment naturally, or can be generated by man, such as ^{131}I-iodine,^{123}I-iodine,^{99m}Tc-technetium pertechnetate, or ^{125}I-iodine for use in medicine, and plutonium for use in nuclear reactors (1,4).

The types, characteristics, and sources of ionizing radiation (1,4) are summarized in Tables 1 and 2.

Units of Measure

There are two current systems to express the units of measure for radiation exposure (Table 3). Some older units continue to be used in clinical medicine, and knowledge of the conversion from these units to the *System Internationale* (SI) units is necessary for the proper interpretation of the literature for patient-care purposes because the old units continue to be reported routinely.

The concepts for radiation exposure that are the basis for their use are classified broadly as the energy absorbed by tissue from γ rays and X rays and the activity for radiation emission from radionuclides. The current terminology for radiation units is the SI Units (1,4,14). The Gray (Gy) replaces the rad, the Sievert (Sv) replaces the rem, and the Becquerel (Bq) replaces the Curie (Ci) (1,4,14).

The energy absorbed from γ rays and X rays is known as the rad, or *radiation absorbed dose*; $1\,\text{Gy} = 100\,\text{rad}$, or $1\,\text{cGy} = 1\,\text{rad}$. The rem, or *roentgen equivalent man*, is a unit of measure of absorbed energy that is based upon a greater biological effectiveness (RBE) of doses from particulate radiation; $1\,\text{Sv} = 100\,\text{rem}$. This unit of human exposure to radiation is in contrast to the rad that is a unit of energy deposited by any type of radiation to any type of tissue or material. Thus, the rem equals the rad times the quality factor, RBE, a factor determined by the type of radiation and tissue (human, animal) or material (metal, wood). For instance, the quality factor for β particles, γ rays, and X rays is 1, whereas for α–particles is 20, or $1\,\text{rad} = 20\,\text{rem}$ (Table 3).

The activity for radiation emission from radionuclides is the Curie (Ci), the traditional measure of radioactivity and measured by radioactive decay. The SI unit, the Becquerel (Bq), is defined as the *decay events per second* where, $1\,\text{Bq} = 1$ *disintegration per second* [*dps*] and $1\,\text{Ci} = 3.7 \times 10^{10}$ *disintegrations per second* (Table 3).

Table 1 Exposure to Ionizing Radiation: Definitions

Unstable atoms emit energy in the form of ionizing radiation and become stable
High-frequency particles and electromagnetic energy causes adverse biologic effects:
Damage to DNA
Production of free radicals
Disruption of chemical bonds
Production of new macromolecules
Radionuclides are elements that emit ionizing radiation. They occur naturally (uranium) or are man-made (plutonium)

Table 2 Types of Ionizing Radiation

Alpha (α) particles = helium atom nucleus (2 protons + 2 neutrons); source: nuclear weapon detonation
Beta (β) particles = electrons (high-speed particles); source: nuclear reactors and radioisotopes of iodine
Gamma (γ) rays = photons (visible light); high energy and penetrance, external radiation hazard; sources: nuclear reactors and weapon detonation
X rays = energy emitted from electrons; unlikely source of ionizing radiation from disasters
Neutrons = powerful and very damaging to tissues; emitted only from a nuclear weapon detonation

Human Exposure

In humans, radiation exposure can occur by internal radiation by inhalation, ingestion, or injection, and external (whole or partial body) radiation. The health effects can be mediated by direct radiation to target tissues, or indirectly through the production of free radicals or other harmful molecules (1–4,12,15). Of greatest concern is the carcinogenic effect of radiation exposure (16).

The health effects may also vary according to the sensitivity of the target tissue, which correlate with the rate of cell division of the tissue and inversely with the degree of cell differentiation. These effects are modulated by age of the exposed subject, radiation dose, the type of radiation, and the production of chromosomal breaks. The latter are associated with an increased rate of cancers, especially in the presence of inactivating mutations of DNA repair genes. Tissue sensitivity differs from the most to least, as follows: Lymphoid greater than gastrointestinal greater than reproductive greater than dermal greater than bone marrow greater than nervous system. The sensitivity of the thyroid is inversely related to age—the fetus and very young infant are very sensitive to the carcinogenic effect of radiation, whereas the adult is not (17–19). The higher radiation doses cause thyroid cell death. Moderate sublethal radiation doses are associated with an increased risk for thyroid neoplasia (thyroid carcinoma, thyroid adenoma), and autoimmune thyroiditis, most likely in predisposed individuals. Autoimmune-mediated insulin-dependent diabetes

Table 3 Radiation Exposure Units of Measure

Energy absorbed from γ rays and X rays			
	Old units	SI units	Conversion
Radiation absorbed dose	Rad	Gray (Gy)	1 Gy = 100 rad 1 cGy = 1 rad
Roentgen equivalent mass	Rem	Sievert (Sv)	1 Sv = 100 rem
Activity for radiation emission of radionuclides			
Unit of decay	Old units	SI units	Disintergrations/sec (dps)
Curie	Ci	–	1 Ci = 3.7×10^{10} dps 1 Ci = 37 MBq
Becquerel	–	Bq	1 Bq = 1 dps 1 MBq = 109 dps

Source: From Refs. 1,4,14.

mellitus (IDDM), like thyroiditis, may be triggered in children and adolescents, who are predisposed genetically.

Exposure to natural radiation is not considered to be harmful in the usual doses of exposure, although the concern about radon exposure remains controversial (Table 4) (15). Higher doses from therapeutic radiation are associated with the development of various benign and malignant tumors, especially in genetically predisposed individuals with mutations in DNA repair genes, Radioactive isotopes of iodine are often used in therapeutic radiation exposures and potential sources of radiation for terrorist activities. The radiation dose from radioiodines is dependent upon the uptake and concentration of iodine by various tissues, as affected by body stores of iodide (Table 5). Health consequences relate to dose and tissue sensitivity (1,4,15,16).

Table 4 Environmental or Natural Radiation Exposure

Average annual exposure

360 mRem or 0.0036 Sv

Sources: Cosmic radiation and radon, cigarette smoke, medical devices, home appliances, pharmaceutical agents

Specific exposures

5–10 mRem	Flight from New York to Los Angeles
5–10 mRem	Chest radiograph
5000 mRem (0.05 Sv)	Computed tomography scan

Environmental or natural radiation exposure

Radon

- Radon is a naturally occurring radioactive gas The main radon and its daughters (decay products) exposure concern is the development of lung cancer. An increased risk of lung cancer has been clearly documented in uranium and certain other miners exposed to radon and its daughters, as well as in experimental animals
- Radon and its daughters are absorbed into the lungs by inhalation after becoming attached to microscopic particles of environmental airborne dust. Inhaled dust particles with attached radon daughters are distributed in the lungs, where they may stick to the moist bronchial epithelial lining
- Mucociliary clearance may not be rapid enough to prevent ionizing radiation (alpha particles) released from the decay of the radon daughters, polonium-218 and polonium-214, from affecting several types of pulmonary cells and eventually leading to cancerous transformation
- Uranium ore has particularly high concentrations of radium. Other types of ore (zinc, lead, fluorospar, tin, niobium, and iron) containing uranium and radium can also release radon and its daughters. When ventilation is not adequate, miners can be at risk for an increased incidence of lung tumors. Cutting uranium metal may release dust containing radon and its daughters
- Radon exposure is thought to be an important environmental cause of death
- The U.S. EPA and the National Cancer Institute estimate that there are 15,000 deaths annually in the United States from radon-induced lung cancer. Of the 164,100 cases of lung cancer diagnosed each year, approximately 14% are attributable to radon exposure. After cigarette smoking, indoor radon is the second leading cause of lung cancer
- Smokers are at greater risk for the development of lung cancer. The risk of lung cancer in cigarette smokers is 10 times that of nonsmokers
  - Lifetime exposure to the EPA recommended guideline of 4 pCi/L is estimated to pose a 1% to 5% risk for developing lung cancer depending if a person is a nonsmoker or smoker
- Radon is odorless, colorless, tasteless, and not irritating; there is no way to detect its presence other than sampling and laboratory measurement
- The dose of ionizing radiation received by the bronchial epithelium in the general population from radon is far in excess of the dose received by any other organs from natural background radiation

Source: From Ref. 20.

Table 5 Radiation Exposure from Iodine-131

Dose of radioiodine-131: 150 μCi/g (5.5 mBq/g)	
Thyroid	12,000 cGy/g
Parathyroid	140–750 range
Stomach	14.0 cGy/organ
Bone marrow	6.8 cGy/organ
Liver	4.8 cGy/organ
Gonads	2.5 cGy/organ

Low-Level Radiation and Radiation Phobia—Controversies over Biological Impact

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The fuel for effective terrorism is the fear and anxiety of the victims who do not understand when or if they will be attacked, and whether or not they will die or be permanently disabled. Radiation exposure adds to these unknown factors because it cannot be detected by any of the human senses, as one does not see, hear, taste, smell, or feel radiation. Most have very little knowledge of its effects, and their only information input is what they learn from the media, which tends to present the worse case scenario on interesting news.

There clearly are distinct differences of opinion about the effects of low-level radiation on health (21–23). The debate became very evident in a review article on radiation risk (23). A subsequent review article presented an opposing opinion (22) that is supported by an editorial response in the same issue of the journal (21) and based on a wealth of data from numerous scientific studies. A letter (24) in reply to the editorial by the author of the earlier review (22) defends three points:

1. The A-bomb survivors represent the best source of data for risk estimates of radiation-induced cancer.
2. It is clear that children are 10 times more sensitive than adults to the induction of cancer.
3. There are no assumptions, and no extrapolation indicated.

As for the experimental data available on radiation risk, a single study suggested a higher risk of cancer from low levels of radiation (25) involving a retrospective study of 11,000 children treated for tinea capitis. The incidence of thyroid cancer was higher, especially in children younger than five years. This study was retrospective and the dose range was estimated to be 4.5 to 50 rem. Retrospective studies have significant limitations as well as the considerable inaccuracies encountered in estimation of radiation exposure. A large proportion of these children received calculated doses greater than 10 rem.

All other published studies have not reached similar conclusions. A study of 14,624 infants less than 16 months of age, treated with external beam irradiation for hemangioma, did not reveal an increased incidence of cancer (26). Similarly, a Finnish study of one million children, who may have been exposed to radiation after the Chernobyl accident, did not reveal a higher incidence of cancer (27). Hjalmars et al. (28) also reported no change in cancer incidence in a study of 1.6 million children in Sweden in response to the same incident. The study by Rallison et al. (29), looking at the radiation fallout in Utah reported similar conclusion. Finally, a study of 35,074 patients who received diagnostic dose of I^{131} did not find a higher incidence of cancer (30) in irradiated people than in a comparable cohort.

In reviewing conclusions stemming from studies involving background radiation, a study in China of 73,000 persons comparing radiation doses of 96 mrem/year versus 231 mrem/year detected no difference in cancer incidence. Similarly, a study by Amsel et al. (31) compared the incidence of cancer in a population of 825,000 patients living at an altitude of 1000 ft. to a population of 350,000 persons living at 3000 ft. and did not find a difference in the incidence of cancer despite differences in radiation exposure. Another study compared four groups living at different altitudes and actually disclosed a negative dose–risk correlation (32). In the United States, a study looking at the radiation exposure of people who lived in 1730 counties also found a negative dose–risk correlation (33). A study of indoor radon exposure did not find any positive correlation (34) between radiation exposure and risk.

As for the experimental evidence from medical exposure to radiation, the analysis by Saenger et al. (35), who evaluated 33,888 patients with Graves' disease treated with either surgery or with I^{131}, revealed fewer complications in patient treated with I^{131}. Similar conclusions were reached in a study of 10,552 patients (30) and a study of 46,000 patients treated with a diagnostic dose (36) of I^{131}. None of these analyzes disclosed a higher incidence of cancer.

Concerning occupational exposure to radiation, data collected from approximately 200,000 persons (37–39) have not revealed an increased incidence of cancer, and in one of these studies the mortality rate from cancer was lower in patients who were radiated. The International Association for Research on Cancer study of 95,673 monitored radiation workers in the United States, United Kingdom, and Canada found 3830 deaths for all cancers except leukemia but no deaths exceeding those expected (40). These analyzes provided no support for the linear no-threshold theory. Finally, several studies reported that workers who inhaled plutonium had lower lung cancer mortality rates than those not thus exposed (41–43). Contrary to impressions generated by the media, no record exists of cancer deaths resulting from human exposure to plutonium. Probably the most significant data on low-level radiation exposure in humans are still being investigated (44). In Taipei and other areas of Taiwan, 1700 apartment units were built using steel contaminated with cobalt-60, exposing 10,000 occupants for 16 years to an average, according to preliminary estimates, of 4.8 rem in the first year and

33 rem in total (45). From national Taiwan statistics, 173 cases of cancer and 4.5 of leukemia would be expected from natural sources, and according to the linear no-threshold theory, there should have been 30 additional cases of leukemia. However, only five cases of cancer and one of leukemia have occurred among these people (45).

There are no statistically sound, well-designed studies that have validated the applicability of the linear no-threshold model at low doses (23). On the contrary, there is a suggestion that low-level exposure may have a beneficial effect (the so-called hormesis effect). A study of human lymphocytes showed a protective effect of exposure to low-dose ^{3}H to subsequent exposure of 150 rem of X rays (46). Shadley and Dai (47) found that preexposure of human lymphocytes to 5 rem reduces the number of DNA aberrations induced by 400 rem. Sanderson and Morley (48) found a decrease in mutagenesis. Kelsey et al. (49) found fewer mutations from 300 rem if human lymphocytes are subjected to prior exposure of 1 rem of radiation. Shadley and Wolff (50) found a decrease in the number of DNA breaks if cells are irradiated with less than 20 rem. Fritz-Niggli and Schaeppi-Buechi (51) found lower embryonic mortality rates when *Drosophila melanogaster* eggs are exposed to 200 rem. Finally, ingenious experimental techniques have been developed for observing the effects of a single alpha particle hitting a single cell. Miller et al. (52) found that the probability for transformation to malignancy from n particle hits on a cell is much greater than n times the probability for transformation to malignancy from a single hit. This is a direct violation of the linear no-threshold theory, indicating that estimated effects based on extrapolating the risk from high exposure, represented by n hits, greatly exaggerate the risk from low-level exposure as represented by a single hit.

Increasingly the view has developed that risk estimates in the low-dose region based on the linear no-threshold theory are exaggerated grossly (44). The data regarding leukemia among atomic bomb survivors (53) strongly suggest a threshold of 20 cSv (44). The evidence presented in that review leads to the conclusion that the linear no-threshold theory fails badly in the low-dose region because it grossly overestimates the risk from low-level radiation (44).

We believe that the press may have misconstrued the following statement: ''A recent analysis of A-bomb survivor data suggested a linear model down to 50 mSv and that this is the lower dose linked to a statistically significant radiogenic risk'' (54). In other independent analyzes of the same data, a curvilinear dose-response also provided a satisfactory fit to the Japanese data (55). Heindenreich, using the same data but different analytical methods (56), did not find any evidence for increased tumor rates below 200 mSv. Finally, if error bars are ignored (44), the data suggest a linear relationship with intercept near-zero doses. The data alone give no statistically significant indication of an increased risk of cancer for doses less than 25 cSv. In fact, considering the three lowest dose points alone, the slope of the dose-response curve has a 20% probability of being negative (risk decreasing with increasing dose) (44).

In some types of tumors, there is actually a decrease in cancer frequency with exposure to radiation (57). The rates of cancer in most populations exposed to low-level radiation have not been found to be increased detectably, and that in most cases the rates have appeared to be decreased (57). The same report asserts that low-dose epidemiological studies are of limited value in assessing the dose–response relationship and have produced results with sufficiently wide confidence limits to be consistent with an increased effect, a decreased effect, or no effect.

There are also medical-legal ramifications involving low-dose exposure to radiation. A recent U.S. federal court dismissed all 2100 lawsuits against GPU Nuclear Corporation that claimed radiation injury from the 1979 Three Mile Island (TMI) accident because of lack of evidence that anyone had received doses greater than 100 mGy (58). The court determined that there is consensus with the scientific community that ''at doses below 10 rem (100 mGy), the casual link between radiation exposure and cancer induction is entirely speculative.'' The Health Physics Society recommends against quantitative risk assessment of radiogenic health effects below individual doses of 50 mGy in one year (58).

In summary, risk perception is intricate as it involves fear and anxiety as well as objective hazard (59). However, an oversimplified algorithm is likely to prevent the empirical application of radiation that would (and should) be a benefit to our fellow citizens. Besides the large number of aforementioned studies, there are numerous other scientific groups (60–70) that question the validity of the linear no-threshold model. The issue of the effects of low-level radiation exposure is obviously intricate and often analyzed with considerable impact from emotional influences. Typically, the mass media exploits the fear of the unknown upon the lay public, as has been most evident among the populations exposed to ionizing radiation after the Chernobyl nuclear power plant accident in 1986.

DISPERSAL OF RADIOACTIVE SUBSTANCES WITH CONVENTIONAL EXPLOSIVES—DIRTY BOMBS

The dirty bomb is a radiation dispersion device that creates a risk from exposure to radiation and a risk of injury or death from conventional explosives, such as dynamite. By definition, a dirty bomb contains a mixture of an explosive and powder or pellets containing radioactive materials. Its purpose is two fold: to disseminate radioactive smoke, dust, or other material into the surrounding area to cause radioactive contamination of buildings and people, and to

instill fear in people who cannot see, taste, smell, or feel radiation (1,4,10).

The sources of the radioactivity used in dirty bombs may be from nuclear facilities that contain high levels of radioactive materials, or from hospitals, construction sites, and food irradiation plants, where low-level radioactive materials are available. The higher the level of radiation, the greater the problems in transport and construction of the device because of the direct radiation exposure to those preparing the device. To prevent acute radiation illnesses, the radiation source must be shielded, and to do so requires special materials and transport devices that are rather difficult to hide. Any surveillance mechanism, such as radiation detection instruments, or Geiger counters, will readily detect such radiation whenever there is routine screening or suspicion about shipment containers. It is important to know that dirty bombs are not nuclear, atomic, or neutron bombs, which are entirely different and more lethal explosive devices.

The dangers of dirty bombs are the direct effects of the explosive blast and the effects of radiation exposure. High-level radiation causes radiation sickness and severe illness within days to weeks, often causing death. More likely would be the use of low-level radiation that would not cause severe illness. The effects would depend on the dosage, type, and duration of exposure of the radioactive isotopes released. Transient bone marrow depression might occur with greater radiation exposure; an effect on the endocrine system or metabolism is unlikely, for to do so would involve the use of mCi to Ci amounts of radioactive iodine that injure the thyroid of the fetus and young children (neoplasia or atropy), or cause activation of autoimmune processes in genetically susceptible individuals.

From a historical perspective, the previous use of a dirty bomb was described in a U.N. report that Iraq tested a device in 1987. The use of the device was abandoned because the radiation levels were too low to cause significant damage.

The details for clinical management after exposure to an explosion of a dirty bomb are found online and in the literature (1–10,71,72). Briefly, humans likely will not know initially if radioactive materials were present when they are exposed to fallout from an explosion, because humans cannot see, smell, feel, or taste radiation. Exposed individuals will learn later that radiation is detected by emergency personnel at the scene of the detonation. If individuals are not injured severely, they should (i) leave the area by going to the nearest building and remain inside; (ii) remove clothes, at least the outer garments, and place them into sealed bags for testing and to avoid further contamination; (iii) shower or wash themselves as soon and as thoroughly as possible; and (iv) maintain contact with emergency medical personnel for information and advice. These procedures should help to reduce injury from chemicals and radiation.

A potentially new therapy for acute radiation syndrome (ARS) with an immune-regulating hormone was reported in the lay press as Neumune (HE2100) (73–75). Both HE2100 and an analogue (HE3204) were reported in the medical literature as immune-regulating steroid hormones that exhibit anti-inflammatory properties in a murine model that may be candidates for future therapy for autoimmune diseases (76). Studies in monkeys (macaques) using HE2100 showed a 90% survival when exposed to ''potentially fatal doses of radiation'' and treated with Neumane compared to a 55% survival for macaques that were not treated, or treated with placebo (73).

ATOMIC BOMBS

Six Decades After the First Atomic Explosion

In the six decades since the atomic bomb detonations in 1945, we have gained most of our knowledge about the acute and long-term biological effects from radiation exposure (Table 6). The magnitude of radiation exposure from the two explosions was enormous and the largest in the history of the civilized world (11). In addition to the early effects of the ARS from direct radiation exposure, late effects caused by radiation exposure have been studied for the past six decades (Table 7).

The most common late effects are malignancies that appear as early as the first decade after exposure for the radiation-sensitive thyroid gland of the fetus, infants, and young children (Figs. 1 and 2) (12,77,78), and as late as 20 to 30 years for the more common malignancies, the most frequent being breast cancer after a 20-year latent period (Fig. 3) (79). Benign parathyroid adenomas increase significantly 30 years after radiation exposure (Table 7). Cancer mortality reflects the severity of the more common malignancies, and breast cancer, is among the three with the highest risk per Sv of exposure (Fig. 4) (80).

Table 6 Atomic Bomb Detonation in Nagasaki on August 9, 1945 at 11:02 am

	Distance from hypocenter	
	500 m	1000 m
Radiation		
Gamma rays	70–80 Gy	9–10 Gy
Neutrons	7–8 Gy	0.9–1 Gy
Heat energy	111.5 Cal/cm^2	42.2 Cal/cm^2
Wind pressure	19.0 ton/m^2	8.7 ton/m^2
Wind velocity	280 m/sec	160 m/sec
Deaths before December 1945	73,884	
Atomic bomb survivors	110,716 in 1978 88,249 in 1995	
Total population in Nagasaki City	210,000 in 1945	

Source: From Ref. 11.

Table 7 Late Effects from Atomic Bomb Exposure

Diseases	Increase suspected (yr)	Increase confirmed (yr)
Thyroid adenoma	3	5
Leukemia	3	10
Thyroid cancer	7	10
Breast cancer	10	20
Lung cancer	10	20
Gastric cancer	15	30
Colon cancer	15	30
Multiple myeloma	23	30
Parathyroid adenoma	–	30

Source: From Ref. 11.

The causal relationship between exposure to radiation at doses that may be as low as 0.1 Gy (10 rad) is established through many studies (11–13,78,81–83). Exposure to external radiation is the most established pathogenetic factor in association with thyroid cancer (78,81,82), as first reported by Duffy and Fitzgerald in 1950 (84). A large percentage of the 28 cases of thyroid cancer in children and adolescents in their series had a history of external radiation therapy for benign conditions of the head and neck. The increased risk continues for decades after exposure (81). Atomic bomb detonations produce large-scale radiation exposure that increased the incidence of many different cancers. The exposure led to whole-body radiation from highly destructive neutrons and highly penetrant γ rays (Table 6) (1,11), causing the subsequent development of papillary thyroid carcinoma within as early as 7 to 10 years after exposure (77–80,82).

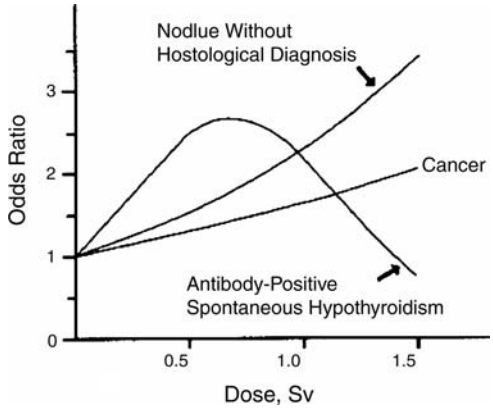

Figure 2 Thyroid disease and atomic bomb radiation. *Source*: From Ref. 77.

In addition to thyroid cancer, the risk to develop benign thyroid tumors (adenomas) increases as the radiation dose increases; however, because the radiation dose increases above 0.7 Sv, the risk for antibody-positive spontaneous primary hypothyroidism (APSPH) begins to decline steadily (Fig. 2) (77). In recent studies nearly six decades after radiation exposure, there is not a linear dose-response relationship with the development of autoimmune thyroiditis, Graves' disease and APSPH (83). In this same study, however, there is a significant linear radiation dose response for malignant and benign nodules in a population exposed to one brief exposure to radiation (83). Thus, the risk following radiation exposure during childhood seems to last for life, though it seems to decline in magnitude many years later (83).

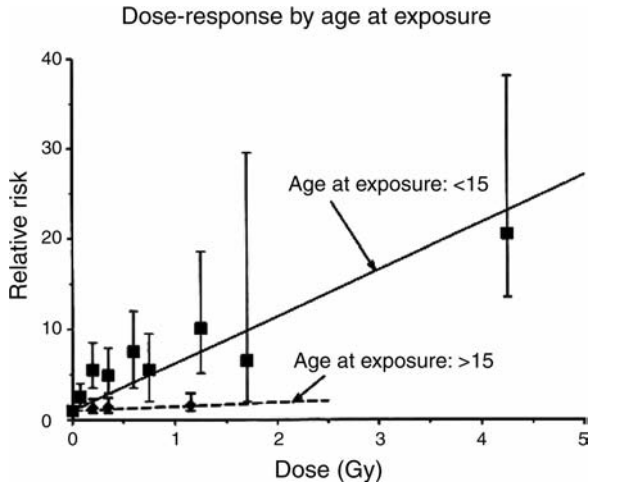

Figure 1 Relative risk for thyroid carcinoma by dose and age at radiation exposure. *Source*: From Ref. 78.

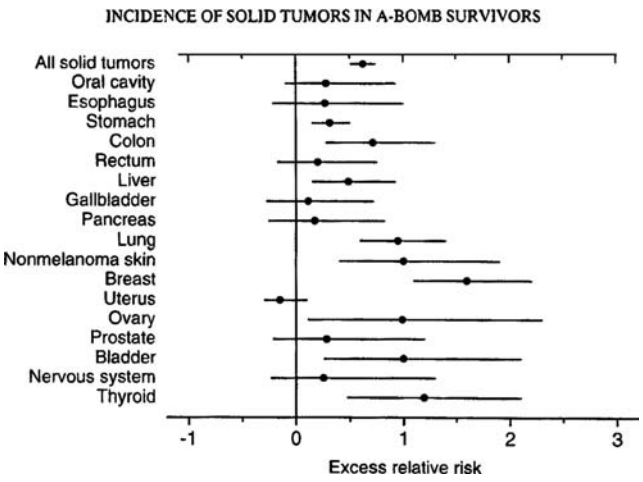

Figure 3 Incidence of solid tumors in atomic bomb survivors. *Source*: From Ref. 79.

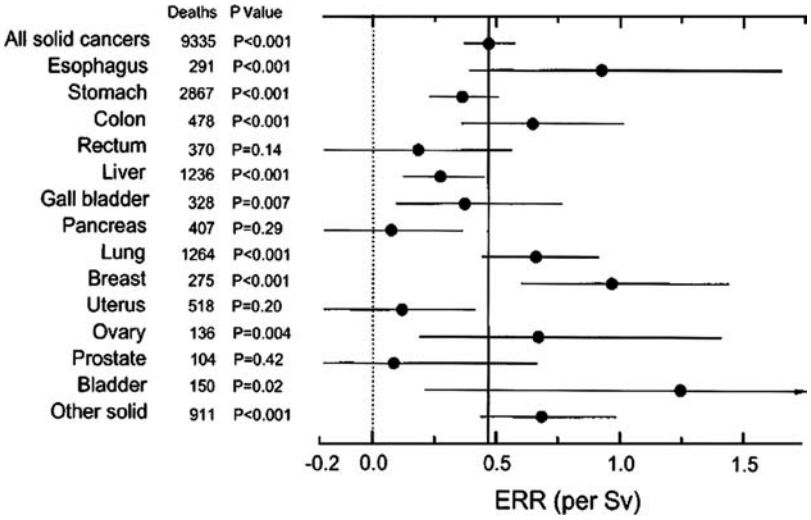

Figure 4 Atomic bomb survivors: cancer mortality 1950–1997. *Source*: From Ref. 80.

Among atomic bomb survivors who developed solid thyroid nodules, the long-term risk of thyroid cancer compared with nodule-free controls was high (7.3% compared to 0.3% in controls) (85). These data suggest that irradiated atomic bomb survivors with benign thyroid nodules need continuous observation for the development of thyroid cancer.

Following a minimum latent period of 20 years, the relationship between age at exposure to radiation and the onset of breast cancer indicates there is an increased risk when exposure to radiation from the atomic bomb occurred at a younger age (Fig. 5) (86). These data are confirmed when they are compared to the risk for breast cancer after exposure to other sources of radiation (Fig. 6) (86). Further studies examined the roles of age and reproductive history as modifiers of radiation-related breast cancer risk (Fig. 7) (87). Of 1093 breast cancers among 1059 breast cancer cases diagnosed during 1950 to 1990, a linear and statistically highly significant radiation dose response was found, confirming earlier reports. Using a modified isotonic regression approach that required only that the dose-specific (1 Sv) estimated relative risk (ERR_{1Sv}) be monotonic in age indicated that both age at exposure and attained age are important modifiers of dose response. For instance, exposure before age 20 years was associated with a higher ERR_{1Sv} when compared to older ages, which may be because these women died before they could develop breast cancer. Furthermore, with increasing attained age at exposure, the ERR_{1Sv} declined and the largest decrease was observed around age 35 years. Double primary breast cancer developed in 34 of 1059, or 1 in 31 women (3.2%) either diagnosed at the same time, or months to years later. The proportion of women who had double primary breast cancer and who were

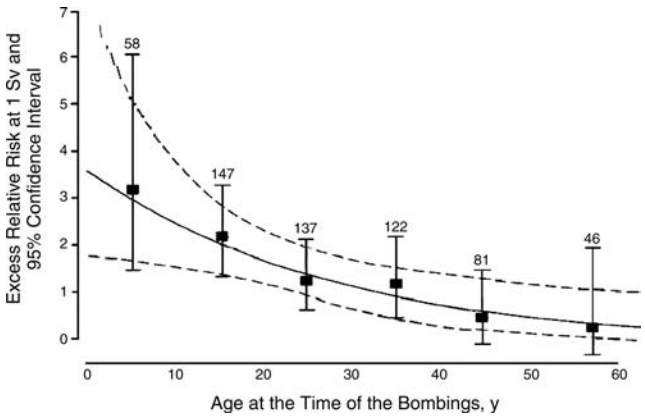

Figure 5 Risk of breast cancer versus age at exposure to radiation. *Source*: From Ref. 86.

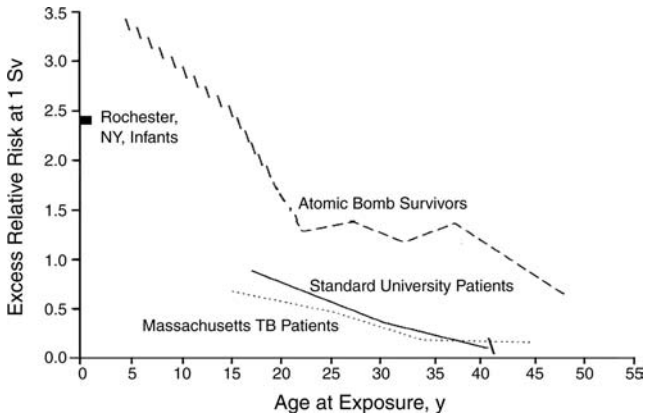

Figure 6 Age at radiation exposure and breast cancer. *Source*: From Ref. 86.

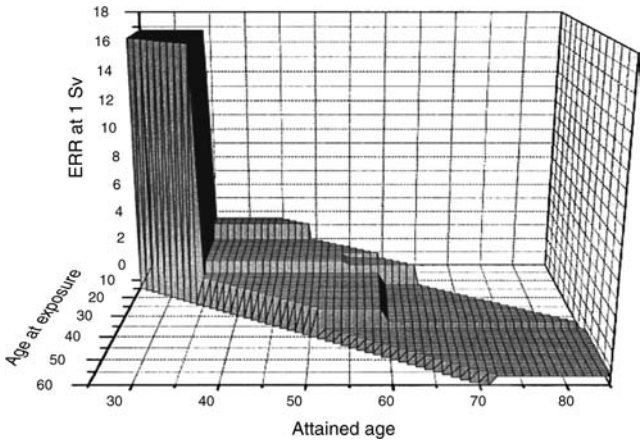

Figure 7 Atomic bomb survivors: breast cancer, 1950 to 1990. *Source*: From Ref. 87.

less than age 20 years at the time of exposure was dose-dependent. The proportion of women with double primary breast cancer who were age 20 years and older was not dose-dependent; these data suggest an increased sensitivity at younger ages of exposure (87). Therefore, exposure to radiation from an atomic bomb is associated with a much greater risk for breast cancer, especially when exposure occurs during the first two decades of life. These data are in agreement with several reports of an increased risk of breast cancer following other types of radiation exposure (Fig. 6) (16,86–88).

In summary, large populations in Japan were exposed to a single release of primarily external radiation from atomic bombs that dispersed neutrons and γ radiation. Of those who survive the initial explosion, the long-term effects on endocrine and metabolic function are predominantly focused on malignancies of the thyroid gland and breast, activation of autoimmune diseases of the thyroid, and the development of primary hypothyroidism, especially for those exposed at younger ages (Figs. 1–3) (77,80,85,87,89).

Table 8 Hypothetical Atomic Bomb Detonation in New York City

A 150-kiloton bomb constructed by terrorists is detonated in the heart of Manhattan, at the foot of the Empire State Building. The bomb goes off without warning at noon. It is a clear spring day with a breeze to the east

1 sec after detonation: blast wave 0.4 mile, fireball thermal effects 0.2 mile
4 sec after detonation: blast wave for 1 mile, buildings destroyed
6 sec after detonation: blast wave for 1.5 miles, thermal effects and fires
10 sec after detonation: blast wave extends 4 miles, damage to buildings
Long-term fallout pattern from a 150 kiloton surface burst, with a uniform 2 mph wind from the east, extends at least as far as 6 miles to the west

Clinical effects of fallout

Rem	Effects
5–20	Possible late effect; possible chromosomal damage
20–100	Temporary reduction in white blood cells
100–200	Mild radiation sickness within a few hours: vomiting, diarrhea, fatigue; reduction in resistance to infection
200–300	Serious radiation sickness effects (as above) and hemorrhage; lethal dose (LD) to 10% to 35% of population after 30 days (LD 1–35/30)
300–400	Serious radiation sickness; also bone marrow and intestinal destruction; LD 50–70/30)
400–1000	Acute illness, early death; LD 60–95/30
1000–5000	Acute illness, early death in days; LD 100/10

Source: From Ref. 9.

Terrorist Atomic Bomb

There are concerns in the scientific and lay literature that a nuclear device could be assembled and detonated by terrorists in areas of the world including the United States. Several selected terrorist-related radiation events have occurred since 1987, though none caused a large number of causalities (90,91). The sequence of events are described for the detonation of a hypothetical 150 kiloton nuclear device that is left on a street in New York City (Table 8). Within a matter of 10 seconds a large population would experience a high mortality from the explosion that stretches for four miles, depending upon the wind velocity, and major morbidity over a six-mile region from the effects of radiation fallout (92). The clinical effects relate to the amount of radiation exposure (Table 8). The shock wave, thermal radiation, and initial and one-hour residual ionizing radiation exposure from nuclear detonations are estimated from available data (Table 9). Dermatological changes, clinical symptoms,

Table 9 Approximate Distance from the Detonation Site at Which a 50% Fatality Rate Might be Expected, According to the Size of a Nuclear Weapon[a]

Yield	Shock wave	Thermal radiation (m)	Ionizing Initial	Radiation Residual[b]
0.01 kt	60	60	250	1270
0.1 kt	130	200	460	2750
1 kt	275	610	790	5500
10 kt[c]	590	1800	1200	9600

[a]Data are from Management of terrorist events involving radioactive material. NCRP report no. 138, Bethesda, MD: National Council on Radiation Protection and Measurements.
[b]Data are for residual radiation (mostly fallout) in the first hour.
[c]At yields exceeding 10 kt, the lethal range of the fireball extends several times farther than the lethal range of either the blast of the initial radiation.
Source: From Refs. 4,93.

survival, and prognosis from whole body radiation are based on the direct dose-effect in Gy from the amount and type of radiation exposure (Tables 8–12) (10–12).

Specific therapy and the degree of internal contamination will vary with the type of radiation sources based upon the specific radioisotopes emitted (Table 13). There are controversies about therapeutic recommendations, such as delineated in Table 13. For instance, there are no data that validate the use of intestinal decontamination as therapy for these patients. Clearly, ipecac-induced emesis is ineffective, may be even contraindicated. Activated charcoal adsorbs substances with a molecular weight of 100 to 1000 kilodaltons; therefore, it will not be effective with most radioactive materials. If the absorption of a radioactive substance was prevented, the substance would still be in the intestinal tract and still be exposing the patient to radiation. Furthermore, emesis or fecal matter (from the use of whole bowel irrigation or cathartics) would then expose the caregivers to the radioactive substance and also necessitate emergency department and personnel decontamination. Also, it would present significant disposal issues. The consequences on health are devastating, but well-organized emergency medical centers with properly equipped and trained personnel using state-of-the-art therapeutic modalities will greatly improve survival and lessen morbidity (1–10).

NUCLEAR POWER PLANTS

Three Mile Island Accident 1978 in Central Pennsylvania

A release of small amounts of radioactive xenon-133 gas and radioisotopes of iodine occurred after an accident on March 28, 1979 at the TMI nuclear power plant in central Pennsylvania just south of Harrisburg (Fig. 8) (95–103). An estimated average amount of whole-body dose from gamma-ray radiation was calculated to be 0.09 mSv, or 9 mrem; the maximum amount of whole-body dose from gamma-ray radiation was estimated to be 0.25 mSv, or 25 mrem, and a wide range of gamma radiation exposure from 1 to 170 mrem was estimated (101). These data are compared to the average annual effective dose of radiation from natural sources in the United States as approximately 3 mSv, or 300 mrem (101).

Table 10 Skin Changes After a Single Acute, Localized Exposure

Absorbed dose	Change
3–4 Gy	Epilation in 2–3 wk
10–15 Gy	Threshold for erythema; appears 18–20 days after exposure at lower doses; may appear within a few hours at higher doses
20 Gy	Moist desquamation, possible ulceration
25 Gy	Ulceration with slow healing
30–50 Gy	Blistering, necrosis at 3 wk
100 Gy	Blistering, necrosis at 1–2 wk

Source: From Refs. 4,94.

Table 11 Dose-Effect Relation After Acute Whole-Body Radiation from Gamma Rays or X Rays

Whole-body absorbed dose (Gy)	Effect
0.05	No symptoms
0.15	No symptoms, but possible chromosomal aberrations in cultured peripheral-blood lymphocytes
0.5	No symptoms (minor decreases in white-cell and platelet counts in a few persons)
1	Nausea and vomiting in approximately 10% of patients within 48 hr after exposure
2	Nausea and vomiting in approximately 50% of persons within 24 hr, with marked decreases in white-cell and platelet counts
4	Nausea and vomiting in 90% of persons within 12 hr, and diarrhea in 10% within 8 hr; 50% mortality in the absence of medical treatment
6	100% mortality within 30 days due to bone marrow failure in the absence of medical treatment
10	Approximate dose that is survivable with the best medical therapy available
>10–30	Nausea and vomiting in all persons in less than 5 min; severe gastrointestinal damage; death likely in 2 to 3 wk in the absence to treatment
>30	Cardiovascular collapse and central nervous system damage, with death in 24 to 72 hr

Source: From Refs. 4,94.

A TMI Registry was organized shortly after the accident and enrolled an original cohort of 35,946 individuals living within a five-mile radius of the power plant. The cohort has been followed to determine if low-level radiation exposure from the accident might increase the risk for neoplasia and nonneoplastic radiation-related disorders (100,101,103). There appear to be no statistically significant increases in thyroid or breast cancer, and no increases in autoimmune diseases, though there was little focus on the latter (103). In the months after the accident, there was a small cluster of cases of congenital hypothyroidism in the Lancaster County region of Pennsylvania that is southeast of the accident. However, most cases were familial thyroid dyshormonogenesis, and the plume from the accident traveled north from TMI. Although the latent period for neoplasias may be too early for a definitive conclusion, the TMI accident with low-level radiation emissions seems to have no adverse effects on endocrine diseases during the first two decades after the accident.

The Chernobyl Nuclear Power Plant Accident, April 26, 1986, in the Ukrainian Socialistic Soviet Republics

The worst nuclear accident in history and the largest peacetime exposure to radiation occurred at the Chernobyl (Chornobyl in Ukrainian) Nuclear Power Plant in the Union of Socialistic Soviet Republics (USSR), or the Soviet Union (Fig. 9). The power plant is located in

Table 12 Prognosis According to the Lymphocyte Count Within the First 48 Hours After Acute Exposure to Penetrating, Whole-Body Radiation

Minimal lymphocyte count (per mm^3)	Approximate absorbed dose (Gy)	Extent of injury	Prognosis
1500–3000 (normal range)	0–0.4	No clinically significant injury	Excellent
1000–1499	0.5–1.9	Clinically significant but probably nonlethal	Good
500–999	2.0–3.9	Severe	Fair
100–499	4.0–7.9	Very severe	Poor
<100	≥ 8.0	Most severe	High incidence of death even with hematopoietic stimulation

Source: From Ref. 4.

the Kyiv Oblast in the north central Ukrainian SSR, now Ukraine, and very close to the southern border with the Belarusian SSR, now the Republic of Belarus (Fig. 10). There is an abundance of scientific literature on the cause: The radioactive, political, and social consequences and the diseases that developed from the accident (12,13,17,18,27,81,104–128). In the prestigious journal, Science, there are 1431 citations that mention Chernobyl alone (as of 2/16/06) in original articles, commentaries, and letters to the editor.

According to the official Soviet statements, the explosion occurred at 1:23 am on Saturday, April 26, 1986, in reactor number 4 of the Chernobyl Nuclear Power Plant. It was operating at low power prior to a planned shutdown. An ''unexpected power surge'' as described by the Soviet Premier, Mikhail Gorbachev, 18 days later, was blamed for the incident (117). The sudden generation of heat in the graphite core of the reactor caused a surge in the output of heat from a 6% capacity to 50% in only 10 seconds. When some of the pressure tubes that circulate water around the fuel rods ruptured, there was no longer any cooling system to dissipate the heat. The fuel elements heated up rapidly and resulted in what is known as a ''meltdown.'' Large amounts of a highly combustible mixture of radioactive gases along with a rapid production of steam under enormous pressure were generated, causing the first of several explosions over a 10-day interval (12,104–107,117).

As a result of the weather conditions at the time of the explosions, most of the radioactive contamination affected the southeastern and southern areas of Belarus, western Russia, and northern and northwestern Ukraine (Fig. 10). A 30-km area in Belarus and Ukraine surrounding the power plant was evacuated during the 10 days following the initial explosion, for these areas received much of the contamination from various volatile radioactive isotopes and radioactive fallout (Fig. 10, Table 14) (12,123). Radiation exposure to humans and animals was caused by direct external irradiation, inhalation of volatile radioisotopes, and ingestion of radioactive contaminated foods, milk, and water.

The radioisotopes of iodine and cesium are present in very large quantities in a nuclear reactor (Table 15). When a meltdown and explosion occurred, these isotopes were released into the atmosphere in large quantities (12). Because certain endocrine tissues concentrate iodine, the β–radiation-emitting isotopes of iodine are of special importance, especially these potent short-lived isotopes of iodine: ^{132}T–tellurium (decays to ^{132}I),^{133}I,^{134}I, and ^{135}I constitute 40% of the radioiodines released during the first few days after the initial explosion; the remaining isotope was ^{131}I (Table 15) (123). The short-lived radioiodines and ^{137}Cs-cesium are the most potent isotopes that cause endocrine pathology, especially for the thyroid gland (12,13,18,114,115), breast (12,13,17), and other tissues that are susceptible to autoimmune diseases, such as Type 1 diabetes mellitus (Table 16) (116) and autoimmune thyroiditis (114,115).

Diseases that increased during the first decade after the accident included thyroid neoplasia (benign adenomas and differentiated thyroid carcinomas, predominately papillary) and autoimmune thyroiditis (Fig. 11) (12,113–115). In those infants and children who were the youngest at the time of exposure, thyroid cancer began to rise as early as four years after the accident; (Fig. 11) (12,113) furthermore, the younger the age at exposure, the greater number of

Table 13 Specific Therapy for Internal Contamination[a]

Radionuclide	Therapeutic approach
Tritium	Dilution (force fluids)
Iodine-125 or iodine-131	Blockage (SSKI or potassium iodide), mobilization (antithyroid durge)
Cesium-134 or cesium-137	Reduction of gastrointestinal absorption (Prussian blue)
Strontium-89 or strontium-90	Reduction of absorption (aluminum phosphare gel antacids), blockage (strontium lactate), displacement (oral phosphare), mobilization (ammonium chloride of parathyroid extract)
Plutonium and other transuranic elements	Chelation with zinc or calcium diethylenetriamine pentaacetic acid (investigational agents)
Unknown	Reduction of absorption (emetics, lavage, charcoal, or laxatives) in cases of ingestion

Abbreviation: SSKI, Saturated solution of potassium iodide.
Source: From Refs. 4,94.

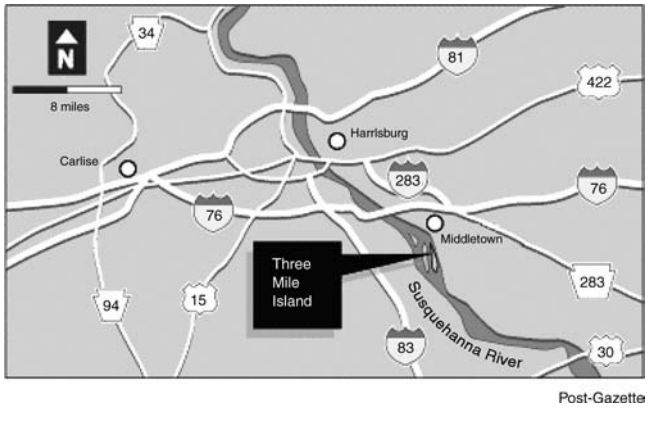

Figure 8 The Three Mile Island nuclear power plant location.

Figure 10 Greatest radiation contamination from Cesium-137 in Belarus, Russia, and Ukraine after the Chernobyl Nuclear Power Plant accident.

cases were seen. The effect of radiation exposure on the age and sex distribution of patients with thyroid carcinoma becomes quite evident when the children exposed to the radiation from the Chernobyl accident (Fig. 12) are compared to children without a history of radiation exposure (128) (Fig. 13).

There are several reasons that have been proposed to explain why the thyroid glands of the youngest children at the time of exposure are most susceptible to the neoplastic effects from radiation (12,127). The radiation dose to the thyroid gland of the fetus, infant, and young child is greater because the iodine uptake of the late-fetal/early infant gland is higher than at any other age; an even greater iodine uptake by the thyroid is found in iodine deficient populations, as existed in the radiation exposed areas of Belarus and Ukraine; and there was a proportionally greater ingestion of milk in young children. Because radioiodine, like stable iodine, is concentrated in the milk of herbivorous animals such as cows and especially goats, the radiation dose to the thyroid and breast of infants is greater (12). Furthermore, the young thyroid gland has a greater inherent sensitivity to radiation because of the growth patterns of the fetal, infant, and early childhood thyroid gland (127). Because most mutations occur during cell division and the number of cell divisions in thyroid follicular cells declines rapidly during childhood, there are very few thyrocytes that continue to replicate. They reach replicative senescence in adult life, and mutations are much less likely to occur (12,127). Thus, as Sir Dillwyn Williams, Emeritus Professor of Pathology at Cambridge University so elegantly described a few years after the accident, ''The considerable postmutagen follicular cell growth in irradiated children will allow the sequential changes leading to malignancy to occur while the limited postmutagen growth in adults with Graves' disease, together with the damage from the dose of radiation used, will greatly limit the possibility of the development of malignancy. It has been shown that external radiation to the thyroid is much more

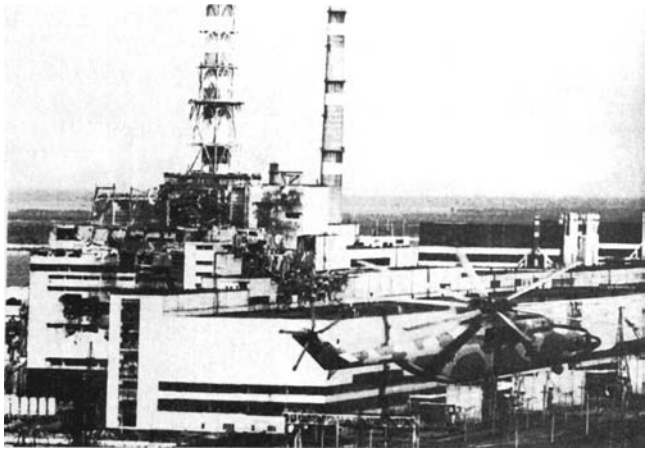

Figure 9 The Chernobyl nuclear power plant in 1986 after the accident. *Source*: From Ref. 120.

Table 14 Data from the Chernobyl Nuclear Power Plant Accident

Date	Saturday, April 26, 1986 at 01:23:48
Location	Chornobyl Nuclear Power Plant, Ukraine, USSR
Total radioactivity released by the accident	$50–100 \times 10^6$ Ci
Total radioactivity released into Belarus	70%: $35–70 \times 10^6$ Ci
Radioactive chemicals released	I, Cs, Sr, Co, Xe, Kr, Pu, etc.

Source: From Ref. 12.

Table 15 Iodine Isotopes Hazardous to Humans

Iodine isotopes which are potential hazards				
Mass number	Half-life	Yield[a] (%)	Saturation inventory[b] Ci/megawatt (thermal)	Contribution to total thyroid dose in first few days[c] (%)
131	8.05 d	2.9	2.5×10^4	60
132	2.3 hr	4.6	3.8×10^4	–
133	20.8 hr	7.2	5.6×10^4	30
134	0.88 hr	10.0	6.6×10^4	–
135	6.7 hr	8.4	5.1×10^4	–

[a]Sum of direct and decay yields.
[b]Curies of isotope present in the reactor at equilibrium per megawatt energy produced.
[c]^{132}I, ^{134}I, ^{135}I contribute the remaining 10%. After a few days, almost all of the dose is from ^{131}I.
Source: From Ref. 123.

carcinogenic in infants than in adults, and it seems likely that the same is true for radiation from Iodine-131'' (127).

Therefore, populations at greatest risk for the development of thyroid neoplasia after exposure to ionizing radiation are the fetus (after fetal age of approximately 11 weeks of gestation when iodine transport becomes expressed) and infants with iodine deficiency who are exposed to greater than 1 cGy of radiation (Table 17). It should be noted, however, that very high doses of radiation cause thyrocyte damage, apoptosis, and hypothyroidism.

The carcinogenic effects from radiation after release from a nuclear power plant seem to be the result of exposure to the volatile, potent short-lived isotopes of iodine, especially ^{133}I, but also ^{132}I,^{134}I, and ^{135}I by inhalation and ingestion, and ^{131}I, present in the greatest quantity, by inhalation, ingestion, and some external radiation exposure (Table 18). Chronic amounts of Cesium-137 in the environment cause exposure by external radiation and ingestion through contaminated foods and water. Because specific tissues concentrate iodine, a higher radiation dose to iodine-concentrating tissues causes radiation-induced molecular changes and an increase in the incidence of thyroid adenomas and carcinomas (13,18). Carcinoma of the breast statistically increased among atomic bomb survivors after a 20-year latent period (11,79,87); the time following the Chernobyl accident is as yet insufficient to know if there will be an increase in breast cancer among those whose breast tissue was exposed to the greater doses of radiation (Table 17) (12), though recent data suggest that to be the case (129).

Table 16 Type I Diabetes Mellitus After Exposure to Radiation from the Chernobyl Accident

DM-I in Gomel Oblast, Belarus, age <15 yr	
Incidence before 1986	3.8/100,000
Incidence after 1986	5.7/100,000
Incidence ages 10–14 after 1986	9.3/100,000
Average yearly increase after 1986	8.9%
Note: Average increase/incidence ratio was the highest compared to bordering countries	

Source: From Ref. 116.

Radiation carcinogenesis and genome instability have been reviewed in depth (12,16,17,52,66,81,88, 130–137), molecular mechanisms are reported in detail for radiation-induced thyroid papillary carcinoma after the Chernobyl accident (Table 19) (12,16,17, 19,81), and investigations in the basic molecular mechanisms for radiation-related breast carcinogenesis are described in detail (138–141). Most of the Chernobyl-related molecular abnormalities are chromosomal rearrangements that cause constitutive activation of the tyrosine kinase (TK) receptor domain (Fig. 14) (142–144). The mechanism of radiation-induced rearrangements occur between a TK receptor and an activating gene, the signal peptide (Fig. 15) that leads to formation of chimeric transforming sequences (Fig. 16A,B) (142,143). Constitutive activation of the tyroxine kinase receptor contributes in part to transformation from normal cell replication to neoplastic transformation, associated with benign neoplasia and malignancy. The resultant chimeric RET protooncogenes associated with papillary thyroid carcinoma (PTC) are

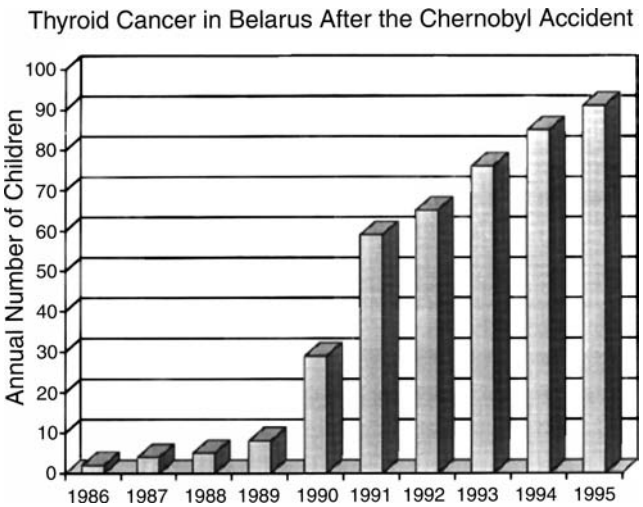

Figure 11 Thyroid cancer in Belarus after the Chernobyl accident. *Source*: Courtesy of Foley TP.

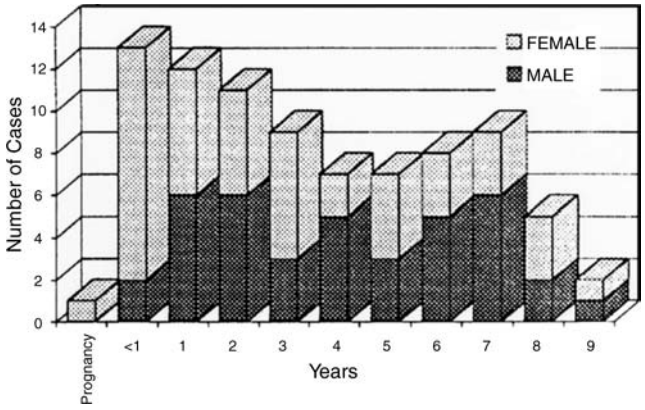

Figure 12 Age and sex distributions of patients of the time of the Chernobyl accident who subsequently developed thyroid cancer. *Source*: From Ref. 128.

expressed in transformed thyroid cells as RET/PTC subtypes (144), and specific RET gene rearrangements, RET-PTC1, and RET-PTC3 (Figs. 16 and 17A,B) are found in PTC specimens from those exposed to radiation from the Chernobyl accident as children and adolescents (Table 19) (12,145–149).

The nuclear power plant accidents at Three Mile Island in Pennsylvania and at Chernobyl in northern Ukraine have had a dramatic adverse effect on the nuclear power plant industry (122). The similarities of the two accidents emphasize the importance of the skill, training, security, and operational procedures of power plant personnel (122). The public is educated poorly about the peaceful use of nuclear energy, about the need to promptly switch from dependence upon fossil fuels to nuclear sources of energy, and, thus, about the critical importance of nuclear energy for the future of our global society. The nuclear energy generated by new reactors is clean, efficient, safe (because there are new and

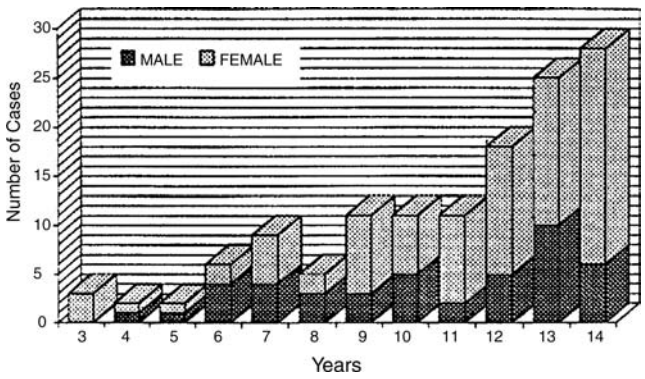

Figure 13 Age and sex distributions in children with thyroid cancer without a history of prior radiation from a collection of 131 cases in the literature. *Source*: From Ref. 128.

Table 17 Populations at High Risk for Thyroid Neoplasia from Exposure to Radiation

Fetus after 12 wk of gestation in April 1986
Number of cases dramatically decreased after 2000
Children ages <6 yr in April, 1986
Children ages 0 to 1 yr have the highest risk
Children living in areas with iodine deficiency
Greater radioiodine uptake increases the dose of radiation exposure to the thyroid gland
Children exposed to >1 cGy of radiation
Rapid thyroid growth in young children occurs with chromosomal rearrangements (PTC1 and PTC3)

Abbreviation: PTC, papillary thyroid carcinoma.

advanced nuclear reactor designs), and requires no use of fossil fuels. Along with solar energy and the energy from fuels derived from renewable products like corn, nuclear energy currently is the most desirable form of energy generation worldwide. There is evidence that the nuclear power energy industry is experiencing a rebound, as there are reports that more nuclear power plants with advanced design for vastly improved safety and efficiency are in production (150). In the meantime, security remains a critical component of design and maintenance in order to avoid terrorist attacks and human error.

MANAGEMENT OF PREGNANT WOMEN AND CHILDREN

The younger the child, the greater the sensitivity to ionizing radiation. This sensitivity relates in part to the rate of cell division and inversely with the degree of cell differentiation (1). Thus, the fetus is the most sensitive human subject, and the sensitivity declines with increasing age (4). Furthermore, the sensitivity of tissues vary (1) from the most sensitive to the least is lymphoid greater than gastrointestinal greater than reproductive greater than dermal greater than bone marrow greater than nervous system. Additional factors that influence the consequences of radiation exposure are dose to tissues and the types of radiation (Table 2) (1,4,15,16). In this review, we are concerned about the effects of ionizing radiation from sources other than natural or medicinal exposures on endocrine and metabolic tissues, and for subjects from fetal life through adolescence. The types of radiation depend upon the specific radionuclides released after a radiation disaster, and are summarized in Table 20 (1–10,104,111,124,126,151–153).

Table 18 The Carcinogenic Effects of Radiation

The isotopes of iodine-131, -132, -133, -133, -135
Chronic exposure to Cesium-137
Higher tissue exposure to radiation occurs in those tissues that concentrate iodine
Thyroid $\rightarrow \uparrow$ incidence of adenoma, carcinoma
Breast $\rightarrow \uparrow$ incidence of carcinoma
Salivary gland
Gastric mucosa

Table 19 Chernobyl Related Thyroid Tumorigenesis

RET gene rearrangements are found in 50% to 90% of the children with PTC from Belarus and Ukraine
RET-PTC1 and RET-PTC3 chimeric proto-oncogene rearrangements
Early affected children usually have the RET-PTC3 rearrangement
Constitutive activation of tyrosine kinase receptor genes
Mutations
No gsp or TSH-R mutations
Rare RAS and TP53 (p53) mutations
Genetic predisposition
? Defective DNA repair
? Second, possibly preexisting, ? germ line mutation
ret/PTC-1
More common in tumors from older children and adults
More common when tumors develop in children >10 yrs after the accident
Less aggressive and have the typical papillary morphology
ret/PTC-3
More common when tumors develop in children <10 yrs after the accident
More common the younger the child at exposure
Aggressive tumor behavior in vivo and in vitro
Atypical solid variant of PTC with less differentiated morphology

Abbreviation: PTC, papillary thyroid carcinoma.
Source: From Refs. 143–148.

Prevention of radiation-induced endocrine and metabolic diseases is directed primarily to blocking radioiodine exposure to tissues from the radioisotopes of iodine that cause external and internal radiation exposure, and, to a lesser extent, external radiation from ^{137}Ce-cesium and ^{60}Co-colbalt. The use of oral stable iodine to block radioiodine uptake by sensitive tissues was reported from the iodine prophylaxis program in Poland within hours after the Chernobyl accident (Table 21) (124). Iodine in the form of potassium iodide (KI) or saturated solution of KI and I_2 (SSKI) should be administered early (within 36 hours of radioiodine release) to reduce the radiation dose to the thyroid and breast. The KI dose varies with age (Table 22) and is effective in reducing radiation exposure when KI and substituted (nonradioiodine containing) milk are used. No serious side effects were observed (Table 21) (124).

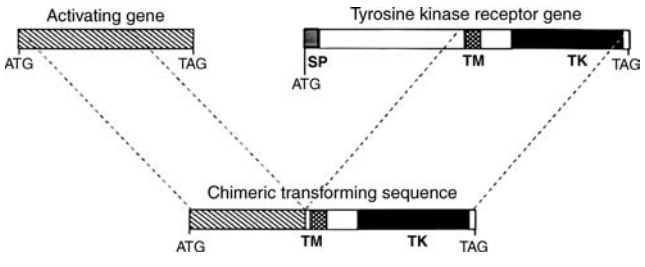

Figure 14 Mechanism of rearrangement between a TK receptor and an activating gene leading to the formation of a chimeric transforming sequence. *Abbreviations*: SP, signal peptide; TM, transmembrane domain; TK, tyrosine kinase receptor domain. *Source*: From Ref. 142.

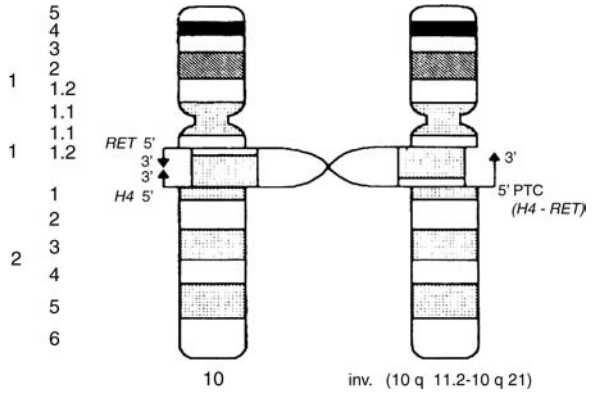

Figure 15 Chromosome 10 RET rearrangements and paracentric inversions = H4-RET chimeric papillary thyroid carcinoma oncogene. *Source*: From Ref. 143.

Procedures to considerably reduce radiation exposure specifically from radioiodines (1,4,7–10,104, 111,124,127,152,154) and from other sources of ionizing radiation (2–10,152) are designed to provide priority protection to the fetus and young children. For radioiodines, evacuation and KI are priority (Table 22). Prepackaged and predistributed KI tablets or liquid should be readily available in homes, schools, day care centers, nurseries, and public health distribution centers near nuclear reactors in proper dosage packages (Table 22). On notification by authorities of the probability of radiation exposure, parents should dispense KI, or have given authorization to child care personnel to dispense KI whenever authorities declare a radiation-exposure emergency. Families should have single-dose KI packaged as a solution for infants; if not, there are directions for the preparation of oral solutions of KI at home from 65- and 130-mg tablets for convenient and accurate administration to infants and children who cannot or will not take tablets. These guidelines are published by the U.S. Food and Drug Administration to provide sufficiently accurate solutions that can be prepared at home in a short period of time (1).

Of interest are the observations that continued intake of KI even when given months after the Chernobyl accident when there no longer was exposure to radioiodines reduced the risk of thyroid cancer (18,83). The explanation is unknown, but may be the normalization of iodine nutrition and reduction in endogenous TSH stimulation to thyrocytes damaged by prior radiation exposure.

Toxicity to KI is negligible except in very rare cases of iodinism. However, there are safety issues and procedures that are important for inclusion in educational material for prospective parents, pregnant women, and parents of infants and children (Tables 22–24). Public health organizations, emergency medicine organizations, and the general public should

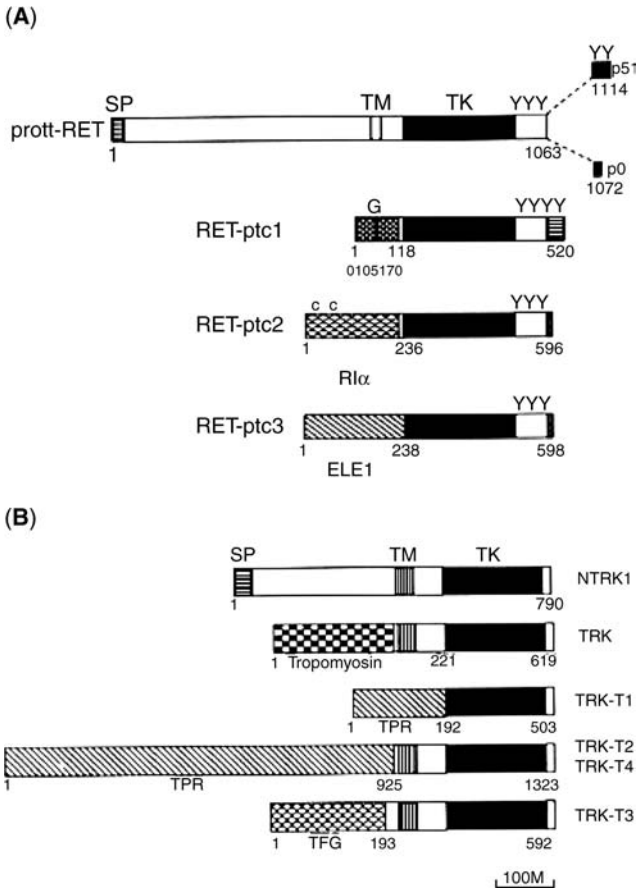

Figure 16 Products from rearrangements: activation of the tyrosine kinase receptor proto-oncogene: (**A**) RET; and (**B**) NTRK1. *Abbreviations*: SP, signal peptide; TM, transmembrane domain; TK, tyrosine kinase receptor domain. *Source*: From Ref. 142.

be informed about the basic principals of preventive procedures in advance of a radiation disaster, especially those living near nuclear facilities (Table 25). Other diagnostic procedures are important for emergency medicine organizations, physicians, and hospital personnel for subjects of any age who are exposed to radiation even if KI has been administered. KI only protects tissues from radioiodines. Exposure from other radionuclides is not affected by KI, and specific measures to assess the magnitude of radiation exposure are very important for clinical management (Table 26).

GLOBAL HEALTH EDUCATION ON RADIATION AND TERRORISM

Program Philosophy and Description

In an effort to provide educational opportunities to learn about radiation and how to prepare and counter the abuse of radiation in the form of terrorism against mankind, we will describe the activities of the Global Health Network Super course Project (158). Super course is not a traditional course, but it is a library of lectures designed to supplement teaching for educators across the world. Both students and faculty members can use the Supercourse to supplement instruction and to learn more about various disciplines within public health, including radiation and terrorism. As of February 2006, The Supercourse has in its network more than 31,000 faculty members from 151 countries who have contributed more than 2500 lectures. Over 100 lectures in the Supercourse collection are dedicated to the topics of terrorism, bioterrorism, radiation, and related issues. Supercourse ''teaches the teachers'' in areas in which they are not familiar, and therefore eases lecture development. Moreover, quality is monitored with statistical quality control of Deming. By using low-bandwidth Internet, the Supercourse can reach scholars in the remotest areas of developing countries. The latest research can speed from journals into the classroom by direct linkage of lectures with journal articles (159). Supercourse has been funded three times by NASA and then a RO1 by the National Library of Medicine (NLM).

Our philosophy is one of sharing our best PowerPoint lectures with each other. Supercourse is a repository of scientific communication, in much the same way as the Library of Congress or your university library. The lectures are graphically presented research communications, much like iconic book

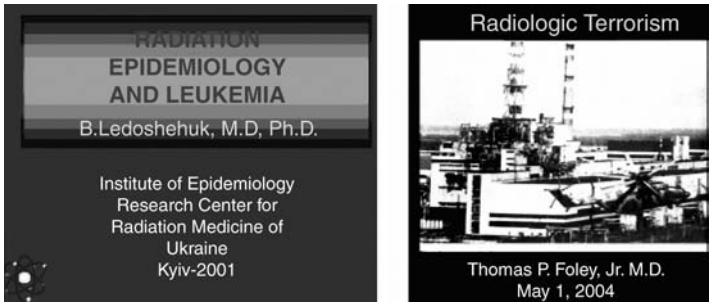

Figure 17 Radiation lectures in the supercourse. *Source*: From Refs. 160,161.

Table 20 Radionuclides Produced after a Radiation Disaster

Symbol	Source	Radiation	Respiratory absorption	Gastrointestinal absorption	Primary toxicity	Treatment
^{241}Am	NWD	Alpha	75%	Minimal	Skeletal, liver deposition, bone marrow suppression	DTPA, EDTA
^{137}Ce	MF	Beta, gamma	Complete	Complete	Whole body irradiation	Prussian blue
^{60}Ce	MF, FI	Beta, gamma	High	$<5\%$	Whole body irradiation	Supportive
^{131}I	NWD, NPP	Beta, gamma	High	High	Thyroid ablation, cancer	Potassium iodide
^{32}P	MF	Beta	High	High	Rapidly dividing cells	Aluminum hydroxide antacids
^{238,239}Pu	NW, NWD	Alpha, gamma	High	Minimal	Lung, bone, liver	DTPA, EDTA
^{90}Sr	NWD	Beta, gamma	Limited	Moderate	Bone-follows calcium	Supportive

Abbreviations: NWD, nuclear weapon detonation; MF, medical and research facilities; FI, food irradiation facilities; NPP, nuclear power plants; NW, nuclear reactor waste sites; DTPA, diethylenetriaminepentaacetic acid; EDTA, ethylene-dinitrillo tetraacetic acid.
Source: From Refs. 1,153.

chapters. The major strength of the research is the network of top scientists that has been established. Supercourse has a growing network of scientists interested in radiation and terrorism. Because of the power of this network, core Supercourse developers in Pittsburgh were able to develop and implement NATO-funded Advanced Research Workshop on the topic of prevention and mitigation of man-made and natural disasters. Scientific networking was highlighted as one of the major means to improved disaster prevention and mitigation. In the Supercourse, strategies are changing continuously and to enhance and to encourage diversity of lecture authors and lecture users. The ultimate goal is to bridge the digital divide and promote information exchange with remote areas of the globe.

Implementation

Supercourse developers always concentrated on building a culturally diverse group of project developers, lecture donors, and lecture users. Our coordinating team has scientists who originally came from India, Pakistan, Iran, Russia, Ukraine, etc. One of the first radiation lectures in the Supercourse came from a Ukrainian investigator and discussed the issues related to epidemiological aspects of leukemia in lieu of Chernobyl nuclear accident (Fig. 17). Reaching underrepresented minority investigators, especially African-American and Latino investigators, is one of the key goals of this project. Specifically, Supercourse has over 4000 contributors from India, over 500 from the countries of the former Soviet Union, over 1000 from Latin-American, and Caribbean countries, etc. with many of them highly interested in seeing more educational materials in the area of radiation and terrorism.

Through the work of the Supercourse, we have seen that networking of scientists from various disciplines is an important aspect of disaster forecast and prevention. Since the break down of the Soviet Union in 1991, man-made disasters became a very real possibility with over 7000 poorly guarded nuclear warheads in Russia and other former Soviet Union (FSU) countries. Collapse of public health infrastructure in the countries of the FSU became a realistic threat to the health of the people in the FSU, Europe, Mediterranean region, and worldwide. There is a

Table 21 Iodine Prophylaxis in Poland

Radiation detected 36 hours after initial release
KI distribution began in the PM on day 3
10.5 M doses of KI given to children
7 M doses of KI given to adults
Exposure to radioiodines in infants age <1 yr
>50 mSv (5 rem) if unprotected from radioiodine
<50 mSv when protected by KI + substituted milk
KI causes approximately 40% reduction in rem dose to thyroid; with early prophylaxis, approximately 60% to 70% reduction in rem dose primarily because inhaled 131I is blocked
Incidence of 0.2% for medically significant, but not serious side effects

Abbreviation: KI, potassium iodide.
Source: From Ref. 124.

Table 22 Guidelines for KI Administration[a]

Patient	Exposure, Gy (rad)	KI dose (mg)
>40 yr of age	>5 (500)	130
18 through 40 yr of age	$\geq$0.1 (10)	130
Adolescents 12 through 17 yr of age[b]	$\geq$0.05 (5)	65
Children 4 through 11 yr of age	$\geq$0.05 (5)	65
Children 1 mo through 3 yr of age[c]	$\geq$0.05 (5)	32
Birth through 1 mo of age	$\geq$0.05 (5)	16
Pregnant or lactating women	$\geq$0.05 (5)	130

[a]KI is useful for exposure to a radioiodine only. KI is given only once to pregnant women and neonates unless other protective measures (evacuation, sheltering and control of the food supply) are unavailable. Repeat dosing should be on the advice of public health authorities.
[b]Adolescents weighing more than 70 kg should receive the adult dose (130 kg).
[c]KI from tablets or as a freshly saturated solution may be diluted in water and mixed with milk, formula, juice, soda, or syrup. Raspberry syrup disguises the taste of KI the best. KI mixed with low-fat chocolate milk, orange juice, or flat soda (e.g., cola) have an acceptable taste. Low-fat white milk and water did not hide the salty taste of KI.
Abbreviation: KI, potassium iodide.
Source: From Refs. 1,154.

Table 23 General Management of Pregnant Women and Children Exposed to Radiation

Priority evacuation
Priority evacuation protocols for pregnant women, infants, and prepubertal children
Priority identification signs should be provided to pregnant women and families of infants and prepubertal children to display on the windshield for priority rapid "HOV-lane" emergency evacuation
Evacuation routes should be defined in advance
Evacuation to an identified location at least 50 miles from the source of radiation
Potassium iodide (KI)
KI tablets or liquid administered on notification by authorities of the possibility of radiation exposure
Dose schedules and negligible toxicity
Distance from radiation source
The radiation plume travels in the direction and at the speed of the prevailing winds
Biologically significant radiation exposure may occur 100–200 miles from the source depending upon the atmospheric conditions

Table 25 General Recommendations for Management of Children Before and After Exposure to Ionizing Radiation

Preparation
Maintain supplies of KI, infant formula, and powered milk
Plan evacuation routes and the locations of radiation-free destinations
Emergency battery-operated communications
Maintain a supply of fully charged batteries
Battery-operated radios
Cellular telephones with automobile charging devices for cigar lighter receptacles
Priority evacuation
Defined priority routes (HOV Routes)
Priority evacuation identification on vehicles (ID Cards for bumpers and windows)
Potassium iodide
Priority 1: Pregnant women and infants
Priority 2:Young children
Monitor TSH in infants and pregnant women receiving KI

Abbreviation: KI, potassium iodide.

growing network of FSU scientists who are interested in the Supercourse and preventing the threat of disasters through the simple means of information sharing. Many of them view the Supercourse as one of the best on-line resources in the area of radiation and disasters. Different strategies that our group is utilizing to ensure diversity of materials on terrorism and radiation in the Supercourse are outlined in the next few paragraphs.

E-Recruiting to Reach Potential Participants Quickly and Effectively

On the Supercourse faculty are some of the world leaders in a new field called e-recruiting, which is defined as a methodology that uses the characteristics of the Internet to rapidly recruit faculty and students into the activity. In little more than five years, the number of members of the Supercourse network jumped from 100 to almost 32,000. In addition, by applying the same recruitment approaches in the Department of Epidemiology at the University of Pittsburgh, there has been close to a 10-fold increase in the numbers of accepted students. To inform the scientific community about these recruitment approaches, the program was reported in depth (162). New and exciting tools for recruitment were developed, such as viral marketing, and these tools will be utilized to increase the numbers of radiation experts in the Supercourse network and lecture library.

Just-in-Time Lectures

The Supercourse also has pioneered the development of what is known as Just-in-Time (JIT) disaster lectures. The JIT lectures represent ''information on demand'' in that the lectures are placed on the web within days of an event. This effort began after a 16-year-old boy crashed his small plane into a bank one week after Sept. 11, 2001. The first JIT lecture was one on airline safety and demonstrated that

Table 24 Issues Regarding Pottasium Iodide

KI toxicity
Acute poisoning is uncommon
Hypersensitivity reactions are rare, but potentially dangerous Angioedema and laryngeal edema
Serum-sickness-like reactions (fever, lymphadenitis, arthralgia, and arthritis)
Chronic exposure
Iodism: Parotid pain and swelling
Goiter and primary hypothyroidism on occasion at any age Contraindicated during pregnancy and infancy
Safety of high-dose KI: Recommended Treatment of Sporotrichosis
Children: 50 mg/dose tid; ? by 50 mg/dose daily
Children: 150–500 mg/dose up to 500–750 mg tid
Older child: 250 mg tid; maximum: 1–2 g/dose tid

Abbreviation: KI, potassium iodide.
Source: From Refs. 152,155–157; courtesy by Dr. Edward Krenzelok, Director, Pittsburgh Poison Center.

Table 26 Recommended Diagnostic Measures to Consider for Victims after Exposure to Radiation

Test	Timing
Nasal swab to identify inhalation[a]	Immediately
Skin swabs to identify external contamination[a]	Immediately and at frequent intervals
Urine and stool analysis to identify internal contamination[a]	Immediately and at 24 hr
Complete blood cell and platelet counts	Daily for 1 wk
Absolute lymphocyte count	Every 12 hr for 3 days
HLA antigen subtyping	Before lymphocyte count decreases
Lymphocyte cytogenetics	Before lymphocyte count decreases

[a]A radiation safety officer or other authority should be consulted in all aspects of management.
Source: From Refs. 1,153.

airline travel was still one of the safest forms of transportation. The second time we employed the JIT lecture methodology was with SARS (163). Within two days after the first SARS case was identified, a lecture was developed on the subject by a faculty member of the Supercourse and updated every two days. The Supercourse group also developed JIT lectures for hurricanes Katrina and Rita, Bam Earthquake, Tsunami in South Asia, and other natural and man-made disasters. JIT lectures have enormous power to educate the public and help to reduce fear and anxiety.

Lecture Translation

Historically, lecture translation for the Supercourse was carried out without cost by the Supercourse faculty members worldwide. Instead of centralized and costly translation efforts, global faculty members worldwide translated the lectures that were considered the most appropriate for teaching in their region. This approach conserved costs and time expenditure for investigators and staff. For example, the Supercourse's ''Golden Lecture of Prevention,'' released in 2003, was translated into 14 languages, including Spanish, French, Russian, Chinese, and others at no cost. The estimated cost, were this lecture translated by NIH, would have been at least $4000. Several of the Supercourse's modules on terrorism were translated into Spanish by our collaborators from Latin America at no cost. Having course content available in multiple languages has encouraged diversity and broadened the audiences of the course.

There clearly is need to assist faculty members whose English proficiency is limited. The power of machine translation is used to achieve this purpose, as discussed in the late 1990s (164,165). Machine translation subsequently underwent significant improvements. The Google search engine currently utilizes powerful machine translation software. The power of the machine translation could be harnessed in the future development of multilingual courses.

Establish a National and Global Distribution System

The best content on global health research and methods in the world is of little use unless it is distributed. The website for the Supercourse (158), has been an essential tool for the dissemination of culturally diverse content, including educational modules about radiation and terrorism. In addition, there are 42 mirrored servers worldwide for access to lectures within each country. However, the Internet covers only about 10% of the world. Thus, Supercourse has established a system of CD distributions for the courses. About 10,000 CDs of the Supercourse have been distributed over the past five years. The CDs are sent to academic scientists worldwide, and they are asked to distribute the CDs to at least individuals whom they know would use and distribute them.

Outcomes

The main achievement of the Supercourse is its multicultural and multilingual network of faculty members. Among the members of the network, the Supercourse has six Nobel Prize Laureates in Medicine, the U.S. Surgeon General, 60 Institute of Medicine members, and other national and international public health and radiation researchers. Among the Supercourse lectures on terrorism, are a series of educational modules from Johns Hopkins University, dedicated to the topic of identification of bioterrorism agents (166).

Students and faculty members utilizing the Supercourse respond with very positive reviews about the Supercourse lectures. Recent studies of the Supercourse project demonstrated that Supercourse is viewed very positively by lay audiences and well-established investigators. In addition to formal evaluations of lectures on-line, the Supercourse receives informal letters from their users.

As a part of the Supercourse development, many important lessons were learned. Fiscal issues are not one of the biggest factors that influence the development of a high-quality educational curriculum. The Supercourse has established one of the largest lecture repositories in public health, terrorism preparedness, and radiation with very little expenditures. Authors were very happy to donate their lectures at no cost. The ability to have lecture materials exposed to thousands of students and faculty members around the globe became a strong drive for lecturers to donate their teaching materials.

SUMMARY

Globalization of Research—The Need for a Multidisciplinary Approach

We are locked into the silos of our occupations with little transdisciplinary work and little diversity. With an approach toward distributed collaboration in public health, our programs encourage diversity in public health education around the globe. Diversity in this context will include the encouragement of collaborative research educational efforts among public health professionals and radiation researches.

Increasingly, health concerns are global and diverse. The health of humans is impacted by a range of boundary-crossing risk factors: Destruction of the ozone layer might be related to melanomas in Auckland and California; global migrations bring new patterns of disease; terrorism is a risk in many areas; SARS showed its potential to spread quickly; and world trade of tobacco spreads health risk across cultures. Despite the globalization of disease, research, and education in the area of radiation and terrorism preparedness have not kept pace. Simple preventive approaches, such as encouraging the development and exchange of educational modules in the area of

radiation and terrorism, are just as important as high-tech research.

The Internet is the most powerful tool for global research, communication, and teaching and the translation of radiation research in the classroom. It is a transparent and cost-effective medium that is becoming ubiquitous. It is a user-friendly medium, breaking down the hierarchy between professor and student, no matter where the student or the teacher is. With the power of the Internet, the best scientists of the world are recruited to foster improved information exchange in the area of terrorism preparedness and radiation. By utilizing successful approaches of networking, Supercourse became one of the largest repositories of knowledge and diverse educational approaches in the area of public health. The Supercourse library of lectures (158) provides and promotes educational modules to the Global Health Network.

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32

Using the Web to Obtain Information on Genetic and Hormone Disorders

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INTRODUCTION

New findings on the genetic basis of endocrine disorders are reported at an ever-increasing rate in a growing variety of books, journals, and other periodicals. Unfortunately, access to this and other current information on clinical and laboratory findings of familial endocrine disorders, and labs that perform genetic tests, cannot be found in a single journal or text (1). Only electronic databases can provide medical professionals rapid access to the bulk of current information and data. These electronic databases can also be searched in an interactive way to identify conditions that cause symptoms and signs, to permit generation of differential diagnoses that will often include rare or recently discovered disorders that many endocrinologists probably have never encountered. Use of these databases can enable all endocrinologists to more frequently diagnose cases and be aware of subtleties that differentiate alternative diagnoses. This chapter provides information on how to use the World Wide Web (www) for information about genetic and hormone disorders.

Online Mendelian Inheritance in Man

Online Mendelian Inheritance in Man (OMIM) (2) is maintained by the National Center for Biotechnology Information (NCBI). OMIM is available without charge at hypertext transfer protocol (http):// www.ncbi.nlm.nih.gov/Omim. It is updated daily. On January 30, 2006, there were 16,528 entries included. These include 852, which contain information on hormones and 563, which relate to endocrine disorders. A wealth of information is contained about the history, signs, symptoms, diagnosis, management, and research findings in these 16,528 genes and genetic disorders, as are detailed gene and disease-focused maps. Access through hyperlinks to a variety of Web sites is another important strength. These hyperlinks include pubmed, genome databases, locus specific mutation databases including androgen receptor mutations database, glycogen storage disease type II (Pompe disease) mutation database; model organisms, and phenotypes and clinical resources including geneclinics, genetests, genetic alliance, and human gene mutation database, and additional resources including Cancer Genome Anatomy Project, coriell cell repositories, Entrez: integrated access to MEDLINE, nucleotide sequences (including GenBank), protein sequences, three-dimensional structures, and genomes; Mitomap: A human mitochondrial genome database and online Mendelian inheritance in animals. The utility of these databases and their hyperlinks in clinical applications will be illustrated in this chapter.

Frequently Used Terms Related to the Web

Knowing the definitions of terms that will be helpful in using the www is a good first step in learning how to use Web (3).

E-mail: Abbreviation for electronic mail, which is used as a network to send and receive messages.

HTML: Abbreviation for hypertext markup language, which enables authors to insert hyperlinks. Clicking on a hyperlink displays another HTML document. Therefore, in a hypertext system one can navigate by clicking hyperlinks, which produces a display of another document that also contains selected hyperlinks.

http: This is the Internet standard supporting exchange of information on the www. Http enables the embedding of hyperlinks in Web documents. Http also defines the process by which a Web client uses a Web browser program to generate a request for information and send it to a Web server, which is a program designed to respond to http requests and provide the desired information.

Hypertext: A computer text form that allows readers, by clicking on the hyperlink, to display another HTML document that may also contain hyperlinks to other related documents.

Internet: The worldwide system of linked computer networks that facilitates data communication services such as remote log on, file transfer, e-mail, the www, and news groups. The Internet assigns

every connected computer a unique Internet address so that any two connected computers can locate each other on a network and exchange data.

Netscape Communicator: A package including a popular Web browser called Netscape Navigator that is available for Microsoft Windows, Macintosh computers, and a variety of Unix workstations.

Surfing the Net: Exploring the www by following a series of hyperlinks of interest to the surfer.

URL: Abbreviation for uniform resource locator. On the www, URLs are a string of characters that precisely identifies an Internet's resource types and locations. The following fictitious URL identifies a www document (http://www.genetic. edu). http:// indicates the domain name of the computer on which it is stored: (www.genetic. edu), fully describes the document's location. In addresses, small letters (www and http) are used. In abbreviations not pertaining to addresses, capital letters may be used (www, http, and HTTP).

Web (WWW): A global hypertext system that uses the Internet-linked computer network to facilitate data communication.

Web Browser: A program that runs on an Internet-connected computer and provides access to the www. An example is Google, which is available at http:// www.google.com

Web Server: A program that accepts Http-formatted requests for information. The server processes these requests and sends the requested document to the connected computer requesting the information.

Web Site: A set of related documents making up a hypertext presentation on the www. A Web site usually has a welcome or home page that serves as the initial document. By following instructions on the home page, one can select and gain access to the information and data included in the web site.

USING THE WEB TO OBTAIN INFORMATION FOR DYSMORPHIC PATIENTS

A newborn baby is suspected of having some form of dwarfism. Ventriculomegaly and short limbs, as detected by fetal ultrasound, were noted at 20 weeks gestation. Chromosome studies from amniocentesis revealed a 46, XY pattern without any abnormalities noted. The fetal head size at 30 weeks' gestation as noted on ultrasound was stated to be 35 weeks. The ventriculomegaly had resolved, and the limb lengths were those expected at 29 weeks. Physical examination detected macrocephaly, macroglossia, downward, slanting palpebral fissures, cataracts, and syndactyly of the second and third fingers. Blood glucose was 28 mg/dL (low).

The attending neonatologist believes that the baby ''looks funny'' and wants to know if you, the consulting physician, think the baby has a syndrome. He wants to know exactly which syndrome you think the baby has, how to confirm your diagnosis, and what is the expected prognosis?

You, as the consulting physician, are unaware of this constellation of clinical findings and/or what syndrome might be present, but must solve the problem. You decide to carry out a systematic search to obtain a list of possible syndromes that share the infant's signs and symptoms. To do this, the first step is to carry out a keyword search. Because the www might contain helpful information, you initiate a search beginning with the OMIM database. Using the computer in the nursery, you open netscape communicator or any Web browser available on the computer, and type in the OMIM URL or address: http://www.ncbi.nlm.nih.gov/Omim. The OMIM home page appears on the computer screen and you click on ''search the OMIM database,'' and then enter ''macrocephaly'' as a search term and press the ''enter'' key. One hundred and forty-three disorders in OMIM have macrocephaly listed. There are too many items to consider, so you repeat the search using ''cataracts'' as a keyword, and 285 matching entries appear. This number of disorders is also too long, so you search using ''syndactyly.'' Your third search produces 255 matching entries—again too many. You then decide to determine how many disorders have both macrocephaly and cataracts by typing both macrocephaly and cataracts, leaving a space between the two words (macrocephaly cataracts) as a search string. Only 13 entries are listed as having both of these keywords (Fig. 1).

Note that the entry numbers are in hypertext (color) on your screen. By clicking on any of these hypertext numbers, each corresponding entry is automatically opened. To narrow further the number of matches and focus your search, you add ''syndactyly'' and enter ''macrocephaly cataracts syndactyly'' as keywords. Only one OMIM entry is listed for all three of these findings: (i) #312870 Simpson-Golabi-Behmel Syndrome, Type 1, (SGBS1) (Fig. 2) (4).

You have done something remarkable. In less than two minutes, you have logged onto and searched a large electronic database of genetic and endocrine disorders to generate a successive series of progressively refined differential diagnoses. You open the single file (#312870 SGBS1) that matches your search criteria (macrocephaly cataracts syndactyly) by clicking your mouse on it, and the first of several pages of information on SGBS1 appears on the screen for review (Fig. 2). The complete text or the clinical synopsis (left panel under NCBI) can then be reviewed. Note in the text that there is a light bulb symbol after each paragraph. If you click on these symbols, you will automatically see related publications. For example, the one after paragraph one links to 30 publications. To see the abstract of any of these, you can click on the author's name in hypertext. Using these hypertext links, you can obtain, select, and print the abstracts of any of the numerous articles that constitute the published knowledge about this disorder.

Because SGBS1 is a rare disorder that you may have not seen previously, you want to know if there are laboratories that can provide confirmatory tests.

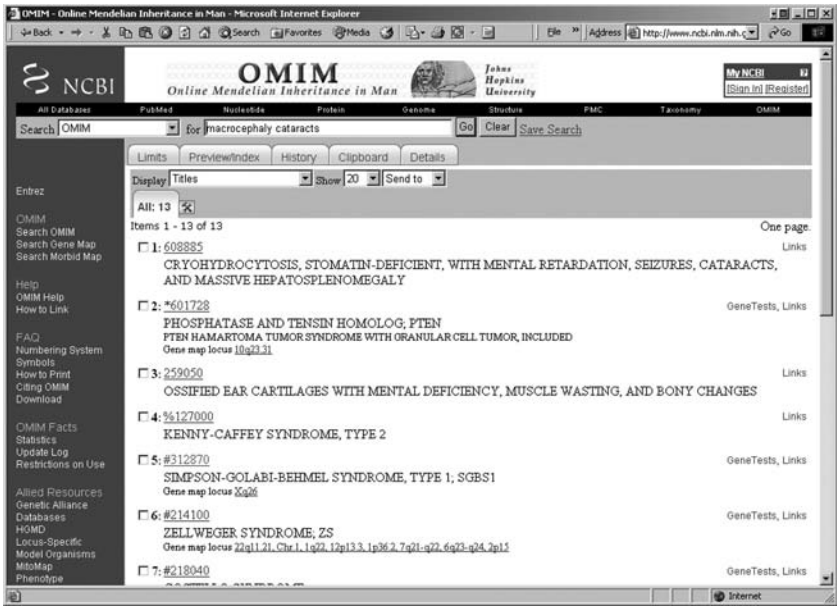

Figure 1 First 6 of 13 OMIM entries obtained using "macrocephaly cataracts" as keywords.

The GeneTests database available at http://www.genetests.org (5) contains a directory of laboratories that provide testing for genetic disorders (see list of selected Web sites below). You can access and search the GeneTests database for SGBS to find labs that provide either clinical or research testing for this disorder. In a matter of a very few minutes, you have generated a working diagnosis (SGBS1); obtained information about the pathogenesis, mode of inheritance, and findings associated with SGBS1; and gained access to a lab that can help confirm the working diagnosis. Using this information that you obtained from the Web in just a few minutes, you feel much better prepared to talk with the neonatologist and the baby's parents, who are waiting for your opinions.

HOW TO GENERATE A DIFFERENTIAL DIAGNOSIS FOR A FAMILY HAVING UNUSUAL ENDOCRINE PROBLEMS

A 15-month-old boy is referred to you by his pediatrician, who suspects he has growth hormone (GH) deficiency. The child weighed 3.2 kg at full term

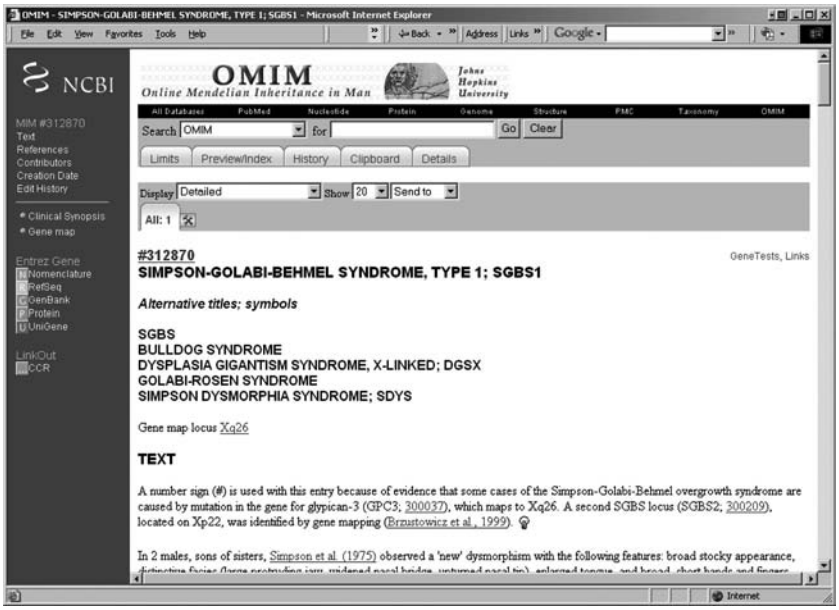

Figure 2 Single OMIM entry (#312870 SGBS1) obtained using "macrocephaly cataracts syndactyly" as keywords. *Abbreviations*: OMIM, online Mendelian inheritance in man; SGBS1, Simpson-Golabi-Behmel Syndrome, Type 1.

following an uncomplicated pregnancy, labor, and vaginal delivery. His height SDS is now −3.2, and his length since five months has become progressively retarded. Your examination shows the child is proportionate and his height and weight are commensurate with each other. You note that his extremities appear normal in length, and you do not detect any kyphosis, limitation of joint motion, or dysmorphic features. His bone age is delayed greater than −2 SD. You see no skeletal abnormalities. The serum thyroxin level is abnormally low, but the levels of electrolytes, glucose, urea nitrogen, bicarbonate and anion gap, calcium, phosphorous, and urine pH are all within normal limits. A 16-year-old full sister reportedly had ''panhypopituitarism'' and she ''did not go through puberty.'' She was treated with ''GH, thyroid hormone, and other shots to make her grow and go through puberty.'' The results of previous endocrine blood tests on the 15-month-old show low serum levels of gonadotropins and thyroxin. Combined pituitary hormone deficiency (CPHD) is a logical diagnosis for the 15 month old, because there is failure of response to GH-releasing hormone, thyroid-releasing hormone, and luteinizing hormone-releasing hormone, and the magnetic resonance imaging study reveals a hypocellular pituitary. This working diagnosis of CPHD also fits with the information you know about his 16-year-old sister.

You decide to perform a keyword search to produce a differential diagnosis. To utilize the www: to obtain information on familial hormone deficiencies, you carry out this search of the OMIM database. To do this, you log onto the OMIM Home Page by entering the URL: http://www.ncbi.nlm.nih.gov/Omim. You then click on ''search the OMIM database'' with the mouse and enter ''GH'' as a search term. Using ''GH'' as the search term gives 90 hits. If you search using both GH and thyroid (GH thyroid), only 18 disorders match. Finally, if you add gonadotropin to your keyword search (GH thyroid gonadotropin) only seven OMIM entries match: (i) 174800 McCune-Albright Syndrome, (ii) 173110 POU Domain, Class 1, Transcription Factor 1; POU1F1, (iii) 262600 Pituitary Dwarfism III, (iv) 601538 Prophet of PIT1, Paired-Like Homeodomain Transcription Factor; PROP1, (v) 103580 Albright Hereditary Osteodystrophy (vi) 133430 Estrogen Receptor 1 and (vii) 600262 prostaglandin-endoperoxide synthase 2 (Fig. 3) (6).

The second entry (173110 POU Domain, Class 1, Transcription Factor 1; POU1F1) contains information about the PIT1 transcription factor. It includes the following interesting paragraph under clinical features ''Mutations of the POU1F1 gene in the human and PIT1 in the mouse are responsible for pleiotropic deficiencies of GH, prolactin (PRL), and thyroid-stimulating hormone (TSH), while the production of adrenocorticotrophic hormone, luteinizing hormone (LH; 152780), and follicle-stimulating hormone (FSH; 136530) are preserved. On the other hand, patients with CPHD due to homozygosity or compound heterozygosity for inactivating mutations of PROP1 (601538) cannot produce LH and FSH at a sufficient level and do not enter puberty spontaneously (7). This latter entry sounds like a very good match to the signs and symptoms of your patient.

The third entry (262600 pituitary dwarfism III) contains interesting information in its first paragraph. ''Many patients classified as exhibiting panhypopituitarism probably have CPHD with sparing of adrenocorticotropin. Mutations causing CPHD have

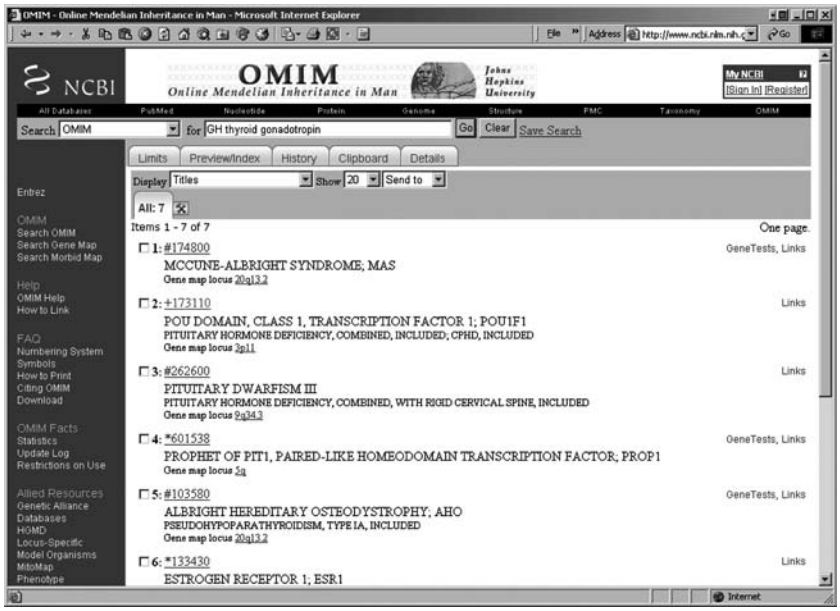

Figure 3 First six of seven OMIM entries obtained using ''GH thyroid gonadotropin'' as keywords. *Abbreviations*: OMIM, online Mendelian inheritance in man; GH, growth hormone.

been described in the *PIT1* (173110), *PROP1* (601538), *HESX1* (601802), and *LHX3* (600577) genes. In addition to manifestations of the deficiency of pituitary hormones, the *LHX3* mutations are associated with rigid cervical spine, and the *HESX1* is associated with septooptic dysplasia (182230).''

The fourth entry (601538 Prophet of *PIT1*, Paired-Like Homeodomain Transcription Factor; *PROP1*) includes, as its first paragraph, *PROP1* has both DNA-binding and transcriptional activation ability. Its expression leads to ontogenesis of pituitary gonadotropes, as well as somatotropes, lactotropes, and caudomedial thyrotropes. Inactivating mutations of *PROP1*, which have an autosomal recessive mode of inheritance, cause deficiencies of LH; 152780, FSH; 136530, GH (139250), PRL (176760), and TSH (188540).

Thus the fourth entry sounds like the best match for the signs and symptoms of your patient. Because the signs and symptoms of the boy and his sister match those reported for individuals with *PROP1* mutations, you decide to review the 1998 article by Wu et al. On the Web, you can obtain a copy of the abstract of this paper by clicking the mouse on either of the following: Wu et al., 1998 hypertext at the end of the paragraph cited above; or Wu et al., 1998 hypertext in either the *PIT1* (173110) or *PROP1* (601538) entries. Then click on the PubMed ID (9462743) that follows the reference that appears. You will then see the abstract of the reference on your screen and you can print it. Because this is a PubMed document, you can also save it as a file on your computer. If you do a PubMed search for ''gh thyroid gonadotropin familial,'' you will immediately found nine related articles, of which four of the first five contain information that you may find helpful in your further evaluation and treatment of your new patient.

As in the first case, you can carry out a genetests search to find a current review that was updated Nov 21, 2005. Interestingly, in this review you find a table on Molecular Testing that states that 55% of familial cases of *PROP1* related combined pituitary hormone deficiency are due to a 301–302delAG two-base pair deletion. On the right side of this table is a hyperlink under test availability to two labs that offer clinical testing for *PROP1* mutations. By clicking on the hypertext for clinical testing you can obtain detailed information on one of these labs that is in the United States (Fig. 4).

Now you have a working diagnosis (*PROP1* defects), information on the pathogenesis, mode of inheritance, the findings associated with the disorder, and access to a CLIA approved lab that can be used to confirm your working diagnosis. Obviously, with this information in hand you feel better prepared to talk with your patient's parents and answer their questions.

HOW TO OBTAIN INFORMATION ON A CASE OF ENDOCRINE NEOPLASIA

You are asked to see a 42-year-old woman who has a widely metastatic pheochromocytoma for advice about etiology and treatment. By history, her father was diagnosed with medullary carcinoma of the thyroid at 20 years of age. His thyroidectomy was complicated by severe hypertension, which led to the discovery of his also having had a pheochromocytoma. You perform a keyword search to produce a differential diagnosis. You carry out this search of the OMIM database using the terms ''medullary carcinoma thyroid pheochromocytoma'' and obtain nine matching entries (Fig. 5). The first matching entry

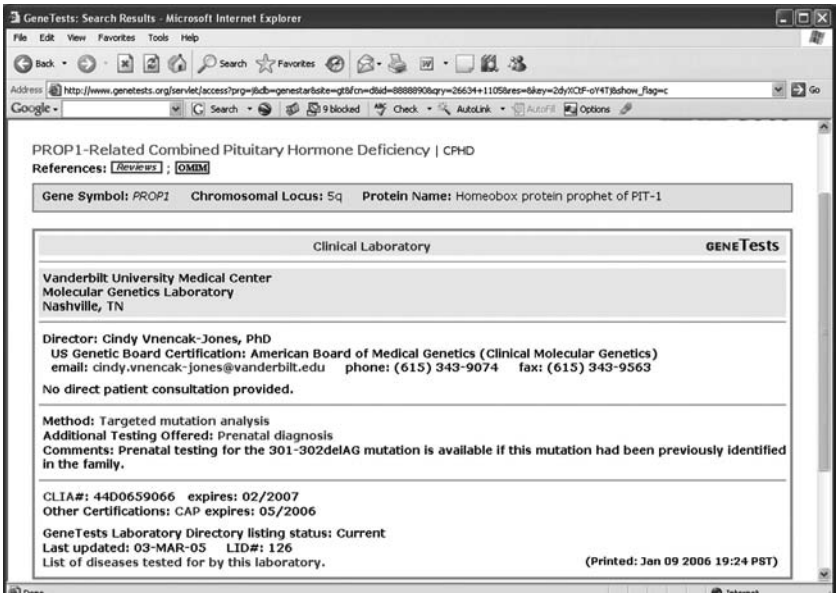

Figure 4 Information on CLIA approved lab offering molecular analysis of PROP1 genes for the common 301–302delAG deletion. *Abbreviation*: CLIA.

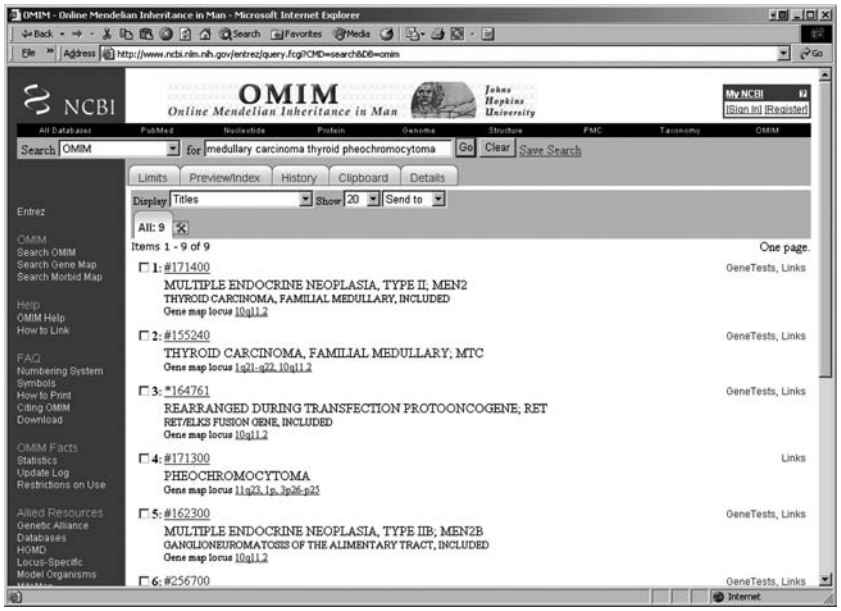

Figure 5 First five of nine OMIM entries obtained using medullary carcinoma thyroid pheochromocytoma as keywords. *Abbreviation*: OMIM, online Mendelian inheritance in man.

(#171400 multiple endocrine neoplasia, Type II; MEN2) contains a number sign (#) in its introduction because evidence indicates that MEN2A results from mutation in the RET oncogene (164761). It also states that ''MEN2A is an autosomal dominant syndrome of multiple endocrine neoplasms, including medullary thyroid carcinoma (MTC), pheochromocytoma, and parathyroid adenomas.'' All this suggests that you should consider MEN2 as a diagnosis, and consider testing to confirm your diagnosis.

The third matching entry (*164761 RET proto-oncogene; RET) begins with the following information: ''Mutations in the RET gene are associated with the disorders MEN2A (171400), MEN2B (162300), Hirschsprung disease (aganglionic megacolon; 142623), and MTC (155240).'' Because a RET gene mutation seems likely, before you see your patient you want to know if there are laboratories that can provide confirmatory tests. To get this information you access and search the genetests database for MEN2 and find the addresses, phone, and fax numbers of several labs that provide testing for this disorder. This testing involves sequencing of exons 10, 11, 13, 14, and 16 of the RET proto-oncogene, which include the sites of common mutations that cause MEN2.

To obtain information about treatment available for MEN2 you decide to query the NIH Clinical Trial database available at http://www.clinicaltrials.gov (8). You enter the search term MEN and obtain information on clinical trials that are recruiting participants (Fig. 6). You print out contact information on several of these studies to share with the physician, who has requested your consultation.

In a matter of a very few minutes, you have generated a working diagnosis (MEN2); obtained information concerning the pathogenesis, mode of inheritance, and the findings associated with MEN2; and gained access to a lab that can help confirm your working diagnosis. In addition you have obtained descriptions of clinical trials that are recruiting participants, who have MEN2. Having this information you feel much better prepared to talk with your new patient to address her questions about the risk of her children having a genetic predisposition to MTC or pheochromocytoma. If she is found to have an identifiable RET mutation, testing of her children who are at 50% risk to have inherited her RET mutation, could be offered to help guide their management. If they test positive, prophylactic resection of their thyroids as well as frequent screening for pheochromocytomas could be recommended. Finally, you also have information about clinical trials of experimental therapy that may be of great interest to her and her primary physician.

SELECTED WEB SITES ON GROWTH AND HORMONE DISORDERS

American Diabetes Association: http://www.diabetes.org (9) is useful for professionals and lay individuals. http://www.childrenwithdiabetes.com/index_cwd.htm (10) is the online community for kids, families, and adults.

Chromosomal Variation in Man: http://www.wiley.com/legacy/products/subject/life/borgaonkar/access.html (11) is a catalog of chromosomal variants and anomalies that includes citations on all common and rare chromosomal alterations, phenotypes, and abnormalities in humans. The database is organized

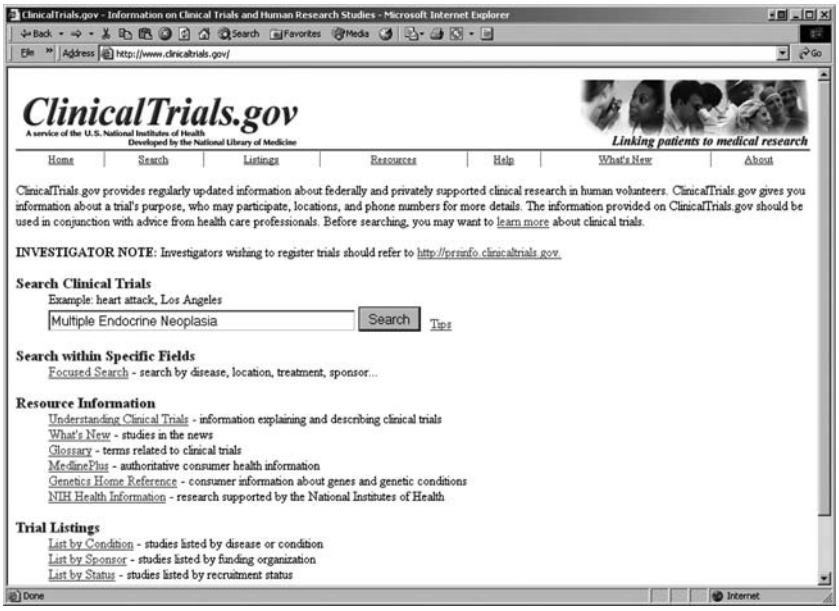

Figure 6 Search of ClinicalTrials.gov database for clinical research trials using "multiple endocrine neoplasia" as keywords.

by variations and anomalies, numerical anomalies, and chromosomal breakage syndromes.

Clinical Trials.gov: http://www.clinicaltrials.gov (8) provides regularly updated information about federally and privately supported clinical research in human volunteers.

Cytogenetic Resources: http://www.kumc.edu/gec/geneinfo.html (12), is a database of normal and abnormal karyotypes, empirical risks for chromosome abnormalities, and maps of genes on chromosomes.

Endocrine Society: http://www.endo-society.org (13) provides information on the Endocrine Society, fellow societies, organizations, and patient education groups as well as resources for scientists and physicians.

Gene Map'99: http://www.ncbi.nlm.nih.gov/genemap (14) includes the locations of more than 30,000 genes and provides a link to genes involved in diseases.

Gene Tests: http://www.genetests.org (5) contains information about genetic tests, genetic diseases (GeneReviews), a genetics laboratory directory, genetics clinic directory, and educational materials.

Genetic Alliance: http://www.geneticalliance.org (15) contains disease information as well as genetic support groups to voice the common concerns of children, adults, and families living with, and at risk for, genetic conditions. Also has a tool for taking family histories.

Genetics Education Center: http://www.kumc.edu/gec (16) useful for professionals, educators, and individuals seeking information on genetic disorders, birth defects, and chromosomal disorders. Contains information on genetic conditions; genetics education center; genetic courses, lectures, and educational materials; ethical, legal, and social implications of the human genome project, and genetic computer resources.

Google: http://www.google.com (17) is a widely used search engine for all types of information as well as images.

Glossary of Genetic Terms: http://www.genetests.org (5) defines over 225 medical genetic terms with illustrations.

Human Growth Foundation: http://www.hgfound.org (18) is a lay organization established for parents and friends of children with various growth disturbances including overgrowth, GH deficiency, and Turner syndrome, along with information about clinical trials.

Information for Genetic Professionals: http://www.kumc.edu/gec/geneinfo (12) contains information on cancer, cytogenetics, genetics, hyperlipid emia, neurogenetics, single-gene disorders, support groups, and genetic tests.

I*nternational Society for Pediatric and Adolescent Diabetes:* http://www.ispad.org (19) contains news, membership roster, and meeting dates.

Lawson Wilkins Pediatric Endocrine Society: http://lwpes.org (20) contains news, job listings.

Magic Foundation: http://www.magicfoundation.org (21) is a lay organization established for parents and friends of children with various growth disturbances including overgrowth, GH deficiency, Turner syndrome, and others.

March of Dimes: http://modimes.org (22) provides information on birth defects and newborn screening for professionals and families.

MEDLINE PubMed: http://www.ncbi.nlm.nih.gov (23) provides access to a cornucopia of scientific

and medical publications in a searchable format. It is available on the Web site of NCBI.

National Association for Rare Disorders: http://www.NORD-rdb.com (24) is a database of rare disorders that includes symptoms, causes, diagnostic tests, and treatment for families and professionals.

National Human Genome Research Institute: http://www.nhgri.nih.gov (25) contains information on the Human Genome Project and ethical, legal, and social implications.

NCBI Education: http://www.ncbi.nlm.nih.gov/Education/index.html (26) contains PubMed and other tutorials; genes and disease and glossary of genetic terms.

NCBI Site Map: http://www.ncbi.nlm.nih.gov/Sitemap (23) contains over 60 links to databases including genes and diseases, gene maps, mutation databases, OMIM PubMed, resource guide, and educational sites.

Children's Tumor Foundation: http://ctf.org (27) contains information about neurofibromatosis and contacts for related resources.

OMIM: http://www.ncbi.nlm.nih.gov/Omim (2) contains textual information, pictures, and reference information on genes and genetic disorders containing clinical findings, references, and gene maps. OMIM has many links to NCBI's Entrez database of MEDLINE articles and sequence information, and many links to other databases.

Policy Statements from the American Academy of Pediatrics: http://www.aap.org/policy/pprgtoc.html (28) contains policy statements and guidelines on diagnosis and treatment of genetic disorders as well as newborn screening.

Policy Statements from the American College of Human Genetics: http://www.acmg.net (29) contains a variety of policy statements about genetic diseases, genetic testing, treatment of genetic disorders, and teaching materials.

Rare Genetic Diseases in Children: http://rarediseases.info.nih.gov/html/reports/fy2000child_ht/ncrr.html (30) supports research and education about a variety of rare diseases that affect children.

Simulated Genetic Counseling Session: http://www.kumc.edu/gec/gcsim.html (31) is an online simulated session that illustrates the process of genetic counseling for a variety of genetic conditions.

CONCLUSIONS

The www is here to stay. It offers access to a wealth of information on genetic endocrine disorders and differential diagnoses for complex genetic and endocrine problems. In addition to information for the physician, the www can also provide access to specialized lab tests; clinical research and clinical trials, and educational materials for patients and their families. It behooves every physician to capitalize on the resources that computer technology can provide. As is true for most things in life, some effort must be expended to develop the expertise to accomplish these goals. We hope that the material in this chapter will help readers to succeed in using the www to obtain information on genetic and hormone disorders.

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10. ttp://www.childrenwithdiabetes.com/index_cwd.htm.
11. http://www.wiley.com/legacy/products/subject/life/borgaonkar/access.html.
12. http://www.kumc.edu/gec/geneinfo.html.
13. http://www.endo-society.org provides.
14. http://www.ncbi.nlm.nih.gov/genemap includes.
15. http://www.geneticalliance.org contains disease information.
16. http://www.kumc.edu/gec useful.
17. http://www.google.com is.
18. http://www.hgfound.org is.
19. http://www.ispad.org.
20. http://lwpes.org contains news.
21. http://www.magicfoundation.org.
22. http://modimes.org provides.
23. www.ncbi.nlm.nih.gov.
24. http://www.NORD-rdb.com.
25. http://www.nhgri.nih.gov.
26. http://www.ncbi.nlm.nih.gov/Education/index.
27. http://ctf.org.
28. http://www.aap.org/policy/pprgtoc.html contains policy.
29. http://www.acmg.net contains.
30. http://rarediseases.info.nih.gov/html/reports/fy2000child_ht/ncrr.html.
31. http://www.kumc.edu/gec/gcsim.html..

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Hormonal Dynamic Tests and Genetic Tests Used in Pediatric Endocrinology

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INTRODUCTION

The approach for evaluation and diagnosis of endocrine disorders has been moving toward molecular testing. Molecular research has increased our knowledge of the genetic basis of endocrine disease. Identification of gene mutations, although still limited to specialized clinics or a research laboratory, is more accessible and enables the physician to provide accurate management and counseling. We have included in this chapter a descriptive compilation of molecular testing that might help the clinician understand the basis of the most used tests in endocrine pathology.

Measurement of hormones continues to have a significant role in the diagnosis of endocrine pathology. Dynamic tests are often required to determine if feedback mechanisms are intact. Stimulation tests are used to document hormonal deficiency by providing a stimulatory agent such as medication. Suppression tests are performed to determine the presence of hormonal excess by evaluating functionality of feedback mechanisms. The individual performing a test is referred to the pertinent chapter in this book for an extensive review of indications, precautions, and interpretations of results.

PRINCIPLES OF HORMONAL MEASUREMENTS

Immunoassays

The most commonly used format for antibody-based assays is displacement analysis (1). With this format, a limited amount of antibody is allowed to bind to a limited amount of specific tracer for the hormone, the antibody-bound tracer is separated from the free tracer, and the antibody-bound tracer is quantitated. If the tracer is a radioactive hormone, then the assay is classified as a radioimmunoassay (RIA). If the tracer is a hormone coupled to an enzyme, then the assay is classified as an enzyme immunoassay. If the tracer is a fluorescent compound, then the assay might be classified as fluorescent immunoassay. In each format, a standard curve is generated by adding known amounts of unlabeled hormone and determining the decrease (displacement) in tracer bound to the antibody. For determination of the serum concentration of a hormone, the observed displacement is compared to the standard curve. The important points to consider are whether there are additional amounts of antibody [or binding proteins (BPs)] present in the serum or there are closely related forms of the hormone in the serum—then the assay result is unreliable. In each of these formats, the least analytic precision occurs at the lowest concentration of analyte. Thus, alternative methodology must be used if the clinically important analyte concentration is at the lowest range of ligand concentration. In the enzyme-linked immunosorbent assay (ELISA) format, a small amount of hormone is prebound to each well of a 96-well plate (alternatively to a plastic or glass tube); then, the standards and unknowns are added. The hormone-specific antibody is added and allowed to react with both the prebound and free hormone. More is bound if there is less free hormone present. The amount of antibody bound is then quantified by a suitable technique, typically by eliminating all unbound proteins and adding a second antibody that is specific for the first antibody and to which an active enzyme is bound. Finally, the amount of active enzyme is specifically determined in each well and compared to the known amount of hormone added and to a standard curve generated for comparison with the unknowns. This format is best used for assays to detect the presence of important compounds. In the immunoradiometric assay (IRMA) format, one hormone-specific antibody is attached to the solid support. The standards and unknowns are added, and a second hormone-specific antibody is added. Note that the second antibody used in IRMA is ligand specific rather than specific for the first antibody, as would be the case in RIA or ELISA. The second antibody is labeled with radioactive tracer, fluorescent or other nonradioactive tracer, or an enzyme. After a suitable incubation period, all unbound materials are washed away. Only when the

desired analyte forms a bridge between the (first) antibody bound to the solid support and the soluble (second) antibody is the tracer or enzyme bound to the solid support and available for detection. It should be noted that if a pair of epitopes are very closely spaced on a ligand of interest, then the combination of antibodies cannot be used in an IRMA. As a consequence of the format, the amount of tracer bound to the bridge is approximately proportional to the amount of ligand present. At high ligand concentrations, the amount of ligand can exceed the amount of either one of the antibodies. This leads to a ''high-dose hook effect'' and results in low estimations of ligand concentration. IRMA reagents are generally more expensive than reagents suitable for displacement analysis because of the requirement for two matched specific antibodies. As a compensating advantage, however, in contrast to displacement analysis, the IRMA format has its greatest analytic precision at the lowest analyte concentrations. The improved sensitivity at low concentrations and the improved specificity inherent in the method has led to widespread replacement of kits for displacement analysis (including RIA) for all analytes large enough for the generation of suitable antibodies. In the immunochemiluminescent assay (ICMA) format, a chemiluminescent reaction occurs between substrates and enzymes that act as labels. Several chemiluminescent substrates and enzymes are available and sensitivity has been improved by adding enhancers including aromatic compounds and phenol derivatives. This assays provides high analytic sensitivity in measurements of glycoproteins such as thyroid stimulating hormone (TSH) and luteinizing hormone (LH).

Assay Specificity

Several factors can affect the sensitivity and specificity of immunoassays including cross-reaction, BPs, autoantibodies, rheumatoid factors, heterophile antibodies, high-dose hook effect, and matrix effects. Cross-reaction interferes in the sensitivity of testosterone assays in the presence of unusual amounts of different androgens in conditions such as congenital adrenal hyperplasia. Even if the testosterone antibody had only 1% cross-reactivity with 17-hydroxyprogesterone, contribution of 17-hydroxyprogesterone to the apparent testosterone concentration could result in abnormal testosterone levels for a prepubertal child. Different techniques are available to overcome this problem, but the physician should be aware of these when ordering the appropriate test.

There are two different mechanisms by which BPs can also contribute to the lack of clinical utility of a particular assay. First, if the affinity constant of the BP is comparable to the affinity constant of the antibody, then it may interfere with the assay by providing additional binding sites. This would probably lead to inappropriately low hormone levels. Second, because the definition of a hormone includes passage through the blood and control of the function of a second organ, serum-BPs can interfere with or supplement the activity of the parent hormone. For example, some BPs increase the half-life of a short-lived hormone (insulin-like growth factor, IGFBP-3); others increase the amount present in the serum by increasing the solubility of a lipophilic compound (testosterone-estradiol binding globulin); others seem to have hormonal functions of their own (corticosteroid binding globulin). Changes in BP concentrations can lead to large changes in total hormone levels without corresponding changes in free levels, which are presumably the active form. Although there is no mysticism in immunoassay, there are many places for error, and no single laboratory value should be considered diagnostic without confirmation.

General Considerations in Interpreting Test Results

Consideration of the units and reference values given by the laboratory are of importance when interpreting test results. First, the units reported can be divided into two types: (i) mass-based units, usually used for small molecules such as steroids and thyroid hormones, and (ii) standard preparation-based units, usually used for proteins. Both of these have specific problems. Most small molecules are generally reported in mass units (e.g., ng/dl). Some laboratories report results as ng/dl; however, others use ng/ml.

The System International (SI), is an effort to standardize scientific nomenclature. Small molecules are now being reported in SI units (moles per liter). Reference values including conventional SI values and conversion factors are available in Table 1.

Second, the reference values are provided by the laboratory and are used for comparison with a test result rather than to identify normal from abnormal values. To have statistical validity, reference values should be established from a sample of 100 to 200 individuals without disease. The reference range is calculated considering 95% of all values ± 2 SD. It is assumed that the sample population has a normal Gaussian distribution. If a test result is not between the reference parameter, different possibilities such as disease, individual variation, different populations, or use of medications may be considered to determine if further action is needed.

PRACTICAL CONSIDERATIONS

Meticulous attention to details, both in the selection of laboratory tests and in the test room, is the key to a successful procedure. In particular, the most important details to evaluate are the sample size requirements, the type of tubes [e.g., serum, ethylenediaminetetraacetic acid (EDTA), or heparin] used to collect the blood, and the specific sample-processing requirements, including whether serum samples

Table 1 Reference Table for the Conversion of Current Units to SI Units

Substance	Conventional unit (CU)	Conversion factor (CF)	SI unit (Cu × CF)
Aldosterone	ng/dL	0.027	nmol/L
Androstenedione	ng/dL	0.0349	nmol/L
Androsterone	ng/dL	0.0349	nmol/L
Corticosterone	ng/dL	2.89	nmol/L
Cortisol	μg/dL	27.59	nmol/L
18-OH-corticosterone	ng/dL	27.9	pmol/L
DHEA	ng/dL	0.0347	nmol/L
DHEAS	μg/dL	0.026	μmol/L
11-Deoxicorticosterone	ng/dL	0.0303	nmol/L
11-Deoxicortisol	ng/dL	0.02886	nmol/L
Dehydrotestosterone	ng/dL	0.0344	nmol/L
17-β–Estradiol (E2)	ng/dL	36.71	pmol/L
Estriol (E3)	μg/L	3.47	nmol/L
Estrone (E1)	pg/mL	3.70	nmol/L
FSH	MIU/mL	1	IU/ml
Glucose	mg/dL	0.0555	mmol/L
Growth hormone	ng/mL	1	mg/L
17-Hydroxyprogesterone	ng/dL	0.03029	nmol/L
Insulin-like growth factor-I	ng/mL	0.1307	nmol/L
LH	MIU/mL	1	IU/ml
Parathyroid hormone intact	pg/mL	0.1053	pmol/L
Progesterone	ng/dL	0.0318	nmol/L
Renin (plasma rennin activity)	ng/mL/hr	1	mg/L/hr
Testosterone	ng/dL	0.03467	nmol/L
Testosterone, free	pg/mL	3.4673	pmol/L
Thyroglobulin	ng/mL	1	mg/L
TSH	μIU/mL	1	mIU/L
Thyrotropin releasing hormone	pg/mL	2.759	pmol/L
Thyroxine total	μg/dL	12.9	nmol/L
Thyroxine free	ng/dL	12.9	pmol/L
Transcortin	Mg/dL	10	mg/L
Triiodothyronine total	ng/dL	0.0154	nmol/L
Triiodothyronine free	pg/dL	×0.01536	pmol/L
Urine			
Catecholamine fractionated			
Norepinephrine	Mg/24 hr	5.911	nmol/24 hr
Epinephrine	μg/24 hr	5.458	nmol/24 hr
Dopamine	μg/24 hr	6.528	nmol/24 hr
17-Hydroxycorticosteroids	Mg/24 hr	2.759	mmol/24 hr
17-Ketosteroids (urine)	Mg/24 hr	2.76	μmol/24 hr

Abbreviations: TSH, thyroid stimulating hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

should be separated and if they could, should, or must be frozen. In consultation with the laboratory, one should determine the amount of serum required for each analyte and prepare a table listing the exact time of sampling, the analytes for that time point, and the amount of blood required by the laboratory. The proper number and type of blood collection tubes should be collected. Recall that if a mistake is made and inappropriate or inadequate blood samples are obtained, the protocol will probably have to be repeated. Finally, arrangements must be made to transport samples to the laboratory in a manner that does not lead to degradation.

Mechanics

Successful testing is accomplished primarily through organization before the test. A tray should be prepared to hold completed laboratory slips, the correct number and types of tubes, labels, syringes, alcohol, arm board, and tape. This allows methodical sampling throughout the test. Normal saline lock is most often used and permits an indwelling line for both withdraw of blood samples and delivery of medication with minimal discomfort to the patient. When using heparin lock flush (10 units/mL) a maximum of 1 mL is recommended. Withdraw and discard 1.0 to 1.5 mL from the line before sampling; after sampling, the line may be cleared by injection of an equal volume of the normal saline or heparin solution flush. An intravenous (IV) setup is a suitable alternative, but leaves the patient somewhat less comfortable during the protocol. In a child younger than four years of age, it is appropriate to maintain a separate IV line with normal saline, in addition to the heparin lock, for emergencies during potentially hazardous testing, such as insulin-tolerance protocols. Although this may cause some added discomfort to the patient, loss of a line is common in young children and a patent line is essential to address any untoward events.

The size of the heparin-lock needle must be selected on the basis of its intended use. A 24-gauge angiocatheter needle in a scalp vein may be adequate for infusion for an infant or young child; however, it is futile to attempt to obtain multiple blood samples from such a small needle or vein. Generally, a 22- or 23-gauge angiocatheter is adequate for both infusion and sampling.

Many of the protocols for tolerance tests include an overnight fast. Because the nothing-by-mouth order starts after midnight, a snack should be given just before midnight if the child is awake at that time. Otherwise, the snack should be given at bedtime. For infants and very young children, however, an overnight fast may be too long. Therefore, the fast should conform to the child's eating patterns (i.e., an infant may be on a three- to four-hour feeding schedule). In general, to avoid unnecessary fasting by young children, most tolerance tests should be started as early in the morning as possible. If the test must be postponed, the child should be fed and refasted. However, it should be noted that if a protocol is delayed, diurnal variation must be considered in the interpretation of the results.

In most cases, patient medications that might interfere with the test should be discontinued for at least one week. If this is not possible, the effects of the medication on the tolerance test must be considered when interpreting the results. Other factors such as extreme agitation or exercise can also affect the results and should be noted.

A critical factor when conducting a tolerance test is the total amount of blood that must be obtained if, as is usually the case, multiple samples are required.

Within a two-week period, the usual guideline is a maximum of 5% of the total blood volume, which is calculated by multiplying the body weight (kg) by 80 mL/kg. Remember to include any other testing planned for the same time or within two weeks of the tolerance test. If the amount required is more than 5% of the patient's total blood volume, the protocol must be modified.

Person-to-Person Considerations

One of the greatest challenges is informing the parents and the child about the purposes and mechanics of the test. This must be done in terms understandable both to the parents and to the child. Allow sufficient time for questions and answers. While you are describing the test, judge whether the parents should stay in the room with the child during the test. In deciding whether to allow the parents to stay, exercise discretion on an individual basis.

Time-of-Day Considerations

Most protocols are usually performed in the morning. Almost all of these tests should be performed while fasting although there might not be any physiologic rationale for food restriction. As a consequence, normal and expected values are all based on testing in the morning. It should be noted that there are circadian rhythms in many hormonal secretion patterns that can be superimposed on other patterns. For example, LH, and follicle-stimulating hormone (FSH) are both secreted episodically with 90-minute cycles but the amplitude of the cycle is increased in the morning. As a consequence, pituitary responses to gonadotropin-releasing hormone (GNRH) may be different and basal testosterone levels are higher in the morning and one cannot measure an acute response to human chorionic gonadotropin (hCG). Adrenocorticotropic hormone (ACTH) is also secreted episodically with a 90-minute cycle, but episodes of secretion occur more frequently in the morning and the ratio of cortisol to adrenal androgen secretion also changes with time of day. Hence, if testing is or must be performed at times other than morning, care must be used in comparing observed values to expected values. The exception to this requirement is in young infants in whom circadian patterns have not yet been established.

PRACTICAL PROTOCOLS FOR DYNAMIC TESTING IN CHILDREN

Dynamic Tests for Growth Hormone Deficiency

Screening Tests for Growth Hormone Deficiency

Background

Insulin-like growth factor–I (IGF-I), IGF-binding protein-3 (IGFBP-3) and acid labile subunit (ALS) production depends on growth hormone (GH). However, the most used screening tests (2,3) to evaluate GH deficiency are serum IGF-I and serum IGFBP-3 levels. Serum levels of IGF-I are age-, sex-, and nutrition-dependent in normal children with a sharp increase at the time of puberty. IGF-1 levels are low in patients with GH deficiency, GH receptor and/or postreceptor defects, thyroid disorders, delayed puberty, diabetes, and malnutrition. Serum IGFBP-3 has less age, sex and nutrition dependence than IGF-I levels. Although different percentages in terms of sensitivity and specificity are reported, diagnosis of GH deficiency is unlikely in presence of normal IGF-I/IGFBP-3 levels. Evaluation of immunoreactive forms of ALS, although less used, could contribute to diagnosis of GH deficiency or insensitivity (4,5).

Indications

The diagnosis of GH deficiency in childhood must be based on auxiological criteria (Vol. 2; Chaps. 1 and 2). Candidates for evaluation of the GH-IGF-IGFBP axis include: (i) children in the lowest fifth height growth or bone age percentiles, when proper consideration is made for family history; (ii) children with syndromes associated with short stature; (iii) children who have acute changes in their growth charts; and (iv) children who have had possible insults to the pituitary such as chemotherapy, radiotherapy, or physical injury to the head.

Preparation and Medication

None are needed.

Sampling

A single sample is obtained at the time of a routine patient visit. There is no time of day or dietary restrictions. Note: Some laboratories require plasma, rather than serum, for IGF-I assays. Check with the laboratory before collecting a sample. IGFBP-3 levels are determined from serum. Thus, both serum and plasma may be needed.

Normal and Expected Values (Insulin-like Growth Factor–I and Insulin-like Growth Factor-Binding Protein-3 Levels)

Different references values are used because the assays are not standardized. IGF-I and IGFBP-3 levels are adjusted for age, sex, and pubertal stage (6).

General Considerations

Although a major screening tool for diagnosing GH deficiency, IGF-I has limited sensitivity because of significant overlap with normal values. Low levels of IGF-I may be found in normal children, mainly less than five years of age. As well, about 50% of low levels of IGF-I are not associated with GH deficiency but with receptor and postreceptor defects. Furthermore, low serum IGF-I levels with normal GH secretory dynamics may be indicative of other disorders associated with growth failure such as: (i) nutritional inadequacies, (ii) inadequate spontaneous GH secretion, and

(iii) psychosocial growth failure. Low serum IGFBP-3 levels are suggestive of GH deficiency; however, up to 43% of normal short children were reported to have low IGFBP-3 levels. In summary, IGF-I and IGFBP-3 are helpful tests in the diagnosis of severe GH deficiency, but their sensitivity and specificity are still suboptimal.

Basic Physiology of Growth Hormone Secretion

GH is secreted episodically with most episodes occurring during rapid eye movement sleep. Random serum concentrations are typically below the sensitivity of most conventional assays (< 1–2 ng/mL) (7) and cannot be used to evaluate GH deficiency. A variety of pharmacological agents have been identified to induce GH secretion, and suitable dynamic test procedures have been developed. There are two factors necessary for the evaluation of the response to pharmacological stimuli for GH secretion: first, knowledge of normal and inadequate responses to the particular protocol and, second, laboratory selection of methods and reagents for the evaluation of serum GH levels. The pharmacodynamics of GH secretion and metabolism determine the design of the serum-sampling protocol. Episodes of active GH secretion by the pituitary last about 5 to 10 minutes, and the half-life of GH is about 20 to 30 minutes. Thus, the specific protocol for a tolerance test must collect serum every 20 to 30 minutes to detect an episode of secretion.

Normal Values

Pediatric Population. Conventionally, a serum GH concentration over 10 ng/mL indicates adequate GH response to pharmacological stimulation. A single value over 10 ng/mL is sufficient to evaluate the response; there is not usually a second serum sample with a concentration over 10 ng/mL because GH concentration will decrease by half before obtaining the next sample. At the present time, the generally recognized criteria for GH deficiency are responses of less than 10 ng/mL (or 10 μg/L) to two different pharmacological stimuli for GH secretion. However, the interpretation of these values must be made in accordance with the child data (Vol. 2; Chaps. 1–3). The stimuli can have their effects at the level of the hypothalamus, the pituitary, or both. High serum glucose levels inhibit GH secretion. Thus, each protocol must also include a significant period of fasting before the test.

Adult Population. All patients receiving GH due to GH deficiency will need reevaluation of the GH status after reaching final height (Vol. 2; Chap. 4). Exception to retesting would include severe congenital or acquired panhypopituitarism. Severe GH deficiency in the transition period and adulthood would be considered with a peak response of less than 5 and 3 mcg/L, respectively (8).

Expected Frequency of Inadequate Growth Hormone Secretion in Response to a Tolerance Test

Children with Prader-Willi syndrome, Russell-Silver syndrome, Down syndrome, Turner syndrome, or other syndromes associated with short stature, or a history of cranial irradiation or of treatments for leukemia have a very high frequency of inadequate responses to GH testing. Cancer survivors present the highest failure rates (Vol. 2; Chap. 30). Thus, there should be a high index of suspicion for a diagnosis of GH deficiency if a child also has one of these syndromes. Children with GH deficiency frequently continue to cross growth lines on growth charts and have significant bone-age delay.

General Considerations

The use of growth-hormone stimulation tests has dropped significantly because new genetic and neuroimaging methods offer an alternative approach for evaluation of GH deficiency. The following sections describe protocols for dynamic tests to evaluate the GH secretory capacity of the pituitary gland. These tests are expensive, not free of side effects, and require special conditions. There are two major groups of GH tests: screening tests that include exercise, levodopa, and clonidine, and definite tests that comprise arginine, insulin, and glucagon tests. Two different dynamic tests, sequentially or simultaneous, are required to confirm the diagnosis of GH deficiency. Although there are few children with responses classified as false negative, there are many children who have responses classified as false positive. A false positive response is a patient who fails to secrete GH in response to insulin but secretes GH in response to other pharmacological stimuli. In contrast, a false negative response occurs if a patient secretes GH in response to insulin but does not secrete GH in response to other pharmacological or physiologic stimuli. Finally, pharmacological tests involving the use of potent medications may mask the diagnosis of partial GH deficiency. Caution must be taken in interpreting results in obese children who undergo provocative testing for GH secretion because of a negative impact of adipose tissue on GH secretion. Table 2 shows the protocols most commonly used in the assessment of GH secretion. The most used GH stimulation tests will be described in this section.

Sex Steroid Priming

During the immediate period of prepuberty, discrimination between GH deficiency and constitutional growth delay is difficult (Vol. 2; Chap. 11). Sex steroid priming with testosterone or estrogen administered for Tanner Stage I or II is recommended before GH testing (9,10). One protocol for androgen priming is the administration of 100 mg depot testosterone between 7 and 10 days before the actual GH tolerance test. For girls and boys, some endocrinologists prime

Table 2 GH Stimulation Tests

Test	Dose	Timing peak of GH	Side effect
Arginine	Arginine hydrochloride 0.5g/kg/i.v. to a maximum of 40 g over 30 min	30–60 min	Late hypoglycemia
L-Dopa	L-Dopa 125 mg if body weight < 13.5 kg 250 mg > 13.5 < 31.5 kg 500 μg > 31.5 kg		Nauseas, emesis, headache
GHRH	1 or 2 mg/kg, i.v. bolus	15 or 30 min	Flushing
Glucagon	0.03 mg/kg to a maximum of 1 mg IM/SC	2–3 hr	Late hypoglycemia
ITT	0.05–0.1 IU/kg, i.v. bolus	30–60 min	Severe hypoglycemia requires i.v. glucose Not recommended in newborn or small children
Clonidine	5 μg/kg to a maximum of 250 μg	60 min	Drowsiness, hypotension

Abbreviations: GH, growth hormone; ITT, insulin-induced hypoglycemia; GHRH, growth hormone releasing hormone.

with ethynil-estradiol. The protocol we use is 0.02 mg for children with less than 50 pounds (23 kg) and 0.05 mg for those over 50 pounds (23 kg) given 18, 12, and 1 hour prior to starting the test.

Arginine and Combined Arginine-L-Dopa Test for Growth Hormone Secretion

Indications

The arginine stimulant apparently works by inhibiting somatostatin release and dopamine acts through α-adrenergic mechanisms to stimulate GHRH release. The combined test (11–13) thus stimulates GH secretion by two separate mechanisms. Perhaps as a consequence, the combined protocol has fewer false positive results than when either agent is administered alone. The test is used primarily when a second pharmacological test for GH secretion is required. Arginine may be used as single stimuli to assess growth-hormone secretion in the same way as described here but without the addition of L-Dopa.

Preparation

Nothing by mouth after midnight or following bedtime snack. Have arginine prepared for administration. Plan ahead: not all pharmacies have stocks of arginine, and it may have to be especially ordered in advance. In addition, levodopa is not available in the United States. Few studies have evaluated the use of carbidopa and levodopa on growth-hormone secretion (14–16). Carbidopa inhibits the peripheral destruction of levodopa. If carbidopa is used, the preparation with the lowest concentration should be used (1 : 10 carbidopa/L-Dopa)

Medications

After the baseline serum sample is obtained, arginine HCL (0.5 g/kg to a maximum of 40 g) is administered IV over a 30-minute period. If the combined arginine-L-Dopa protocol is used, L-Dopa is administered orally immediately after the baseline blood sample is obtained. Then, arginine is administered. The dose of L-Dopa should be as follows: (i) 125 mg for children less than 13.5 kg; (ii) 250 mg for children between 13.5 and 31.5 kg; and (iii) 500 mg for children over 31.5 kg.

Sampling

Blood for GH assay should be sampled at 0, 30, 60, 90, and 120 minutes.

Normal Expected values

GH release peaks 60 minutes after starting the Arginine infusion and mean response has been reported as 11.1 ± 1.2 (±SEM) μg/L. After L-Dopa, GH reaches maximum response at about 45 minutes although timing varies, and mean response has been reported as 13 ± 1.1 (±SEM) μg/L (17).

Special Considerations

The L-Dopa protocol often produces nausea and vomiting, particularly in toddlers. Be prepared. Do not stop taking blood samples. Children should be recumbent and may be given water throughout the test.

Clonidine Stimulation Test for Growth Hormone Secretion

Specific Indications

Clonidine is an alpha 2-adrenergic agonist (18–20) that increases the growth hormone releasing hormone (GHRH) secretion, and inhibits somatostatin releasing factor. This agent is probably the best choice for avoiding false positive results. Children who fail to secrete GH in response to pharmacological doses of clonidine seldom secrete GH in response to any other test.

Preparation

The patient should receive nothing by mouth for at least four to six hours before the test and is generally off all other medications.

Medications

Administer clonidine, 5 μg/kg, after baseline sample is drawn, to a maximum of 250 μg.

Sampling

Blood for GH assay should be drawn at 0, 30, 60, and 90 minutes and blood for cortisol assay at 0 and 90 minutes.

Normal Expected Values

Usually, the 60-minute sample has the highest amount of GH, the 90-minute sample being about 30% lower. GH peak response usually is higher than peak response to other tests. Mean GH level in normal children has been reported as $13 \pm 1.8\,\mu g/L$ (17).

Special Considerations

Clonidine is an agent that lowers blood pressure. Blood pressure should be monitored at 0, 30, 60, and 90 minutes. In young children, clonidine frequently causes drowsiness, which lasts for several hours. Parents should be aware of this possible, transient side effect. Patients should have a place to lie down and may sleep or lie quietly throughout the procedure. Drowsiness may prolong fasting period and may cause hypoglycemia. Patient must be encouraged to eat or drink after the test is finished. Water may be given freely throughout the test period.

Growth Hormone Releasing Hormone Test for Pituitary Reserve of Growth Hormone Secretion

Indications

Administration of GHRH evaluates the ability of the pituitary to secrete GH directly (21–24). Due to fluctuations in somatostatin secretion, there is great variability in the GH response. Thus, inhibitors of endogenous somatostatin such as pyridostigmine and arginine have been used to enhance the GH response and to reduce the intra- and interindividuality variability. If a patient secretes GH in response to GHRH but not to pharmacological stimuli that function in the hypothalamus, then a defect in the hypothalamus is indicated.

Preparation

The test should be performed in the morning after an overnight fast.

Medication

IV inject human pituitary GHRH with a dose of 1 μg/kg over a period of one minute. The patient may experience some flushing immediately after the infusion.

Sampling

Serum samples for evaluation of GH should be obtained at 0, 15, 30, 45, and 60 minutes. Earlier protocols also collected a late sample at 90 minutes, but this does not seem to be needed because the peak generally occurs within the first hour after administration of GHRH.

Normal and Expected Values

The peak serum level usually occurs in the 15- or 30-minute sample. The mean peak GH level in normal children has been reported as $28 \pm 2\,\mu g/L$ (SEM) (17).

General Considerations

Most individuals with idiopathic GH deficiency have a defect in hypothalamic regulation of pituitary secretion of GH. Hence, most patients secrete GH in response to GHRH but do not secrete GH in response to normal physiologic processes.

High endogenous (or exogenous) levels of somatostatin block the effect of GHRH.

Combined Growth Hormone Releasing Hormone–Arginine

Indications

The combination of these two medications results in a potent growth-hormone release. This test is used as an alternative to the insulin tolerance test in children and adults (25,26).

Preparation

Fasting after midnight.

Medication

GHRH1-29 1 μg/kg of body weight IV at time 0 and Arginine 0.5 g/kg infusion IV at time 0 to 30 minutes with a maximum of 40 g.

Sampling

Serum samples for evaluation of GH should be obtained at 0, 15, 30, 45, 60, 90, and 120 minutes.

Normal and Expected Values

This combined test stimulates GH to a higher extent than the traditional test. In normal children, growth-hormone range response has been reported as 19 to 120 μg/L (17). In adults, a GH peak below 4.1 μg/L has a 95% specificity and sensitivity for a diagnosis of growth-hormone deficiency (26).

Specific Considerations

This GHRH–Arginine combined test has a great value in children with multiple pituitary growth-hormone deficiency when the appropriate cutoff value (19 μg/L) is applied. Further evaluation is needed in children with growth-hormone deficiency of a different etiology (25).

Glucagon Test for Growth Hormone Secretion

Indications

This test is the best choice in young children and infants (27,28). Glucagon induces GH secretion by stimulating endogenous insulin secretion to compensate for elevated serum glucose levels. It is a good

substitute for the insulin tolerance test that could be risky in newborn and small children.

Preparation

Nothing by mouth after midnight. Patients must have normal glucose reserves at the start of the test.

Medications

After baseline sample is drawn, glucagon is administered intramuscularly or subcutaneously at a dose of 0.03 mg/kg to a maximum of 1 mg.

Sampling

For evaluation of GH secretion, serum samples should be obtained at 0, 1, 2, 2.5, and 3 hours after administration of glucagon. For other indications of the glucagon tolerance test, different sampling protocols are required. Be sure to collect the last samples.

Normal and Expected values

At least one sample with a GH concentration over 10 ng/mL of GH secretion usually occurs between two and three hours after glucagon administration.

Specific Considerations

The administration of glucagon causes a temporary increase in serum glucose levels. As part of the rebound process, insulin is oversecreted and serum glucose levels decrease. Hence, a glucagon tolerance test cannot be used as a stimulus for GH secretion in individuals with a limited ability to secrete insulin. Young children frequently develop nausea and vomit during the course of this test. Be prepared.

Insulin-like Growth Factor-I (Somatomedin C) Generation Test

Indications

This procedure examines GH-receptor function by evaluating its ability to increase serum IGF-I levels (29,30). This test is useful for identifying patients with short stature related to a degree of GH insensitivity (Vol. 2; Chaps. 2 and 3). There is major variability in GH dosing, timing of samples, and cutoff levels of the normal IGF-I response.

Preparation

No specific preparation is necessary, but an adequate diet must be maintained.

Medications

Daily doses (four- or seven-day protocol) of GH (0.025–0.05 mg/kg/day 7 days or 0.1 mg/kg/day 4 days) are given subcutaneously. Parents or guardians may administer the additional GH injections.

Sampling

A blood sample should be obtained on days 1, 5, and 8 when using 0.025 to 0.05 mg/kg/day. If 0.1 mg/kg/day GH dose is used, blood should be obtained before the first GH injection and 8 to 16 hours after the fourth injection. Samples on intermediate days are often helpful, but are not required.

Normal and Expected values

Selva KA, reported in normal subjects a mean delta IGF-I of 230 ng/mL and 322 ng/mL for a low (0.025 mg/kg/day) and high dose (0.05 mg/kg/day), respectively. Response of delta IGF-I and IGFBP-3 in patients with idiopathic short stature was lower when compared to normal subjects. Previous criteria for GH insensitivity include a response of delta over baseline < 15 ng/mL for IGF-I and 400 ng/mL for IGFBP-3 (31,32).

General Considerations

The GH should be administered at the same time each day, either in the morning or in the evening. The dose administered is equivalent to the normal daily secretion. There are no reported side effects. Children who have GH insensitivity are not good candidates for GH therapy.

Insulin Stimulation Test for Growth Hormone Secretion

Specific Indications

This test is generally considered the ''gold standard'' (33,34), but is risky and must be done under appropriate surveillance. This test is currently used infrequently for the clinical assessment of GH status, whereas in the past it was almost always employed for the diagnosis of GH deficiency. The mechanism of stimulation is the counter-regulatory response to insulin-induced hypoglycemia. False positive responses may be caused by insulin insensitivity, which leads to an inadequate induced hypoglycemia. The reserve of the adrenal cortex for cortisol secretion can also be confirmed during this protocol. If cortisol reserve is adequate, then at least one sample will have a cortisol level over 20 μg/dL. This test is neither recommended for newborns or small children because they are more sensitive to insulin, nor for those with suspected hypopituitarism with risk of adrenal insufficiency.

Preparation

Patient should be fasting after midnight. Calibrate and prepare for use a bedside device for rapid serum glucose measurement. Prepare a 50%-glucose solution, and fill a 25 mL syringe (fill two syringes if the patient is larger than 25 kg) and an IV line with saline solution should be established.

Medications

In children over four years of age, to start the protocol, 0.1 unit/kg of regular insulin should be administered IV. For younger children, a dose of 0.05 unit/kg is usually sufficient. However, if used for infants, the dose must be one tenth (0.01 unit/kg) and must be administered under careful observation. It is preferable to use glucagon or a different provocative test.

Sampling

Serum samples should be obtained before insulin administration and then at 15, 30, 45, and 60 and 90 minutes later. Serum glucose levels must be evaluated at the bedside at each time point during the protocol. Glucose levels must decrease by 50% of the initial value or to less than 40 mg/dL. However, more severe hypoglycemia must be avoided because it can lead to seizures, coma, or death.

Monitoring and Dangers of Hypoglycemia

At the bedside, each blood sample must be immediately evaluated for serum glucose levels. It is not sufficient to send the sample to the hospital laboratory. If a child develops symptoms of hypoglycemia (blood glucose level less than 40 mg/dL, rapid pulse, diaphoresis, warm skin, and lethargy) and the signs do not improve by the next scheduled blood sampling, the 50%-glucose solution should be administered (1 g/kg) from the previously prepared syringes. If this occurs, do not stop collecting serum samples according to the protocol. Upon completion of the protocol, either the glucose solution (0.5–1.0 mL/kg) is administered or a good meal is given to the patient. The patient must be monitored until serum glucose levels return to normal. Water should be provided as requested.

Normal and Expected Values

About 20 minutes after the glucose nadir, there should be an episode of GH secretion. The peak level should be above 10 ng/mL. In some patients, the response is delayed. Thus a 90-minute sample is recommended. Children with GH deficiency have a response of less than 10 ng/mL. About 20% of children with short stature and severe bone-age delay can have a false positive response.

Special Considerations

For the test to be valid, serum glucose levels must decrease more than 50% from the baseline or to less than 40 mg/dL. If signs of severe hypoglycemia occur, administer glucose but continue to collect serum for GH assay.

Children with GH deficiency frequently have enhanced response to insulin, thus making them more likely to have an episode of severe hypoglycemia. Hence, this test requires the presence of either an experienced nurse or a physician.

Shah et al. (33) reported three cases of iatrogenic illness as a result of tolerance tests for GH deficiency (two with insulin and one with glucagon). Two of the three children died as a result of hyperglycemic hyperosmolar coma, perhaps as a result of inappropriate management after the test.

L-Dopa Stimulation Test for Growth Hormone Secretion

Indications

This test is frequently used (35,36) as the second test necessary to confirm the diagnosis of GH deficiency. However, Levodopa is no longer available in the United States. Few studies are available about the effects of carbidopa/levodopa on growth-hormone secretion (15,16). As stated above, L-Dopa is not available as a single medication and when the carbidopa/L-Dopa preparation is used, the lowest ratio of carbidopa/L-Dopa is recommended.

Preparation

Nothing by mouth after midnight on the night before the test.

Medications

L-Dopa is given orally immediately after the baseline blood sample is obtained. The dose is as follows: (i) 125 mg for children less than 13.5 kg; (ii) 250 mg for children between 13.5 and 31.5 kg; and (iii) 500 mg for children over 31.5 kg. The carbidopa/L-Dopa (1:10 preparation is the available medication (as mentioned above).

Sampling

Draw blood for GH assay at 0, 30, 60, 90, and 120 minutes.

Normal and Expected Values

At least one serum value should be above 10 ng/mL. Usually, the samples with high levels of GH are the last samples collected during the protocol. If there is no sample with a concentration of GH greater than 10 ng/mL, then the test is diagnostic for GH deficiency.

Special Concerns

Nausea and vomiting frequently occur in toddlers. Be prepared. Do not stop taking blood samples. Children should be recumbent and may be given water throughout the test.

Overnight Test for Spontaneous Growth Hormone Secretion

Indications

This procedure is used to evaluate spontaneous GH secretion rather than secretion in response to pharmacological stimulation (35,37). This is the diagnostic test necessary for documentation of inadequate

spontaneous GH secretion or a neurosecretory defect in GH secretion. This test is not used as often as it was used in the past due to the cost of admitting the patient to the hospital overnight.

Preparation

Patients can be tested in the hospital or at home with the aid of an experienced home care service. In either case, patients should go to bed at the usual time but not later than 11 P.M. A heparin lock can be used to cause the least disturbance in sleep pattern.

Medication

No medication needed as part of the tolerance test.

Sampling

Serum samples should be obtained every 20 minutes from 8 P.M. to 8 A.M., a total of 37 samples over 12 hours.

Normal and Expected Values

There are two criteria for evaluating the adequacy of overnight GH secretion: (i) mean levels and (ii) number of episodes and height of peak secretion. The mean level is the simple average of the 37 samples collected. This is a representation of the total amount of GH secreted during the 12-hour period. Most reports suggest a normal lower limit of the mean, about 3 ng/mL. Means below this limit probably indicate inadequate physiologic secretion.

The second method of evaluating results is after deconvolution analysis using the Veldhuis and Johnson cluster analysis program. The program permits the evaluation of the number of episodes of secretion and the half-life of serum GH. There should be 6 to 10 episodes of secretion, with at least four peaks over 10 ng/mL. Fewer peaks and lower peak height are consistent with the diagnosis of inadequate spontaneous secretion or neurosecretory defect.

Special Concerns

In view of the large number of samples collected and the general limitation of using no more than 5% of total blood volume for laboratory testing in any two-week period, it is frequently necessary to limit the amount of serum obtained in each sample. Hence, it is necessary to discuss with the laboratory the absolute minimum amounts of blood necessary for each sample. This test is difficult to perform, expensive, and unspecific. No normative data for comparison purposes are available yet.

Combined Hormonal Stimulation Test

Indication

This hormonal stimulation test is used for evaluation of pituitary function by combined sequential hormonal administration in children with pathological short stature (38). Patients with GH deficiency may have associated pituitary deficiencies in about 30% of the cases. This combined hormonal stimulation test (CHST) includes simultaneous assessment of the following axes: growth, thyroid, gonadal, and adrenal. In prepubertal children, the assessment of the gonadal axis can be eliminated unless LH and FSH deficiencies are suspected.

Preparation

Nothing by mouth after midnight on the night before the test.

Medications

It includes sequential administration of insulin, thyrotropin-releasing hormone (TRH), GNRH, and levodopa. First, 0.1 unit/kg of body weight of regular insulin is infused IV over 90 seconds. This is followed by 100 μg of gonadorelin IV (10 μg/kg, max 100 μg), and protirelin (7 μg/kg to a maximum of 400 μg IV) over 90 seconds. Afterward, L-Dopa is given orally at the dosage of 125 mg for children below 13.5 kg, 250 mg for children between 13.5 and 31.5 kg, and 500 mg for children above 31.5 kg.

Sampling

The following illustrates timing for measurements of glucose, GH, TSH, LH, FSH, and prolactin.

Time	Glucose	GH	Cortisol	FSH	LH	TSH	Prolactin
0′	X	X	X	X	X	X	X
20′	X	X					
45′	X	X		X	X		
60′	X	X	X			X	X
90′	X	X		X	X		
120′	X						

Special Concerns

For detailed information about specific monitoring of each test see the pertinent section described in this chapter. Special attention must be given to hypoglycemia induced by insulin. Additionally, there should be an assessment of the amount of blood required for all these tests and the cost of performing them.

Normal and Expected Values

Normal values for each test are described in each specific section elsewhere in this chapter. The glucose level should drop by 50% from baseline level or below 40 mg/dL. Normal growth-hormone peak response is equal to or greater than 10 ng/mL. Normal cortisol response is considered equal to or greater than 20 mcg/dL. Normal TSH response to TRH is an increment of 5 to 10 times over TSH basal level. Normal prolactin response to TRH is an increment of three to five times over basal level. Basal levels of LH and FSH are less than 2 IU/L after six months

of age and during prepubertal stage. Prepubertal LH response to GHRH test is usually below 7 IU/L. FSH response is greater in females but is not different during prepuberty or puberty.

Special Considerations

There are potential modifications to this test. The first is to perform the full CHST as described but store the samples for TSH, LH, FSH, and prolactin until confirmation of growth-hormone deficiency. The second modification is to select who will undergo TRH and/or GNRH test. This test provides an effective manner of evaluating multiple pituitary functions in two hours.

Tests for Thyroid Function

Calcium–Pentagastrin Test

Indications

Measurement of plasma calcitonin after calcium and pentagastrin provocative testing might be considered for tumor surveillance if there is no detectable RET protooncogene mutation, and for detecting persistent or recurrent medullary thyroid carcinoma (MTC) postoperatively. Screening for DNA mutations of the RET protooncogene is more sensitive in detecting MTC (39,40) (Vol. 2; Chaps. 20, 27 and 28).

Preparation

Patients should be fasted after midnight or bedtime snack; water is permitted as desired. The test should be performed in a supine position.

Medications

Elemental calcium (2 mg/kg) is infused IV over one minute period; pentagastrin (0.5 μg/kg) is administered as a bolus immediately thereafter. The elemental calcium content of some common calcium salts is (i) calcium gluconate, 10%; (ii) calcium lactate, 13%; and (iii) calcium chloride, 27%.

Sampling

Serum samples for calcitonin are obtained at 0, 1, 2, 3, 5, and 10 minutes after administration of both stimulants.

Normal and Expected Values

Normal values should be established in conjunction with the laboratory. An increase of five times over the baseline level during the test is diagnostic of medullary thyroid carcinoma. Test results could be false negative in the presence of early changes of the C cell.

General Considerations

The test protocol leads to some minor discomfort (1). Infusion of calcium may be accompanied by a mild generalized flush or feeling of warmth, the urge to urinate, and a sensation of gastric fullness. These symptoms are self-limited and usually do not last longer than five minutes (2). Pentagastrin may cause some discomfort in the pharynx and substernal and retrosternal areas, a sense of gastric fullness, abdominal cramping, nausea, and dyspepsia. These symptoms also last less than two minutes. It is extremely important to maintain patient IV access during the calcium infusion; infiltration of calcium into subcutaneous tissue can cause tissue necrosis.

Thyroid Suppression Test

Indications

This test is still used in some centers for the diagnosis of autonomous functioning thyroid nodules (41,42). Radioactive iodine uptake by the thyroid gland should be decreased by exogenous thyroid hormone in a properly functioning gland. If uptake continues after treatment with thyroid hormone, then the gland is autonomous and the patient is at the risk of thyrotoxicosis (Vol. 1; Chaps. 18 and 20).

Preparation

Medications that affect thyroid function should be discontinued at least one week before the test.

Medications

Triiodothyronine (75 μg; Cytomel) orally daily (25 μg three times per day) for 7 to 10 days. Alternatively, Levothyroxine 2 mcg/kg/day, for 10 days has also been used.

Sample

Radioactive iodine uptake studies should be performed before and after treatment. Alternatively, [99 mTc] pertechnetate as radiopharmaceutical agent has been used when using levothyroxine.

Normal and Expected Values

Radioactive iodine uptake in the thyroid should decrease by 50% of the initial value. Failure to suppress is indicative of an autonomous gland.

General Considerations

No side effects of this test have been reported. However, the test is contraindicated during pregnancy.

Thyrotropin-Releasing Hormone Test

Indications

TRH tolerance tests are used primarily for evaluation of prolactin secretion or secondary hypothyroidism. TSH is secreted in response to TRH reaching 5 to 10 times the basal TSH level. Thus, during a TRH tolerance test in euthyroid individuals (43,44), the serum TSH increases into the range that could be detected

by the assay even though the basal level could not be detected.

Preparation

The patient should be off all thyroid medication and chronic aspirin therapy for at least one week before the test.

Medication

TRH (7 μg/kg up to a maximum of 400 μg) should be administered IV over 90 seconds.

Sampling

Samples should be collected before the administration of TRH and at 15-minute intervals for one hour after TRH. All the samples should be assayed for TSH and prolactin. Baseline samples should be assayed for triiodothyronine and thyroxine.

Normal and Expected Values

TSH should increase to 5 to 10 times higher than the basal level. Prolactin levels should increase to three to five times over basal levels, with the peak secretion 15 to 30 minutes after TRH administration. Individuals with secondary hypothyroidism do not raise their TSH levels into the normal range. High basal prolactin levels without increase during the tolerance test is suggestive, but not diagnostic, of prolactinoma.

General Considerations

TRH may cause an increase in blood pressure and is contraindicated in patients with hypertension or cardiovascular disease. During the infusion of TRH, subjects may feel a strong urge to urinate. Thus, it is useful to suggest urination before the start of the protocol. Other side effects of TRH infusion are nausea, vomiting, and facial flushing. These effects only last for 30 to 90 seconds. Because of the nausea, an overnight fast or omission of the last meal should be considered, although not directly required for the test.

Tests for Parathyroid Function

The following protocols were used to evaluate parathyroid function before the availability of RIA tests for parathyroid hormone (PTH). At present, these protocols are occasionally used to detect and evaluate minimal degrees of dysfunction, perhaps associated with partial resistance to PTH or partial protein S deficiency (Vol. 2; Chap. 21).

Ethylenediaminetetraacetic Acid Infusion Test

Indications

This test is a direct method for detecting disorders of the parathyroid gland, including both hypoparathyroidism and pseudohypoparathyroidism (45,46). EDTA is a calcium-specific chelating agent. It is metabolized by excretion in urine with its chelated cations, mostly calcium. Thus, the infusion of EDTA leads to a decrease in serum calcium levels, and it is the response to this stimulus that comprises the test. Under the regulation of hormones secreted by the parathyroid gland, normal individuals respond to its stimulus by mobilization of calcium stores and restoration of serum calcium levels within 12 hours.

Preparation

Patient should fast overnight before the test. Patients should be recumbent for the duration of the test.

Medication

IV infusion of 50 mg/kg of trisodium EDTA in 300 mL of 5% dextrose over a one-hour period. To reduce discomfort at the site of infusion, procaine hydrochloride, 1% or 2%, or lidocaine should be added to the infusion. Care should be taken to assure that the tubing is primed with the anesthetic before the administration of EDTA.

Sampling

Draw blood for calcium immediately before EDTA infusion, immediately after infusion, and at 4, 8, and 12 hours after the start of the infusion. Serum samples may also be assayed directly for intact parathyroid hormone and calcitonin to differentiate the basis for the disorder.

Normal and Expected Values

Preinfusion values of calcium should be within the normal range. Postinfusion levels should fall immediately by 2 to 3 mg/dL. The failure of calcium levels to return to preinfusion levels within 12 hours after the EDTA infusion is indicative of the lack of proper function of the parathyroid hormone. Further tests may be necessary to identify the exact nature of the disorder.

General Considerations

Patients in whom calcium stores may be challenged should be monitored carefully until normal serum calcium levels are restored. Paresthesias of the face and extremities may occur, and patients should be forewarned. Positive Chvostek's and/or Trousseau's signs may be seen at any time during the 24-hour period. Patients should be observed carefully for signs of tetany; appropriate measures should be taken should tetany or seizures ensue.

Ellsworth-Howard Test

Indications

The test is used to differentiate between hypoparathyroidism and pseudohypoparathyroidism (47)

Preparation

All supplemental medications used to treat hypoparathyroidism and pseudohypoparathyroidism such as

Table 3 Expected Rise in cAMP Excretion and Decline in the Tubular Maximum Phosphate Reabsorption after PTH

Diagnosis	Rise in cAMP excretion (nmol/L/GF)	Percentage decline in the tubular maximum phosphate reabsorption (mmol/L/GF)
Normal adults	541 ± 319	0.209 ± 0.061
Pseudohypoparathyroidism	2.7 ± 6.0	0.087 ± 0.064
Hypoparathyroidism	890 ± 452	0.678 ± 0.405

Abbreviations: cAMP, cyclic adenosine monophosphate; PTH, parathyroid hormone.

calcium or vitamin D, should be withheld for 8 to 12 hours before the testing period. Patients should be fasting overnight and over the testing period. Water should be given hourly to assure hydration before and during the test.

Medications

Over a 10-minute period, PTH-(1-34), 3 U/kg with 200 U maximum, is administered IV.

Sampling

Collect urine every 30 minutes three times before and four times after the administration of PTH as well as hourly serum samples for determination of cyclic adenosine monophosphate excretion and tubular maximum for phosphate reabsorption.

Normal and Expected Values

Expected values are listed in Table 3.

Tests for Prolactin Secretion

Dopamine Inhibition of Prolactin Secretion

Indications

Dopamine normally inhibits prolactin secretion. Thus, this test is used when hypersecretion of prolactin is suspected (48).

Preparation

Nothing by mouth from midnight or after the bedtime snack.

Medication

L-Dopa is given orally immediately after the baseline blood sample is obtained. The dose is as follows: (i) 125 mg for children less than 13.5 kg; (ii) 250 mg for children between 13.5 and 31.5 kg; and (3) 500 mg for children over 31.5 kg. Levodopa is not available in the United States. A carbidopa/L-Dopa preparation may be used (1:10). This preparation has been used to evaluate hyperprolactinemia in adults (49).

Sampling

Draw blood for prolactin assay at 0, 40, 60, 90, 120, and 180 minutes after administration of L-Dopa. In view of the method of evaluation of the result, two baseline samples should be obtained, one 15 minutes before and the second just before the administration of L-Dopa.

Normal Values

Prolactin levels should decrease to less than 50% of the baseline value within one to three hours. Lack of suppression suggests autonomous hypersecretion of prolactin.

General Considerations

Nausea and vomiting frequently occur in toddlers. Be prepared. Do not stop taking blood samples. Children should be recumbent and may be given water throughout the test.

Thyrotropin-Releasing Hormone-Induced Prolactin Secretion

Indications

This test was often used for evaluation of prolactin secretion abnormalities (50). However, the access to high-resolution magnetic resonance has replaced the use of this test to distinguish microprolactinomas from functional hyperprolactinemia.

Preparation

The patient should not receive thyroid medication and should not take aspirin therapy for at least one week before the test. For their own comfort, patients should be requested to urinate before the start of the test.

Medication

After collection of a baseline sample, TRH (7 μg/kg up to a maximum of 400 μg) should be administered IV over a 90-second period.

Sampling

Serum should be collected every 15 minutes for one hour after administration of TRH. The samples should be assayed for prolactin.

Normal and Expected Values

In children, prolactin levels should increase three- to five-fold during the test. The peak usually occurs at 15 or 30 minutes. Men have a similar response. In women, the increase can be somewhat larger.

General Considerations

TRH may cause an increase in blood pressure and it is contraindicated in patients with hypertension or cardiovascular disease. Infusion of TRH might cause a feeling of urgency to void; thus, it is useful to suggest urination before starting the protocol. Other side effects of TRH infusion are nausea, vomiting, and

facial flushing. These effects only last for 30 to 90 seconds. Because of the nausea, an overnight fast or omission of the last meal should be considered, although not directly required for the test.

Tolerance Tests for Adrenal Cortex Function

Dynamic endocrine tests for the adrenal cortex are indicated to evaluate secretion of cortisol in individuals with clinically suspected hypercortisolism or hypocortisolism. In addition, tests are useful in evaluating the intermediates steps in cortisol and adrenal androgen production (Vol. 2; Chap. 8). Hypercortisolism is reliably demonstrated by measurements of 17-OH corticosteroids and free cortisol in 24-hour urine. Salivary cortisol level at 11 P.M. has also been used as a screening test.

Low-Dose Dexamethasone Suppression Test

Indications

This test is used as a screening test for Cushing's syndrome or excessive cortisol and/or androgen production (51,52). It can also be used to differentiate between the ovary and the adrenal as the source of excess androgen production. If the source of excess androgen production is the ovary or autonomous adrenal function, then androgen levels are not suppressed by overnight dexamethasone.

Preparation

No specific preparation is needed. The test does not need to be performed in the hospital. Dexamethasone can be provided to the parent and administered at the proper time at home.

Medication

A simple screen is performed by administering a single dose of dexamethasone. For children over 25 kg, prescribe 1 mg of dexamethasone at bedtime (10 P.M.). For children smaller than 25 kg, administer 0.5 mg of dexamethasone at bedtime. However, the preferred test is to administer the Dexamethasone for 48 hours as 0.5 mg every six hours for two days starting at eight or nine in the morning to complete eight doses (1.25 $mg/M^2/24$ hours).

Samples

A serum sample for cortisol is obtained between 8 and 9 A.M. after the single dose of dexamethasone or after the last dose in the 48-hour dexamethasone test.

Normal and Expected Values

Although different cutoff values have been published, a post dexamethasone morning serum cortisol level less than 2 μg/dL or 1.8 μg/dL proved to offer better sensitivity (95–98%) although it increased the number of false positives. In the absence of extenuating circumstances, a serum cortisol level in excess of 2 μg/dL is abnormal. Children with Cushing's syndrome may have cortisol levels after dexamethasone suppression as low as 4 μg/dL in the early course, and this level should slowly increase under the continuing ACTH-induced hyperplasia.

General Considerations

The use of a cortisol cutoff value of 1.8 or 2 μg/dL (more than 2.5 standard deviations above the mean) might result in a few more false positive results, but it leads to fewer false negatives, which is the real purpose of a screening test. The diagnosis of Cushing's syndrome should not be excluded based only on this test.

Low-Dose Dexamethasone–CRH Stimulation Test

This test, also named combined dexamethasone–CRH test, is used to distinguish Cushing's syndrome from pseudo Cushing's. CRH (1 mcg/kg) IV is administered after the two-day low-dose dexamethasone suppression test. Many use a single dose of 100 mcg/dose of IV CRH. Cortisol level is measured before CRH administration and 15 minutes later. Cortisol levels after CRH stimulation for normal individuals or pseudo Cushing's patients remained suppressed (less than 1.4 mcg/dL). Cortisol levels above 1.4 mcg/dL indicate Cushing's syndrome (53).

High-Dose Dexamethasone Suppression Test (Liddle Test)

Indications

This test is used to define further the control of cortisol secretion for individuals who do not have adequate suppression with the overnight dexamethasone test (54,55). Most often, this test is performed as a continuation of the low-dose dexamethasone test described above. It begins on the third day when the low-dose protocol is completed. This test consists of two parts:

Preparation for Part 1

No specific preparation is needed.

Medication for Part 1

Dexamethasone, 20 μg/kg per day, to a maximum of 0.5 mg/dose, is given PO every six hours for two days beginning on day 3 of the test. Older children and adults may be given 0.5 mg every six hours for eight doses ($3.75\ mg/M^2/24$ hours).

Sampling for Part 1

The 24-hour urine collections are started on day 1. Each urine sample should be assayed for 17 hydroxycorticosteroids, urinary free cortisol (UFC), and creatinine. Serum should be collected each morning and assayed for cortisol.

Normal and Expected Values for Part 1

Urinary 17-hydroxycorticosteroid and UFC levels should suppress by more than 50% in normal subjects.

General Considerations for Part 1

Food and water should be available as desired throughout the test period. The test can be done on an outpatient basis. Patients who do not suppress urinary free cortisol levels should be tested with high doses of dexamethasone, part 2 of the protocol. In general, if the results are not available or are ambiguous, part 2 of the protocol should be performed immediately.

Preparation for Part 2

Part 2 should be performed immediately after completion of part 1 on days 5 and 6 of the combined protocol.

Medication for Part 2

Dexamethasone 2 mg/dose is given orally every six hours for two days beginning on day 5 of the test.

Sampling for Part 2

The 24-hour urine samples are collected on days 5 and 6. Each urine sample should be assayed for 17-hydroxycorticosteroids, urinary free cortisol, and creatinine. Serum should be collected each morning and assayed for cortisol.

Normal Values for Part 2

17-hydroxycorticosteroids and urinary free cortisol levels should suppress by more than 50% even in subjects with adrenal hyperplasia or Cushing's disease. In patients in whom they are not suppressed during part 2 of the protocol, the presence of an independent, steroid-producing tumor must be explored.

General Considerations for Part 2

An overnight 8 mg dexamethasone (orally) single-dose test has been used in adults. It provided better accuracy than the 6 day, high dexamethasone dose test by using a criterion of serum cortisol suppression greater than 50%.

Low-Dose Adrenocorticotropic Hormone Stimulation Test

Indications

This test is used to evaluate the integrity of the hypothalamic–pituitary–adrenal axis and adrenal reserve (56,57). A low-dose ACTH test has more sensitivity detecting subtle states of adrenal insufficiency and provides a more physiological concentration of ACTH. It is useful when testing patients who may present with mild adrenal insufficiency due to inhaled steroids.

Preparation

The patient should be not receiving medications that interfere with ACTH secretion (glucocorticosteroids) for at least one week before the test. If the patient has been receiving chronic steroid treatment, the withdrawal process may take a few months.

Medications

In the morning, after collecting baseline serum samples, a single IV bolus of 1 μg of Cortrosyn is administered. Although a 1 mcg-ampule would be desirable, this presentation is not available. We advise dilution by the pharmacist following an established protocol. Dilute the 250 μg-vial with 10 mL of normal saline (25 μg/mL). Take an aliquot of 0.2 mL and dilute it into 1 mL of normal saline; this will yield a final concentration of 5 mcg/mL as stock solution. This solution can be stored at 4°C for seven days. Take 0.2 mL of the stock solution and dilute it into 1 mL of normal saline (1 mcg/mL) for the test. Glass or plastic tubes can be used.

Sampling

Baseline sample and additional sample at 30 minutes will be sent for cortisol measurement. Some authors recommend a single sample at 30 minutes.

Normal and Expected Values

Various cutoff set points of serum cortisol levels have been proposed to indicate normal adrenal function. However, a cutoff of 600 nmol/L (22 μg/dL) is an optimal level with 100% sensitivity and 83% specificity. High sensitivity of a test is preferred in life-threatening conditions such as adrenal insufficiency.

Standard Dose Adrenocorticotropic Hormone Stimulation Test

Indications

This protocol is the standard technique used to evaluate the adequacy of cortisol secretion and adrenal reserve, primarily to eliminate a diagnosis of Addison's disease or congenital ACTH unresponsiveness (58–60). It is the most reliable tool to demonstrate 21-hydroxylase deficiency in patients with CAH. It might be necessary to discern newborns who are detected by the newborn screening program (Vol. 1; Chaps. 8 and 9).

Preparation

The patient should not be receiving medications that interfere with ACTH secretion, especially high-dose glucocorticoids or other steroids. High-dose steroids must be discontinued for at least one week to permit restoration of the normal biosynthetic reserve. If the patient has been receiving chronic steroid treatment, the withdrawal process may take a few months. This test should not be performed during the first 24 hours of life because it will yield ambiguous results.

Medications

In the morning, after collection of baseline serum samples, a single IV bolus of 0.25 mg Cortrosyn is administered. This dose of cortrosyn is used for all age groups including newborns and premature infants.

Sampling

Before the administration of Cortrosyn, a baseline sample is obtained. An additional sample is collected 30 or 60 minutes after administration of Cortrosyn. Each sample is assayed for cortisol, 17-hydroxyprogesterone, progesterone, and 17-hydroxypregnenolone. The exact interval between the administration of ACTH and the second sample must be noted.

Normal and Expected Values

Cortisol levels should exceed 16 μg/dL in either the baseline or post-ACTH sample. Cortisol levels may not be decreased in individuals with 21-hydroxylase deficiency. For evaluation of 21-hydroxylase deficiency, the sum of the increase in progesterone and 17-hydroxyprogesterone concentrations is divided by the time between the samples. Heterozygous individuals for congenital adrenal hyperplasia have responses between 9 and 30 ng/dL/minutes. Responses below 7 ng/dL/minutes are typical of normal subjects. For evaluation of 3 β-hydroxysteroid dehydrogenase deficiency, the ratio of 17-hydroxypregnenolone to 17-hydroxyprogesterone levels is considered. Normal individuals have a ratio of less than 10, and higher ratios are considered diagnostic.

General Considerations

Because of the variable timing of the morning endogenous ACTH-secretory episodes and the secretion of ACTH when a child is frightened, the baseline values are often already stimulated. Therefore, a dexamethasone suppression test should be continued as described below.

Dexamethasone-Suppressed Adrenocorticotropic Hormone Stimulation Test

Indications

This protocol differs from the simple ACTH stimulation test by the administration of a single dose of dexamethasone at bedtime before the test (61,62). This step serves to block the normal morning episodes of ACTH secretion and eliminates the ongoing secretion of cortisol and its intermediates. Without the dexamethasone treatment, the ''white coat syndrome'' (fear of doctors) experienced by many children leads to immediate ACTH secretion and thus to high levels of steroids in the baseline samples. In fact, on many occasions, the baseline samples have higher levels of steroids than the samples obtained after ACTH administration. In contrast, after dexamethasone pretreatment, the presence of steroids in the baseline samples can be attributed either to gonadal secretion or to ACTH-independent pathways. The protocol is used to confirm a suspected diagnosis of complete or partial steroid biosynthetic defects. The dexamethasone-pretreated protocol was specifically developed to overcome the variation in baseline steroid intermediate levels caused by uncontrolled endogenous episodes of ACTH secretion.

Preparation

A single dose of dexamethasone (0.5 mg/m^2) is administered just before the subject goes to bed, usually between 10 and 11 P.M. Patients should be fasted from the time of dexamethasone treatment until the completion of the test. Water may be consumed as desired. Menstruating women should be tested in the follicular phase of the cycle.

Medications

In the morning, after collection of baseline serum samples, a single IV bolus of 0.25 mg Cortrosyn is administered.

Sampling

Two baseline samples are obtained 15 minutes before and right before the administration of Cortrosyn. Additional samples are collected 30, 45, and 60 minutes after the administration of Cortrosyn.

Normal and Expected Values

The test is evaluated by considering the difference between the baseline and stimulated samples of steroid levels. The baseline level is obtained by averaging the two baseline samples; the stimulated level is the average of the two highest samples obtained after Cortrosyn administration. It should be noted that as a consequence of the continued function of the long-acting synthetic glucorticoid, the morning episodes of ACTH secretion do not occur. Thus, baseline levels of steroid intermediates are not elevated.

Expected values are listed in Table 4.

General Considerations

After dexamethasone suppression, there are four common causes of elevated baseline levels of cortisol, androgens, or steroid intermediates: (i) the subject did not take the dexamethasone, (ii) breakthrough ACTH secretion, (iii) gonadal secretion of steroids, and (iv) lack of regulatory control, perhaps caused by Cushing's syndrome. If the subject does not take the dexamethasone, the baseline samples are frequently similar to stimulated values. Ovarian secretion of 17-hydroxyprogesterone, DHEA, and androstenedione is most common in patients with polycystic ovarian disorder or during the luteal phase of the menstrual cycle. The source of excess steroids in

Table 4 Expected Steroid Values

Steroid	Baseline levels	Stimulated levels
Normal individuals		
Cortisol, μg/dL	≤2	12–24
17-Hydroxyprogesterone, ng/dL	≤50	50–150
Androstenedione, ng/dL	≤50	50–200
Dehydroepiandrosterone, ng/dL	≤200	400–800
Heterozygote for 21-hydroxylase deficiency		
Cortisol, μg/dL	≤2	12–24
17-Hydroxyprogesterone, ng/dL	≤50	150–500
Androstenedione, ng/dL	≤50	50–200
Dehydroepiandrosterone, ng/dL	≤200	900–1300
Individuals with 21-hydroxylase deficiency		
Cortisol, μg/dL	≤2	12–24
17-Hydroxyprogesterone, ng/dL		≥2000
Androstenedione, ng/dL	≤50	50–200
Dehydroepiandrosterone, ng/dL	≤200	900–1300

the baseline samples can be attributed to the ovary if the cortisol level is less than 2 μg/dL or less than 5 μg/dL if a woman is taking birth control pills. Finally, with the exceptions noted, baseline cortisol levels in excess of 2 μg/dL are suggestive of Cushing's syndrome. Thus, the addition of dexamethasone pretreatment the night before the administration of ACTH increases the validity of the entire Cortrosyntolerance test procedure.

Metyrapone Test

Indications

Metyrapone inhibits 11 b-hydroxylase, thus blocking the conversion of 11-deoxycortisol to cortisol (63,64). The test is used to assess: (i) pituitary ACTH reserve, (ii) adrenal insufficiency, and (iii) the extent of adrenal suppression for patients on prolonged glucocorticoid therapy. This test may be done as a single- or as a multiple-dose test.

Warning

For patients in whom adrenal insufficiency (Addison's disease) is suspected, an appropriate steroid medication should be kept at the bedside in case of an adverse reaction.

Preparation

The patient should not be receiving medications that interfere with ACTH production, including glucocorticoids, drugs that accelerate the action of metyrapone such as diphenylhydantoin, and drugs that alter the concentration of 17-ketogenic steroids such as penicillin and its variants.

Medication and Sampling

For the multiple-dose test, Metyrapone, 300 mg/m^2 in children or 750 mg in adults, is orally administered every four hours for six doses. Basal 24-hour urine should be collected before the administration of metyrapone and for the next three days. The urine is assayed for creatinine and for 17-hydroxycorticosteroids. Serum should be obtained four hours after the last dose of metyrapone and assayed for ACTH, cortisol, and 11-deoxycortisol.

Normal Values

In normal individuals, 17-hydroxycorticosteroid levels in urine should be greater than 9 mg/M^2/24 hours during the metyrapone treatment. Plasma cortisol levels should be less than 8 μg/dL, and 11-deoxycortisol levels should exceed 10 μg/dL.

Medication and Sampling

The Single-Dose Test is the preferred test. Metyrapone, 30 mg/kg to a maximum of 1 g, is administered as a single oral dose at midnight. Serum should be obtained at 8 A.M. and assayed for ACTH, cortisol, and 11-deoxicortisol.

Normal Values

The results of the single-dose test should be considered in the following order: (i) ACTH levels should be elevated. Inadequate ACTH secretion suggests inadequate pituitary reserve for secretion of ACTH. (ii) Cortisol levels should be less than 8 μg/dL; higher levels suggest inadequate therapy (rapid metabolism) or excessive production of cortisol, perhaps caused by Cushing's syndrome. (iii) 11-deoxicortisol levels should exceed 10 μg/dL; lower levels suggest adrenal insufficiency caused by either lack of recovery from high-dose therapy or Addison's disease.

General Considerations

Hypotension and vomiting may occur during the administration of metyrapone. Transient vertigo may be avoided by administering the drug with milk or a meal. Activity should be mild throughout the day of drug treatment.

Tests for Pheochromocytoma

Dynamic tests in the diagnosis of pheochromocytoma are used when basal levels of catecholamines (plasma metanephrines or normetanephrines/24-hour collection of free or unmetabolized levels of catecholamines) are not diagnostic (Vol. 2; Chap. 10). Patients with pheochromocytomapresent more consistent and relativelylarger increases in plasma free metanephrines and normetanephrines than plasma catecholamines. Age-appropriate reference values are described in Vol. 2; Chap. 10.

Clonidine Suppression Test

Indications

Clonidine suppresses catecholamines arising from the sympathetic neuroendocrine system but not from a pheochromocytoma (65,66). This test is indicated when the biochemical data fail to distinguish and clearly document the presence of a pheochromocytoma.

Preparation

Nothing by mouth after midnight or for 10 to 12 hours before the test. B-adrenergic blocking drugs should be discontinued 48 hours before the test; concomitant administration of these drugs can lead to severe bradycardia and decreased cardiac output. Adequate hydration must be maintained (or restored) before the test because of the possibility of hypovolemia.

Medication

Clonidine, 0.005 mg/kg to a maximum of 0.25 mg, is administered as a single oral dose.

Sampling

Draw blood for free catecholamines (epinephrine and norepinephrine) at 0, 1, 2, and 3 hours after clonidine administration.

Normal and Expected Values

By three hours after clonidine treatment, circulating catecholamines should decrease to less than 500 pg/mL. Failure to suppress the circulating levels is usually diagnostic of pheochromocytoma.

General Considerations

Because of the hypotensive effects of clonidine, blood pressure should be monitored during the protocol and the patient should be in a supine position. Patients may become very drowsy about 30 to 45 minutes after clonidine administration. Various clinical laboratories have different instructions for the handling of blood plasma for catecholamine assays; hence, it is necessary to refer to the specific laboratory you intend to use and their specific protocol. This test has high sensitivity and low specificity.

Glucagon Test

Indications

This test is useful in pheochromocytoma-suspected patients, who present ambiguous results in metanephrine and catecholamine levels and an equivocal response to clonidine-suppression.

Preparation

The patient should rest quietly in a supine position without environmental stress or distraction. A heparin lock should be established at least 30 minutes before the start of the test.

Medication

After collection of baseline blood pressure data and serum, the test is started by the IV administration of 0.5 mg glucagons (67).

Sampling

Blood pressure should be determined 10 and five minutes before glucagon administration and after 5, 10, 20, 25, and 30 minutes of glucagon administration. Plasma should be collected for catecholamine levels at 0, 5, and 10 minutes after glucagon administration.

Normal and Expected Values

In normal patients, there should not be a rise in blood pressure. If a 0.5 mg dose of glucagon does not produce a rise in blood pressure, the test can be repeated with 1 mg glucagon administered as an IV bolus. A significant elevation in blood pressure and catecholamines at 5 to 15 minutes after glucagon administration is indicative of pheochromocytoma.

General Considerations

Patients must be supine throughout the test period. A darkened room without disturbance is often helpful. Various clinical laboratories have different instructions for the handling of blood plasma for catecholamine assay. Hence, it is necessary to refer to the specific laboratory you intend to use and their specific protocol. This test has high specificity and low sensitivity.

Test for Acromegaly

GH hypersecretion is a rare condition related in most cases to a GH-cell pituitary adenoma. Other etiology includes GHRH producing tumors or ectopic tumors. Clinical manifestations result from a long-standing GH secretion and pituitary mass effect. Biochemical diagnosis parameters include measurement of IGF-I and GH response to OGTT. Elevated levels of IGF-I (>2 SD) are considered a good initial screening test suggestive of GH excess. It is important to use age-reference comparative values essentially when evaluating adolescents who have higher levels among all age groups. Elevated α subunit levels are also found in GH-secreting tumors as well as in nonfunctioning pituitary tumor. MRI of the brain is a gold standard image study to precise size and extension of the mass. CT scan might be used if MRI is not available.

Glucose Test for Suppression of Growth Hormone Secretion

Indications

Individuals with autonomous GH secretion do not blunt GH response to hyperglycemia as occurs

physiologically. It should be noted that young infants and diabetics in poor control often have paradoxical GH secretion (68,69).

Preparation

Overnight fast is prescribed before the test.

Medication

After collection of a baseline serum sample, glucose, 1.75 g/kg body weight to a maximum of 75 g, is given by mouth (Glucola, Trutol, or Dextol Cola is commonly used).

Sampling

Serum should be obtained for glucose and GH at the time of glucose administration and every 30 minutes for two hours.

Normal and Expected Values

The initial higher cut of 5 to 10 ng/mL has been reduced to less than 1 ng/mL or even lower, depending on the GH-assay sensitivity.

General Considerations

As desired, patients can drink water and walk during the protocol.

Tests for Diagnosis of Glucose Intolerance

Glucose Tolerance Tests

Indications

This test is useful for the evaluation of impaired glucose tolerance and insulin resistance or hypersensitivity (70) (Vol. 1; Chaps. 3 and 11). The test must include insulin levels to make an accurate diagnosis. A new cut-off point for glucose less than 100 mg/dL is considered the upper limit of normal fasting plasma glucose level (FPG). Impaired fasting glucose (IFG) corresponds to a range of 100 mg/dL to 126 mg/dL. FPG greater than 126 mg/dL indicates a provisional diagnosis of diabetes mellitus, and must be confirmed on a separate day. If the FPG is below 126 mg/dL and there is a high suspicion of diabetes, an oral glucose tolerance test (OGTT) is indicated. The IV protocol should be reserved for patients with gastrointestinal disturbances or intolerance on oral glucose load. The IV protocol may also be preferred when assessing insulin secretion reserve or residual function of β-pancreatic cell in patients at a high risk of developing diabetes or in patients with diabetes if indicated.

Diabetes autoimmunity measurements are useful and should be performed to corroborate the diagnosis of diabetes mellitus (Vol. 1; Chap. 5). Glutamic acid Decarboxylase antibodies (GADA), islet cell autoantibodies (ICA), insulinoma 2 associated autoantibodies (IA-2A) and insulin autoantibodies (IAA) are available for testing in clinical laboratories. MODY testing is also available to distinguish a heterogeneous group of autosomal dominant disorders of insulinopenic diabetes (Vol 1; Chap. 10).

Preparation

For three days before the test, patients should be on a high carbohydrate diet with at least 60% of calories from carbohydrates. Patients should be fasted after midnight or after a bedtime snack the night before the test. Medications that may act as hyper- or hypoglycemic agents should be discontinued.

Medication

After the baseline serum sample is obtained, glucose solution, 1.75 g/kg body weight to a maximum of 75 g, is administered as an oral solution. Trutol, Glucola, and Dextol Cola are commonly used commercial solutions.

Sampling

Draw blood for glucose and insulin at 0, 30, and 60, 90 minutes and at two, three, and four hours after glucose administration. Urine is measured for sugar and acetone at each void throughout the protocol.

Normal and Expected Values

The criteria established by the National Diabetes Data Group in 1979 have been revised by an International Expert Committee sponsored by the American Diabetes Association. (i) Two-hour postload plasma glucose levels less than 140 mg/dL are normal. (ii) Two-hour postload plasma glucose levels greater than 140 but less than 200 mg/dL indicate an impaired glucose tolerance test. (iii) Two-hour postload glucose at levels higher than 200 mg/dL indicate a provisional diagnosis of diabetes mellitus.

Tests for Insulin Resistance/Sensitivity

IFG and IGT are associated with insulin resistance, obesity, dyslipidemia of the high-triglyceride and/or low-HDL type, and hypertension. Insulin resistance is evaluated by simple glucose insulin ratios or by a variety of indexes and/or by sophisticated protocols more suitable for research (Vol. 1; Chap. 11). Insulin sensitivity from data obtained with OGTT may be assessed in numerous ways. The most frequently used are the homeostasis model assessment (HOMA) for insulin resistance (HOMA-IR) and the quantitative insulin-sensitivity check index (QUICKI). HOMA-IR calculation is based on the product of basal insulin level and glucose level divided by 22.5 or 404 when using glucose in nmol/L or mg/dl, respectively. Low values (below 3) indicate good sensitivity. QUICKI is calculated as 1/(log fasting insulin [μU/mL] + log glucose [mg/dL]). The whole body sensitivity index (WBISI) and the insulin sensitivity index (ISI) have been recently validated in children. The WBISI is calculated dividing 10,000 by the square root of the product of fasting glucose and insulin. ISI is

equal to [1.9/6 body weight (kg) FPG (mmol/L)+ 520–1.9/18 body weight x area under the curve for glucose (mmol/h.liter)–urinary glucose (mmol/ 1.8)]/[area under the curve for insulin (pmol/h.liter) body weight). The ISI index has a strong correlation with the euglycemic hyperinsulinemic clamp (71). Other insulin sensitivity indices include belfiore, cederlhom, Gutt, and Stumbul (72).

Direct Methods to Assess Insulin Sensitivity

Intravenous Glucose Tolerance Test

Protocol. After the baseline serum sample is obtained, glucose, 0.5 g/kg body weight, is given as an IV bolus over a three- to four-minute period. It is preferable to have two separate IV lines, one for infusion of glucose and one for obtaining blood samples. The first line may be discontinued after the infusion is complete.

Sampling. Draw blood for glucose and insulin at 0, 1, 3, 5, 10, 20, 30, 45, and 60 minutes after the start of the glucose infusion.

Normal and Expected Values. For quantitative evaluation of the first-phase insulin response (FPIR), the parameter used is the sum of plasma insulin values for one and three minutes [designated insulin $\sum (1' + 3')$]. Normal values for individuals over eight years of age are insulin $\sum (1' + 3') > 100\,\mu U/mL$. Normal values for individuals between three and eight years of age are insulin $\Sigma\ (1' + 3') > 60\,\mu U/mL$. Individuals at high risk for developing diabetes mellitus have a low FPIR ($< 48\,\mu U/mL$).

The disappearance curve of glucose is plotted as a function of time on semilogarithmic paper and the K value (glucose disappearance rate) determined. $K = 0.693\ 100/T_{1/2}$, where $T_{1/2}$ is time in minutes for the glucose to fall into half of its initial value. A K value of less than 1.4 indicates impaired glucose tolerance in persons less than 50 years of age. Insulin levels should reach their peak within 10 minutes of the start of the infusion.

General Considerations. Patients may drink water and move about freely during the test. The test should be postponed until two weeks after any acute illness. There is a risk of hyperosmolality in patients with elevated baseline blood glucose levels.

Hyperglycemic Clamp

The hyperglycemic clamp provides information about insulin secretion and sensitivity (73). It is complementary to the euglycemic hyperinsulinemic clamp, which is mainly used in research because it requires complex equipment and monitoring.

Preparation: Overnight Fasting Medication. An infusion of 25% dextrose is used to raise the glycemic level to 180 to 220 within two minutes and 20% dextrose infusion rate is adjusted to maintain these glucose levels for two hours.

Method. The plasma glucose level is measured every 2 to 2.5 minutes during the first 15 minutes and every five minutes thereafter. The insulin level is measured every 15 minutes during two hours. C peptide is also measured as part of the evaluation of insulin secretion.

Normal and Expected Values. First-phase insulin secretion is calculated as the mean of three measurements during the first 15 minutes and steady phase is calculated as the mean of five levels from 60 to 120 minutes. Insulin sensitivity is calculated as the ratio of the glucose infusion rate divided by the average glucose concentration divided by the mean insulin concentration in each phase. Insulin sensitivity values of first and second phase are decreased in children with type 2 diabetes compared to those with a normal or impaired glucose tolerance test.

Hypoglycemia Workup

Indications

A fasting test might be used in the evaluation of hypoglycemia in children. Hyperinsulinemia, glycogen storage disease, factitious hypoglycemia, carnitine deficiency, fatty acid oxidation disturbance, and inborn errors of metabolism are indications for this test (74–76). The protocol evaluates metabolic changes that occur during a fast (Vol. 1; Chap. 15).

Preparation

The test should be conducted under close supervision. For younger children, maintain a separate IV line with 0.25 normal saline, in addition to the heparin lock, both for hydration and to ensure a patent venous line other than the heparin lock. The patient should be on a high-carbohydrate diet (60% of calories from carbohydrates) for three days. The diet should include frequent feedings. No high-fat foods should be allowed on the evening before the start of the test, and the patient should be fasted after midnight or bedtime snack. Test and prepare bedside device to measure glucose (Dextrostix, Chemstrip bG, or equivalent). In anticipation of hypoglycemia, have at hand syringes prepared with (i) glucagon, 30 μg/kg, and (ii) 50% glucose, 1 mL/kg body weight.

Medications

Glucagon, 30 μg/kg to a maximum of 1 mg is administered by slow IV push.

Method

Consider starting fasting according to age and previous evaluation of glucose levels on a normal diet.

Fasting might start after 8 or 9 P.M. in older children; however, monitoring in neonates or infants might start after last feeding or breakfast, respectively. Check plasma glucose tightly, every four hours until glucose is 80 mg/dL, every two hours until glucose is less than 70 mg/dL, every hour when glucose is 60 mg/dl, and continue every 30 minutes to the end of the test when glucose is less or equal to 50 mg/dL. Indications to terminate the tests are: glucose less than 50 mg/dL, worrisome signs of hypoglycemia or 24- or 36-hour fasting in infants and older children, respectively. If no hypoglycemia occurs, but the patient develops metabolic acidosis (HCO_3 less than 15 mEq/L, with or without normal pH), end the test. If hyperinsulinism is suspected, use glucagon at the end of the test. All urine is collected and evaluated for ketones and specific gravity. If severe symptomatic hypoglycemia and/or convulsions occur at any point, collect the serum sample and terminate the fast at once by administering the 50%-glucose solution, 1 mL/kg IV. The serum should be assayed for the hypoglycemia panel including glucose, insulin, cortisol, GH, lactate, free fatty acids, beta hydroxybutiric acid, and electrolytes. According to the suspected diagnosis, other tests might be included such as plasma ammonia, plasma acyl-carnitine profile, and urinary organic acid profile.

Normal and Expected Values

Glucose levels should remain above 50 mg/dL throughout the fasting. Episodes of hypoglycemia are considered abnormal at any time throughout the test. Episodes of GH secretion greater than 10 ng/mL should follow about 20 minutes after any hypoglycemic episodes. Cortisol levels of 8 to 20 μg/dL are normal under nonstressful conditions. Under the conditions of this fast, levels should increase by 10 μg/dL or exceed 20 μg/dL. In samples obtained during hypoglycemic episode, low glucose–insulin ratios (ratio of 3 versus ratio of 6 as observed in normal individuals) are indicative of hyperinsulinism. The concentration of metabolites must be considered to evaluate the pathophysiology of hypoglycemia in other patients.

General Considerations

Patients must remain quiet but can sit in a chair or lie down, as they desire. They can drink water and use the bathroom freely. The IV infusion rate should be adjusted according to water intake. Parents should be encouraged to remain with the child, if this will lower stress. If the patient fails to develop ketonuria during the test, the possibility of surreptitious food intake should be considered.

Glucagon may cause some mild abdominal discomfort and/or nausea. Hence, it should be administered by slow IV push. Patient should be checked frequently for vital signs and symptoms of hypoglycemia, including convulsions, stupor, tremors, or coma. A flow sheet should be maintained and all data should be recorded.

Tests for Gonadal Function

Acute Response to Human Chorionic Gonadotropin Administration Combined with Dexamethasone

Indications

This protocol is useful to confirm the diagnosis of nonclassic 17-ketosteroid reductase deficiency, typically in a teenage boy with severe gynecomastia (77,78).

Preparation

The test should be performed in the morning. Patients should eat a normal breakfast and can continue to eat and drink as desired and as appropriate.

Medications

At bedtime at home, the patient should take 1 mg of dexamethasone. In the morning (about 9 A.M.), the patient should receive 4000 IU of hCG as an intramuscular injection.

Samples

Serum samples should be obtained before the administration of hCG and 2, 4, 6, 24, and 48 hours later.

Normal and Expected Values

In normal males, the testosterone–androstenedione ratio increases in response to hCG. The final testosterone levels should exceed 300 ng/dL, which are adult normal levels. In patients with partial 17-ketosteroid reductase deficiency, the sum of testosterone and androstenedione concentrations equals the adult normal levels.

General Considerations

The two major sources of androstenedione are the adrenal and the testis. Administration of dexamethasone serves to eliminate secretion from the adrenal and thus permits monitoring of testicular synthesis.

Compared with testosterone, androstenedione is the preferred substrate for aromatization. Thus, the excess production of androstenedione leads to excess production of estrogens and the development of gynecomastia. Many boys at puberty develop mild gynecomastia that disappears as testosterone production increases. In normal individuals, the 17-ketosteroid dehydrogenase is substrate- (androstenedione) and product- (testosterone) dependent, thus leading to complete conversion of androstenedione to testosterone. However, the enzyme of some individuals does not have the proper kinetic properties and does not fully convert all androstenedione to testosterone. The incomplete deficiency disorder is inherited as an autosomal recessive trait. This test helps to identify these patients.

Tonic Response to Human Chorionic Gonadotropin Administration

Indications

This test is used to aid in the diagnosis of vanishing testis syndrome, disorders of steroidogenesis, micropenis, ambiguous genitalia, or other circumstances in which there is a question about normal testicular function (79,80). It is also indicated to distinguish patients with gonadotropin deficiency from those with delayed puberty (Vol. 2; Chap. 11).

Preparation

No specific preparation is needed.

Medication

Four doses of hCG (3000 IU/m^2 per day, max 5000 Units/day) are administered intramuscularly. Alternative protocols use only 3000 IU/m^2 every other day for one to three injections.

Samples

A baseline serum sample and subsequent stimulated samples are obtained 16 to 24 hours after each dose of hCG, and should be assayed for testosterone and androstenedione.

Normal and Expected Values

Testosterone levels should increase to over 170 ng/dL after a single dose, over 200 ng/dL after two doses, and normal adult levels or greater than 300 ng/dL after the third dose. Androstenedione should not exceed one quarter of the observed testosterone level.

General Considerations

Progesterone or other intermediates can be assayed if the testosterone response is inadequate.

Gonadotropin Sleep Study

Indications

This rarely-used test is useful in suspected polycystic ovarian disease and in suspected precocious puberty (81,82).

Preparation and Medication

None are needed.

Sampling

Blood samples are obtained every 20 minutes via heparin lock for LH and FSH, overnight for up to 12 hours. In small children for whom the amount of blood drawn may be a critical factor, the test may be modified by limiting the period of sample collection and increasing the interval to 30 minutes.

Normal Values

Normal, prepubertal children have no nocturnal episodes of gonadotropin secretion.

General Considerations. There are no side effects to this protocol.

Gonadotropin-Releasing Hormone (Factrel) Response Test

Indications

The response to the administration of GNRH is useful for evaluating the role of pituitary dysfunction in children with premature or delayed puberty (83,84). Tolerance test responses can be used to distinguish central nervous system dysfunction from peripheral dysfunction in both premature and delayed puberty. In children with premature puberty, peripheral dysfunction (testotoxicosis, McCune-Albright syndrome, and ovarian follicular cysts) is frequently associated with hypogonadotropism, whereas hypergonadotropism can be caused by central nervous system dysfunction (hamartoma or craniopharyngioma). In contrast, in children with delayed puberty, peripheral dysfunction is associated with hypergonadotropism (Turner syndrome and Klinefelter syndrome) and hypogonadotropism suggests dysfunction in the central nervous system (Kallman syndrome, Prader-Willi syndrome, and constitutional delay). The test is also useful in evaluating the extent of damage caused by radiation or chemotherapy in children with leukemia or brain tumors. In this group, the results can indicate the need for endocrine replacement therapy.

Preparation

Although not required, many physicians request that: (i) the patient fast overnight before the test and (ii) the test be performed in the morning. Certainly, there is time-of-day variation in the secretory pattern for LH and FSH, and most of the normal data have been collected in this manner. Thus, using a consistent protocol eases comparison of results to previous studies.

Medications

Administer an IV bolus dose of 10 μg/kg to a maximum of 100 μg GNRH (Factrel, Ayerst Laboratories, New York, NY).

Sampling

Baseline samples ($n = 2$) should be obtained 15 minutes apart before the administration of the GNRH. Stimulated samples should be obtained 15, 30, 45, 60, and 90 minutes after GNRH administration. For comparison with published values, the baseline samples and the three highest stimulated samples should be averaged. This protocol minimizes slight variations in secretory pattern and in laboratory values.

Normal and Expected Values

The response pattern changes with age, sex, and development. After six months of age and before puberty, basal levels for both LH and FSH are frequently less than 2 IU/L. Stimulated levels less than 5 IU/L are indicative of hypogonadotropism, and levels in excess of 50 IU/L are indicative of hypergonadotropism. With a few exceptions, the increase in both gonadotropins should be similar in magnitude. In girls with precocious puberty, the LH levels rise more than the FSH. Girls within a year of menarche often have FSH levels about twice those of LH. Individuals with mild or partial steroid-biosynthetic defects often have higher LH than FSH levels.

General Considerations

Patients need not remain seated but can walk around during the test. There are many units for LH and FSH. Be sure to check that the units reported by the laboratory are the same units used for reporting normal values.

Alternative LHRH Tests

Due to the unavailability of Factrel, different approaches were developed using Leuprolide Acetate. There has been no general consensus regarding the alternative approaches. Based on a few studies that have been published (83,85), we summarize the method that we are using at our center. The LHRH test is based on the fact that LH reaches its peak three to four hours after the subcutaneous administration of Leuprolide acetate. Leuprolide acetate is given at 20 mcg/kg/dose, with maximum dose of 500 mcg, as subcutaneous injection. Serum gonadotropins are done three hours after the injection. Serum estradiol for girls and testosterone for boys are done 24 hours after the injection. The three-hour gonadotropins will help to determine if the precocious puberty is gonadotropin-dependent. The 24-hour sex steroid level will help to differentiate if the gonadotropin-dependent precocious puberty is progressive. The interpretation of the results is based on the studies done by Ibanez et al. (86) and by Garibaldi et al. (87). LH level above 9.7 IU/L was indicative of gonadotropin-dependent progressive puberty in males and females. Testosterone level above 3.5 nmol/L in males and estradiol level above 2.49 pmol/L in females were indicative of progressive puberty. Practitioners should also pay attention to the current changes of assays for the gonadotropins and sex steroids in order to compare the values of the laboratory used to the published information. This method has limited usefulness for differentiating gonadotropin deficiency from constitutional delay of puberty.

Monitoring Therapy

For patients who are receiving long-acting GNRH analog (depot leuprolide) treatment for central precocious puberty, reevaluation using Factrel test is required for the efficacy of the treatment. Recently, a study about the pharmacokinetic of depot leuprolide indicated a rapid rise of serum gonadotropins after depot leuprolide injection (88). Therefore a single sample of LH obtained at 30 to 60 minutes after depot leuprolide injection could effectively assess efficacy of treatment. There is no special preparation needed. In our center, we reevaluate patients using this method at the fourth injection or as needed thereafter. The proposed cutoff of LH for the treatment efficacy is <3.0 mIU/mL.

Test for Diabetes Insipidus

Water Deprivation Test

Indications

This protocol is useful in the diagnosis of diabetes insipidus and in differentiating neurogenic (hypothalamic central) diabetes insipidus, nephrogenic (renal) diabetes insipidus, and primary polydipsia (inappropriate thirst mechanism or psychogenic diabetes insipidus) (89,90) (Vol. 2; Chap. 29).

Preparation

No special preparation is necessary. Treatment for diabetes insipidus with vasopressin, desmopressin (DDAVP), or other analogs should be discontinued 48 to 72 hours before the protocol. Careful attention to the possibility of dehydration should be given after the medications are stopped.

Method

After obtaining the first pair of urine and plasma samples, water and food are restricted for four hours in infants and up to seven hours in older children. Obtain weight every hour and measure specific gravity in each urine sample. End the test if dehydration of 3% to 5% occurs. If polyuria persists, administer DDAVP at the end of the test. No food or drink may be consumed during the protocol.

Sampling

Collect baseline samples for Uosm (urine osmolality), Posm (plasma osmolality), and plasma electrolytes. Repeat sample at two hours in infants and four hours in older children.

Normal Values

At any time during the protocol, baseline Uosm in excess of 400 mOsm/kg with normal Posm (between 275 and 300 mOsm/kg) eliminates a diagnosis of diabetes insipidus. Baseline Uosm less than 300 mOsm/kg with normal Posm are consistent either with overhydration or with diabetes insipidus. After the water deprivation period, individuals with overhydration or diabetes insipidus may still have Uosm less

than 300 mOsm/kg. However, individuals with overhydration have Posm between 275 and 290 mOsm/kg while Posm remains above 290 mOsm/kg in individuals with diabetes insipidus.

General Considerations

Some physicians have extended the period of water deprivation even longer. However, care must be taken to assure that the patient does not ingest water surreptitiously and/or does not become dehydrated.

GENETIC TESTING

A significant number of pediatric endocrine disorders are in fact genetic diseases. Although the traditional hormonal and dynamic testing still plays an essential role for the clinical diagnosis and guidance of management, the rapid emergence of molecular genetics over the past two decades has provided an opportunity for diagnosis by direct analysis of DNA samples. For all mutations identified on genes causing endocrine disorders, some of them can be tested in commercial laboratories and most of them still can only be tested in research laboratories. There are still a large number of genes and mutations causing endocrine diseases yet to be determined. The advancement of mutation detection techniques leads to significant improvement in the accuracy for detection of both known and unknown mutations. However, the use of these techniques and the interpretation of the results are frequently not straightforward. As all other analytical methods, genetic tests have limitations. We will briefly discuss the techniques that are commonly used to provide general information about the principle usage and limitation of genetic testing.

Based on the information in the medical literature, we summarized the clinical indications for genetic testing: (i) to make a diagnosis when no other conventional diagnostic tests can be applied; (ii) to confirm a diagnosis made by conventional tests; (iii) to make a prenatal diagnosis; (iv) to assess a person's risk of developing a disease; (v) to assess the risk of having an affected child; and (vi) to make a postmortern diagnosis. Carefully choosing of candidates for genetic testing is essential for appropriate interpretation of results.

Several types of mutations from large chromosomal abnormalities to single base pair changes are known. For chromosomal abnormalities such as trisomy or polysomy, rearrangements, and deletions, chromosomal analysis and fluorescent in situ hybridization (FISH) can be performed. For major gene deletion, duplication, rearrangement, and presence of pseudogenes, southern blot analysis and quantitative PCR can be performed. Two new techniques, multiple amplification and probe hybridization (MAPH) and multiple ligation-dependent probe amplification (MLPA), were developed recently for detection of midsize deletion. Both deletion and duplication of multiple-target sequences can be detected simultaneously. We will focus on the rapid development of techniques for detection of DNA variations such as small deletions, insertions, and point mutations. Two main approaches for detection of these types of mutations are applied: direct detection of known mutations and identification of unknown mutations. Sequencing of DNA is considered the gold standard for both known and unknown mutations. However, sequencing of the entire coding region of a gene can never be a routine procedure due to the intense labor, time consumption, and cost. In addition, other alternative methods are required to confirm the sequence variations identified by DNA sequencing. *Guide to Mutation Detection* (91) and *Current Protocols in Human Genetics* (92) provide comprehensive reviews and detailed protocols for the applications of each technique.

Direct Detection of Known Mutations

When the disease-causing gene and mutations have been found and the methods to detect such mutations have been established, direct detection of mutations can be done for patients who have been diagnosed or suspected clinically with the condition. Most of the modern DNA-diagnostic techniques are dependent on the polymerase chain reaction (PCR) and analysis of the PCR products. A pair of specific primers is used to amplify a small region of a gene known to contain a disease-causing mutation. If the mutation is a deletion or insertion of nucleotides, direct analysis of the size of the PCR products can be done. Sometimes, the presence of the PCR product can be diagnostic for the integrity of a specific region of the gene on at least one allele. If a specific mutation (most frequently a point mutation) abolished or introduced a restriction enzyme site, the PCR product can be digested by the restriction enzyme and analyzed on either an agarose gel or an acrylamide gel based on the predicted sizes of the DNA fragments. This method can be used for identification of wild type, heterozygous, and homozygous in one experiment. However, partial enzyme digestion may cause misinterpretation of the results and limit the usage of this method.

Some mutations may not be associated with the changes of restriction enzyme sites. In this situation, allele-specific oligonucleotide (ASO) probes can be used. This method is based on the fact that mutations, as small as a single base mismatch, prevent the ASO probe from annealing to the target DNA. Multiple mutations can be done at the same time. It is difficult to demonstrate the difference between wild type and heterozygote because one copy of the normal target region of a gene will be able to produce the hybridization signal, although using the intensity of the hybridization signals has been suggested. Another PCR-based method used for detection of a specific mutation is the amplification refractory mutation

system. In general, the PCR reaction tolerates a mismatch in the middle or the 5′ end of a primer, but not at the 3′ end of a primer. If a mismatch is located at the 3′ end of a primer, the presence or absence of the PCR product can be indicative of the presence of that mismatch. Hence, when using both wild type and mutant primers in separate PCR reactions, the normal homozygote, or mutant homozygote, or heterozygote can be identified by the presence of the PCR products in each reaction.

The ligase chain reaction (LCR) is another diagnostic tool for detection of point mutation. The principle of this method is based on the ligation of two oligonucleotide probes only when they are directly adjacent. In conjunction with appropriate design of gap filling, LCR can detect point mutations with great accuracy and specificity. This method, however, has limited sensitivity.

DNA microarray is a powerful tool for screening and detecting large number of mutations simultaneously. Also known as DNA chip, microarray is a solid-phase technique that binds a large number of specific oligonucleotides to either glass or silicon slides. PCR product labeled with fluorochrome is then hybridized with the DNA chip. A perfect match or incomplete match or mismatch will be determined by an optimized detection system on computer. Although the homozygous mutation is readily detected, the interpretation of the heterozygous mutation is complicated. To improve the sensitivity and specificity, a modified step called minisequencing primer extension was developed. Using this new cutting-edge technology named tag-array minisequencing, both homozygous and heterozygous mutations can be detected.

Identification of Unknown Mutations

If a patient with a clinical condition does not have any of the known mutations in the disease-causing gene, a search for the unknown mutation on the gene might be considered. For autosomal recessive conditions, patients may have a known mutation on one allele and no known mutation on the other allele, and thus a search of the unknown mutation may be needed. Direct sequencing of the gene is the gold standard to identify the unknown mutation. However, direct sequencing can never be a routine procedure. Several techniques were developed to identify unknown mutations or polymorphisms. These methods are based on the following principles: (i) A small variation of DNA sequence can cause conformational changes of the secondary structure of either single- or double-stranded DNA chains, and mobility changes on electrophoresis as a consequence. Methods among this category are denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), conformation sensitive gel electrophoresis (CSGE), heteroduplex analysis, and single-stranded conformation polymorphism (SSCP). Although DGGE and TGGE can achieve nearly 100% sensitivity, they are labor-intensive and time-consuming. SSCP and heteroduplex analysis are fairly simple but their sensitivity is not as good as the others. (ii) Increased sensitivity of single-stranded regions of DNA or DNA–RNA hybrids to chemical or enzyme cleavage. Methods in this group include chemical cleavage method and enzyme mismatch cleavage. (iii) The ability to separate heteroduplex DNA by denaturing high-performance liquid chromatography (DHPLC). This development has brought the mutational search into a highly sensitive, specific, and automated stage. Nonetheless, the findings from all the above indirect methods require confirmation by direct DNA sequence analysis.

Recently, capillary electrophoresis (CE) was introduced to enhance the sensitivity and reproducibility, as well as the possibility of high-throughout mutation screening. In contrast to the traditional gel-based electrophoresis, CE significantly shortens the time for separation of DNA fragments. Because CE was introduced to DNA sequencing technology, there has been about an eight-fold increase in sequence data production. CE can be applied to many methods used for detection of known and unknown mutations. CE-SSCP and temperature-gradient CE have been described and used for screening of unknown mutations. Furthermore, microchip-based CE may decrease the electrophoresis time further and increase the efficiency of mutation screening.

For situations that the disease-causing gene has not yet been identified or there is no effective way to detect known mutations in a disease-causing gene due to a wide variety of mutations, linkage study remains the method of diagnosis. Linkage study can help to localize a disease-causing gene on a chromosome. There are three types of markers used: restriction fragment length polymorphisms, variable number of tandem repeat, and microsatellite markers. Linkage analysis requires accurate clinical diagnosis for the proband and the participation of critical family members. The interpretation of the results is potentially complex and misleading if the quality of clinical diagnosis is poor.

Virtually all diagnostic testing has certain limitations, including genetic testing. Caution should be used in genetic testing and interpretation of the results. Careful clinical evaluation and use of conventional testing should be applied prior to the consideration of genetic testing. Most genetic testing is limited to the known disease-causing mutations or common mutations. The service for genetic diagnosis is limited and the majority is still in the research stage. Due to the ethical complexities associated with genetic testing, genetic counseling before a test is ordered should be done. Appropriate counseling and support are extremely important specially for patients and family members who are positive for a mutation causing a genetic disease or carry the mutation for a recessive disease. Guidelines should be strictly followed when considering a genetic test for patients and family members (93).

Table 5 List of Current Available Genetic Tests Offered by Laboratories

Genes	Associated conditions	Laboratories
Growth and growth disorders		
POU1F1	Combined pituitary hormone deficiencies involving GH, TSH, or prolactin	All Children's Hospital, Molecular Genetics Laboratory, St. Petersburg, FL Athena Diagnostics
PROP1	Combined pituitary hormone deficiencies involving GH, TSH, prolactin, and gonadotropins, possible late onset ACTH	All Children's Hospital, Molecular Genetics Laboratory, St. Petersburg, FL Athena Diagnostics Vanderbilt University Medical Center, Molecular Genetics Laboratory, Nashville, TN
SHOX	Short stature, Leri-Weill syndrome, Turner variants	Esoterix
Adrenal and sex developmental abnormalities		
ABCD1	X-linked adrenoleukodystrophy	Athena Diagnostics Kennedy Krieger Institute, Genetics Laboratory, Baltimore, MD Mayo Clinic, Biochemical Genetics Laboratory, Rochester, MN Oregon Health and Science University, Biochemical Genetics Laboratory, Portland, OR Reproductive Genetics Institute, Chicago, IL
CYP21	Congenital adrenal hyperplasia due to 21-hydroxylase deficiency	Chapman Institute/Center for Genetic Testing at Saint Francis, Tulsa, OK Comprehensive genetic services, SC, Milwaukee, WI Esoterix Mt. Sinai Laboratory of Pediatric Endocrinology, New York, NY Pediatrix screening, inc., Bridgeville, PA Quest diagnostics Reproductive Genetics Institute, Chicago, IL University of Texas health science center at San Antonio, histocompatibility and immunogenetics laboratory, San Antonio, TX
GNAS	McCune–Albright syndrome	Chapman Institute/Center for Genetic Testing at Saint Francis, Tulsa, OK
HSD11B2	Low-renin hypertension	Athena Diagnostics
LHCGR	Familial male-limited precocious puberty	Athena Diagnostics
NR0B1 (*DAX1*)	X-linked adrenal hypoplasia congenita including complex glycerol kinase deficiency	Athena Diagnostics Baylor College of Medicine, Medical Genetics Laboratory, Houston TX Signature Genomic Laboratories, Spokane, WA
SRY	46 XX sex reversal, 46 XY gonadal dysgenesis	Most of the university medical center cytogenetics and molecular diagnostic laboratories offer FISH or mutational analysis, or both in the United States. Other commercial laboratories offer similar services: DIANON system, Molecular Genetics Laboratory, Stratford, CT GeneDx Inc., Gaithersburg, MD Genezyme Genetics, Santa Fe, NM Quest Diagnostics
Thyroid disorders		
FOXE1, *PAX8*, and *TSHR*	Congenital hypothyroidism	Pediatrix Screening, Inc., Bridgeville, PA
SLC26A4	Pendred syndrome	Boston University School of Medicine, Center for Human Genetics, Boston, MA Chapman Institute/Center for Genetic Testing at Saint Francis, Tulsa, OK Children's Hospital Boston, DNA diagnostic Laboratory, Boston, MA Harvard-Partners Center for Genetics and Genomics, Laboratory for Molecular Medicine, Cambridge, MA National Institute of Health, Laboratory of Molecular Genetics, NIDCD, Bethesda, MD Stanford Hospital and Clinics, Molecular Pathology Laboratory, Palo Alto, CA University of Iowa, Molecular Otolaryngology Research Laboratories, Iowa City, IA
THRB	Thyroid hormone resistance	All Children's Hospital, Molecular Genetics Laboratory, St. Petersburg, FL Chapman Institute/Center for Genetic Testing at Saint Francis,

(*Continued*)

Table 5 List of Current Available Genetic Tests Offered by Laboratories (*Continued*)

Genes	Associated conditions	Laboratories
		Tulsa, OK
		Quest Diagnostics
Calcium and phosphorus metabolism		
CASR	Familial benign hypocalciuric hypercalcemia	Athena Diagnostics
		GeneDx, Inc. Gaithersburg, MD
CLCN5 and *OCRL*	Dent disease including hypophosphatemic rickets, X-linked recessive	GeneDx, Inc. Gaithersburg, MD
CYP27B1	Pseudovitamin D deficiency rickets due to 25-hydroxyvitamin D-1-a hydroxylase deficiency	Connective Tissue Gene Tests, Allentown, PA
		GeneDx, Inc. Gaithersburg, MD
FGF23	Hypophosphatemic rickets, dominant	Athena Diagnostics
		Connective Tissue Gene Tests, Allentown, PA
		GeneDx, Inc. Gaithersburg, MD
GNAS	Albright hereditary osteodystrophy/ pseudohypoparathyroidism	Chapman Institute/Center for Genetic Testing at Saint Francis, Tulsa, OK
		John Hopkins Hospital, DNA Diagnostic Laboratory, Baltimore, MD
PHEX	Hypophosphatemic rickets, X-linked dominant	Athena Diagnostics
		Connective Tissue Gene Tests, Allentown, PA
		GeneDx, Inc. Gaithersburg, MD
Bone diseases		
CLCN7	Osteopetrosis, autosomal dominant type II	Connective Tissue Gene Tests, Allentown, PA
COL1A1 and *COL1A2*	Osteogenesis imperfecta type I, II, III and IV	Athena Diagnostics
		Reproductive Genetics Institute, Chicago, IL
		Tulane University Health Sciences Center, Matrix DNA Diagnostics, New Orleans, LA
		University of Washington, Collagen Diagnostic Laboratory, Seattle, WA
Hypoglycemia		
ABCC8, *KCNJ11*, *GLUD1*, and *GCK*	Familial (congenital) hyperinsulinism	Athena Diagnostics
Diabetes mellitus		
HYMA1 and *PLAGL1* (*ZAC*)	Transient neonatal diabetes mellitus, chromosome 6q24 related	Comprehensive Genetic Services, SC, Molecular Diagnostic Laboratory, Milwaukee, WI
		University of Chicago Genetic Services, Chicago, IL
IPF1, *GCK*, and *KCNJ11*	Neonatal diabetes mellitus	Athena Diagnostics
HNF4A	*MODY1*	Athena Diagnostics
GCK	*MODY2*	Athena Diagnostics
TCF1/HNF1A	*MODY3*	Athena Diagnostics
		Esoterix
IPF1	*MODY4*	Athena Diagnostics
TCF2/HNF1B	*MODY5*	Athena Diagnostics
Diabetes insipidus		
AVP	Neurohypophyseal (Central) diabetes insipidus	Quest Diagnostics
AQP2	Nephrogenic diabetes insipidus, autosomal	Quest Diagnostics
AVPR2	Nephrogenic diabetes insipidus, X-linked	Athena Diagnostics
		Quest Diagnostics
Endocrine neoplasia		
MEN1	*MEN1*	Athena Diagnostics
		Boston University School of Medicine, Center for Human Genetics, Boston, MA
		GeneDx, Inc. Gaithersburg, MD
		Yale University School of Medicine, DNA diagnostics Laboratory, New Haven, CT
RET	*MEN2*	All Children's Hospital, Molecular Genetics Laboratory, St. Petersburg, FL
		Athena Diagnostics
		Children's Hospital of Philadelphia, Molecular Genetics Laboratory, Philadelphia, PA
		Comprehensive Genetic Services, SC, Molecular Diagnostic Laboratory, Milwaukee, WI
		GeneDx, Inc. Gaithersburg, MD
		Henry Ford Hospital, DNA Diagnostic Laboratory, Detroit, MI
		Huntington Medical Research Institutes, Molecular Oncology &

(*Continued*)

Table 5 List of Current Available Genetic Tests Offered by Laboratories (*Continued*)

Genes	Associated conditions	Laboratories
		Cancer Genetics Laboratory, Pasadena, CA
		Mayo Clinic, Biochemical Genetics Laboratory, Rochester, MN
		Ohio State University, Molecular Pathology Laboratory, Columbus, OH
		Quest Diagnostics
		University of Pittsburgh Medical Center, Division of Molecular Diagnostics, Pittsburgh, PA
		Washington University School of Medicine, Molecular Diagnostic Laboratory, St. Louis, MO
		Yale University School of Medicine, DNA diagnostics Laboratory, New Haven, CT
VHL	Von Hippel-Lindau disease	Athena Diagnostics
		Boston University School of Medicine, Center for Human Genetics, Boston, MA
		Children's Hospital of Philadelphia, Molecular Genetics Laboratory, Philadelphia, PA
		John Hopkins Hospital, DNA Diagnostic Laboratory, Baltimore, MD
		Reproductive Genetics Institute, Chicago, IL
Obesity and cholesterol metabolism		
LDLR	Familial hypercholesterolemia	Athena Diagnostics
APOB	Familial hypercholesterolemia type B	Andrology Laboratory Services, Inc. Chicago, IL
		ARUP Laboratories, Inc. Salt Lake City, UT
		Athena Diagnostics
MC4R and *ADRB2*	Familial early-onset severe obesity	All Children's Hospital, Molecular Genetics Laboratory, St. Petersburg, FL
		ARUP Laboratories, Inc. Salt Lake City, UT
		Athena Diagnostics (MC4R only)
Autoimmune endocrinopathy		
AIRE	Autoimmune polyendocrinopathy syndrome type I	Athena Diagnostics
		GeneDx, Inc. Gaithersburg, MD
Genetic syndromes		
BBS1 and *BBS2*	Bardet-Biedl syndrome	Athena Diagnostics
		University of Iowa Hospitals and Clinics, Department of Pathology, Iowa City, IA
CDKN1C, *H19*, *KCNQ1OT1*	Beckwith-Wiedemann syndrome	Baylor College of Medicine, Medical Genetics Laboratory, Houston TX
		Chapman Institute/Center for Genetic Testing at Saint Francis, Tulsa, OK
		Comprehensive Genetic Services, SC, Molecular Diagnostic Laboratory, Milwaukee, WI
		LabCorp Clinical Cytogenetics Laboratory, Research Triangle Park, NC
		Mayo Clinic, Biochemical Genetics Laboratory, Rochester, MN
		Sacred Heart Medical Center, Cytogenetics Laboratory, Spokane, WA
		Shodair Hospital, Genetics Laboratory, Helena, MT
		Signature Genomic Laboratories, Spokane, WA
		Washington University School of Medicine, Molecular Diagnostic Laboratory, St. Louis, MO
PTPN11	Noonan syndrome	Athena Diagnostics
		Baylor College of Medicine, Medical Genetics Laboratory, Houston TX
		Children's Hospital Boston, DNA diagnostic Laboratory, Boston, MA
		Columbus Children's Hospital, Molecular Genetics Laboratory, Columbus, OH
		GeneDx, Inc. Gaithersburg, MD
		Greenwood Genetics Center, Molecular Genetics Laboratory, Greenwood, SC
		Harvard-Partners Center for Genetics and Genomics, Laboratory for Molecular Medicine, Cambridge, MA
		Mount Sinai School of Medicine, Department of Pediatrics, New York, NY
		Oregon Health and Sciences University, Molecular Diagnostics Center, Portland, OR
		Prevention Genetics, Molecular Diagnostics and Biobanking, Marshfield, WI
		Signature Genomic Laboratories, Spokane, WA

(*Continued*)

Table 5 List of Current Available Genetic Tests Offered by Laboratories (*Continued*)

Genes	Associated conditions	Laboratories
		Transgenomic Labs, Omaha, NE
PWCR on chromosome 15q11–13	Prader-Willi syndrome	Most of the university medical center cytogenetics and molecular diagnostic laboratories offer either FISH or methylation analysis, or both in US. Other commercial laboratories offer similar services:
		ARUP Laboratories, Inc. Salt Lake City, UT
		Comprehensive Genetic Services, SC, Molecular Diagnostic Laboratory, Milwaukee, WI
		Genezyme Genetics, Molecular Diagnostic Laboratory, Westborough, MA
		LabCorp, Research Triangle Park, NC
		Quest Diagnostics
		Specialty Laboratory, Valencia, CA

Abbreviations: GH, growth hormone; ACTH, adrenocorticotropic hormone; TSH, thyroid stimulating hormone.

A public website (94) sponsored by the NIH is an extensive resource for information about genetic conditions and DNA testing. Table 5 provides a list of endocrine conditions, genes and mutations that currently can be detected commercially using the study of DNA.

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Endocrinology

about the fifth edition . . .

Celebrating more than twenty years as the single best source in the field, this ***Fifth Edition*** has now expanded into two cornerstone volumes with 53 fully inclusive chapters and 77 renowned contributors that comprehensively address every topic and trend relevant to the identification, diagnosis, and management of endocrine and endocrine-related disorders in children. Substantially revised, ***Volume 2*** offers 33 chapters reflecting the current state of the field and analyzes the frequent clinical endocrine ailments affecting children. The partner volume, ***Volume 1***, contains 20 chapters covering obesity, diabetes, insulin resistance, and hypoglycemia. This text is devoted to the diseases of the endocrine glands, including disorders of growth, the adrenal cortex and medulla, as well as sexual differentiation, thyroid, and parathyroid conditions and fluid balance alterations.

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about the editor...

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