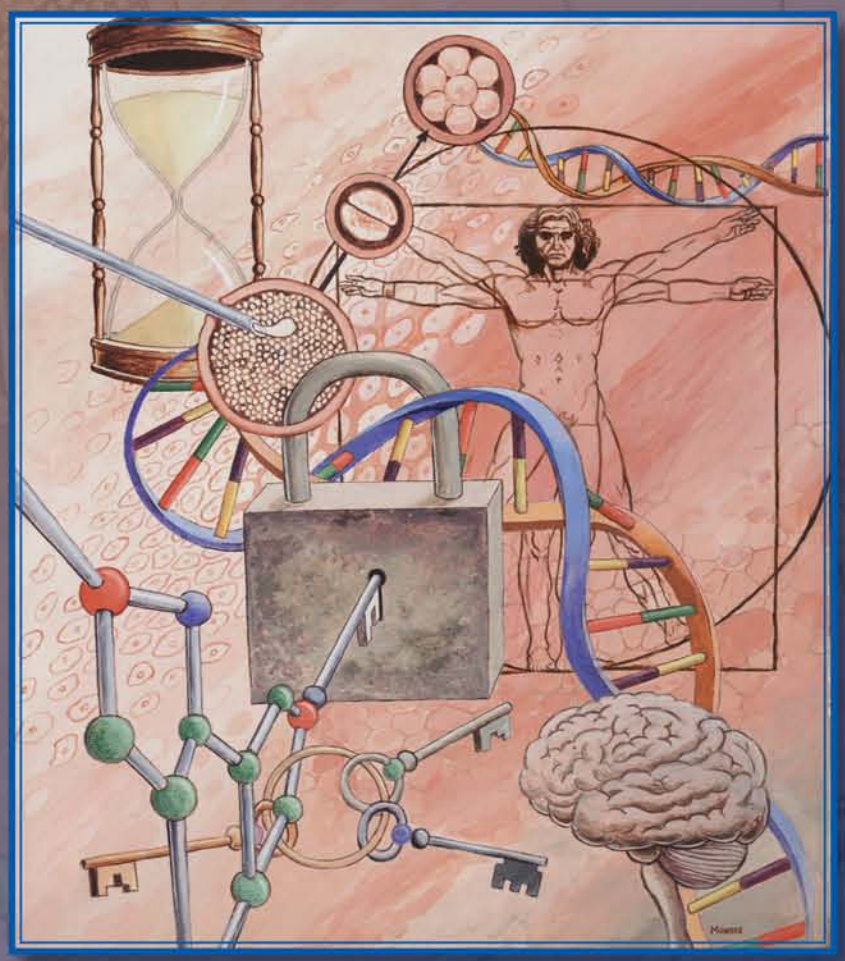
Physiological Basis of Aging and Geriatrics

Fourth Edition



Edited by Paola S. Timiras

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Preface

The publication of the fourth edition of *Physiological Basis of Aging and Geriatrics* comes at a particularly opportune time. The lengthening of life expectancy *at birth and in old age, which began in the twentieth century, has reached both industrialized and, increasingly, developing countries*. Currently, individuals aged 80 years and older appear to be the fastest growing segment of the human population, and centenarians, once rarely seen, are much more frequently encountered. Understandably, these demographic changes are being accompanied by a new way of thinking about aging.

In the early twentieth century, the study of aging focused primarily on biomedical models of pathology, that is, how to diagnose the diseases and chronic disabilities afflicting the elderly, and how best to treat them. It is safe to say that most scientists studying aging before the l980s regarded aging as a rising wall of mortality. Since then, however, due to a multiplicity of factors, social as well as medical, we have witnessed a major effort among researchers to reinterpret aging as a normal, healthy, and even positive feature of the life span. Although aging may make older adults more susceptible to disease, most retain sufficient plasticity and regenerative capability to ensure functional competence, which may determine how successfully, if not how long, aging populations may live. As we now view them, aging and death, much like development and maturation, entail numerous, complex interactions that have encouraged researchers to turn their attention to the physiological basis of aging as well as the genetic and environmental factors that alternately enhance and impair functional competence at molecular, cellular, and organismic levels.

Chapters are grouped into three main parts: In Part I, *General Perspectives*, aging is viewed as an individual's "journey taking place in a community setting." It describes the demographic, epidemiologic, and comparative aspects of aging and discusses molecular and cellular aspects of aging in relation to several theories of aging, thus providing a comprehensive profile of aging in individuals and populations. Part II, *Systemic and Organismic Aging*, surveys the aging of body systems, focusing on maintenance of optimal functioning and adaptation to environmental demands. Part III, *Prevention and Rehabilitation*, presents a synopsis of pharmacologic, nutritional, regenerative, and assistive interventions that promote successful aging and longevity. Using physiology as its unifying concept, the fourth edition contains concise, explicit explanations and numerous comprehensive tables and graphs. Clinical correlations are included as a practical reference for the geriatrician and as a guide to normal aging for the gerontologist.

Comparable books on aging target a professional and/or academic readership; here, the goal is to offer information that will be useful to a broad spectrum of readers from different biological and educational backgrounds. We believe it will not only meet the needs of those preparing for a career in gerontology or geriatrics or interested in aging as a specific topic in biological sciences, but also of older persons themselves, along with their families and caretakers, who seek to better understand the aging-related changes and to gain new insights about this stage of life.

When the elderly are viewed through the eye of the clinician, the emphasis is on the need for assessing, managing, and reducing risk factors. Equally important, as we begin to see aging in a new light, is strengthening physiologic competence and devising appropriate interventions aimed at improving quality-of-life. The concept of "continuity through change" is fundamental to all biological processes. As individuals and society itself age, continuity of prior events may provide "a usable past" that can serve us well in shaping future functions. Indeed, in a 1972 book, *Developmental Physiology and Aging*, (Timiras) identity was shown to be as dynamic a process among the elderly as it is in the young. Slowing or otherwise mitigating the effects of old age by strengthening physiological competence throughout life does not deny the inevitability of death, but it does deny the inevitability of disability, disease, and despair. In this first quarter of a new century, we have every reason to rejoice in the vigorous declaration of the nineteenth century English poet, Robert Browning's, "Grow old along with me/The best is yet to be/The last of life for which the first was made/Grow old nor be afraid!"

Paola S. Timiras

Acknowledgments

I have been greatly encouraged in preparing this fourth edition by the participation of several of the collaborators of the first, second, and third editions and by the equal enthusiasm and expertise of the first-time co-authors. All have willingly accepted the task of reviewing, updating, or preparing anew their respective chapters or sections within stringent deadlines. To them, I offer my most heartfelt thanks for an extraordinarily well-accomplished performance.

Special thanks to Dr. S. Oklund for the many drawings, which effectively illustrate and integrate the complex material presented.

I also wish to thank my assistants, Irene Thung and Brian Bui, who competently prepared the manuscripts according to the specifications of the publisher and dealt with the word processing, editing, and formulation of the many tables that effectively integrate the diverse information.

I thank the editing team of Informa Healthcare and The Egerton Group for their vigilance about the progress of the book and its many drafts. In particular, I would like to thank Sherri Niziolek, Sandra Beberman, Chris DiBiase, and Ginny Faber.

Finally, I would like to recognize the silent encouragement of the many hundreds of students who have taken, over the years, my class, "Physiology of the Aging Process." By their enrollment in the class, interest in the subject matter, their criticism or praise, they have inspired me to continually improve, streamline, and update the course material, a process which has resulted in the subsequent editions of this book.

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Part I

General Perspectives

1

Old Age as a Stage of Life: Common Terms Related to Aging and Methods Used to Study Aging

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THE "LONGEVITY REVOLUTION" AND ITS IMPLICATIONS

Perhaps two of the greatest human achievements to have occurred from prehistoric to more recent times, and, particularly, in the last two centuries, are the increase in the human population worldwide and the extension of human longevity. Thanks to improved living conditions stemming from advances in agricultural practices and advances in industry and technology, the human population today has reached an unprecedented size (1–3). Living conditions have greatly improved due to

1. public health reforms and improved personal hygiene,
2. advances in medical knowledge and practices,
3. vastly increased control over the environment, and
4. rising income and standards of living.

Never in human history have so many humans lived longer: In the United States, for example, the percentage of Americans aged 65 and older, relative to the entire U.S. population, rose from 4% in 1900 to 8% in 1950 and to 13% in 1990, and these increases are even greater in the population statistics of other countries (Chapter 2). Indeed, because of this longevity revolution that owes much to improvements in technology and extraordinary advances in biomedicine, industrialized countries are now faced with a large graying population; individuals in the "healthiest" countries now have a life expectancy at birth of 80 years and longer (Chapter 2). Although it is difficult to predict with certainty what will happen in the future, it is expected that this trend toward increased longevity will continue, at least in the most developed countries. For example, it is predicted that by the year 2030, about one-fifth (18%) of the population of the United States will live to 65 years and longer (4,5).

Today, in several developed countries, the rise in the proportion of the elderly relative to the total population is associated with a decline in the proportion of young people (Chapter 2). This phenomenon has to do not only with the increased longevity of the elderly, but also with the decrease in the number of the young persons. Despite a reduction in infant mortality worldwide, attributable to successful preventive public health and medical interventions, we have seen a concomitant reduction in fertility, which has been attributed to various socioeconomic and lifestyle factors. For example, in Italy, the average number of births in the family has fallen from 4.67 in 1850 to 3.14 in 1900, to 1.88 in 1950, and to 1.3 in 2005 (6,7). To adjust to a population shift where the elderly represent a predominant group, societies must rearrange their economic, technologic, medical, and educational priorities. Such adjustments are as costly as they are difficult to accomplish, for while both younger and older persons have a similar degree of vulnerability, their needs are quite different. Thus any change in priorities must be based on a solid understanding of the fundamental principles that regulate human aging if we are to achieve optimal social, economic, and medical support for both groups.

In regard to older persons, such planning necessarily involves (*i*) repudiating persistent myths and stereotypes of old age (Box 1) and replacing them with a firm grounding in what we now know about the physiology of human aging, and (*ii*) adopting new guidelines compatible with recent demographic changes (Box 2) and incorporating a realistic view of current and future physiotechnological advances. According to the renowned economist Fogel, "health care is the growth industry of the twenty-first century. It will promote economic growth through its demand for high-tech products, skilled personnel, and new technologies" (2).

SIGNIFICANCE OF PHYSIOLOGY IN HUMAN AGING

Why focus on the physiology of human aging rather than on the diseases of old age? Until recently, pathologic processes in old age were studied extensively with the intent of combating diseases specific to this high-risk group; the physiology of aging was not of primary interest. Understandably, it is difficult to isolate "normal function" (the domain of physiology) from "abnormal function" (the domain of pathology); aging, while resulting from a "normal" process, nevertheless leaves us increasingly vulnerable to degenerative diseases.

In these early years of the twenty-first century, however, aging is being regarded anew as a positive and potentially rewarding process, free of the stigma it has had in the past of inevitable decline in health and productivity. With the introduction of the concepts of "successful" or "healthy" aging, many researchers are shifting attention from a primary focus on the diseases of old age to the possibility of strengthening normal functional competence at the molecular, cellular, and organ systems levels over the life span (8–10). During the life span, according to Hayflick, "the level of physiologic capacity reached at the age of reproductive success in living things is taken as the determinant of their potential longevity" (11). While aging is not a disease, it may result from increasing "molecular disorder" in the cells of vital organs (11). The simplistic and hotly disputed statement that "aging is cell aging" links the reduction in or loss of the compensatory and regenerative functional capacity of cells and organs with aging to an increase in susceptibility to disease and death (12) (Chapter 4).

One of the many problems encountered by those who study aging is that, thus far, aging has defied all attempts to

BOX 1 *Myths and Stereotypes About Old Age*

Myths and stereotypes about old age pervade our culture. Not only do they obscure the facts about the later years, they undermine the potential most of us have today for a vigorous and healthy senescence.

According to the writer and painter, Henry Miller[a], "At eighty I believe I am a far more cheerful person than I was at twenty or thirty. I most definitely would not want to be a teenager again. Youth may be glorious, but it is also painful to endure. Moreover, what is called youth is not youth; it is rather something like premature old age".

To take full advantage of the many years remaining at the end of our lives, it is essential that we begin with an accurate picture of physiologic aging. The most common and insidious myths to be disavowed are the following:

- Old age starts at 65 years. (In the words of the painter and sculptor, Pablo Picasso[a], "One starts to get young at the age of sixty, and then it's too late.")
- Old age is a disease.
- Old age always brings mental impairment.
- The elderly are a homogeneous group in terms of their physiologic decline over time.
- Old people are usually poor.
- Old people are powerless.
- Nothing can be done to modify aging; the elderly of the future will have the same problems they have today.
- The United States has been and will always be a youth-focused culture.

With intensive re-education, starting at an early age, these negative images can be reversed. The truth is that:

- Good health during early ages is important as a forerunner of good health in old age.
- Considerable advances have been made in prolonging life expectancy and in preventing and treating many diseases, and similar advances may be expected in the future.
- The quality-of-life in old age depends on biomedical as well as socioeconomic and cultural factors.
- Prevailing attitudes about aging and negative images of the elderly can be turned around through education.

At all ages, a sense of well-being or "wellness"—the overworked but useful term coined in the 1970s—depends on good mental and physical health and a favorable environment. Although elderly persons afflicted by declining function and increasing pathology are more vulnerable to the everyday stresses of life, maintaining and even restoring wellness are possible at all ages. A state of wellness, especially in later years of life, can be secured through a judicious combination of interventions: biomedical and bioengineering along with social and economic (Chapters 22–25).

[a] Sampson A, Sampson S, eds.: The Oxford Book of Ages. Oxford University Press, New York, 1985.

establish objective landmarks, as menarche, does at puberty. Old age in humans is conventionally accepted as the stage in the life cycle that begins at around 65 years of age and terminates with death. However, given the considerable heterogeneity of the elderly population and the complexity of physiological processes, it is difficult to circumscribe the physiologic boundaries of aging in temporal terms. Rather, the onset of aging occurs at some indeterminate point in the mature individual and its progression follows timetables that differ with each person and vary depending on genetic and environmental factors. *Indeed, "physiologic heterogeneity" is one of the consistent characteristics of the elderly population* (Chapter 3).

■ ORGANIZATION OF THE CHAPTERS

This book comprises 25 chapters divided into three parts: *General Perspectives*, *Aging of Systems and Organs*, and *Prevention and Rehabilitation*. Our goal is to provide a book that will be useful to a broad spectrum of readers: those preparing for a career in gerontology or geriatrics, those interested in aging as a specific topic in biology, and older persons (as well as their families and caretakers) who would like to better understand and distinguish age-related changes occurring in the body over time. The joint fields of gerontology and geriatrics are fast expanding, and they attract people from many disciplines, all of whom require a common understanding of the fundamental principles of aging. Using physiology as the unifying concept, this book assimilates and distills information from multiple sources to produce a comprehensive text accessible to a wide audience.

Any consideration of the aging process cannot ignore psychological, social, and economic components, and this is particularly so for the geriatrician, who must deal with the patient from a holistic perspective. Although such factors are vital to a full understanding of this phase of life, an in-depth exploration of their contribution is beyond the scope of this book; the reader will be referred to appropriate publications for further study.

■ THE JOURNEY OF LIFE

Historically, chronological age has been used to assess the transition from one stage of life to the next. In its broadest sense, the human life span can be divided into two main periods:

BOX 2 *The Elderly as an Asset*

Unlike in times past, people turning 50 today have nearly half of their adult life ahead of them. While the quality of their physical life will be considerably better than that of their predecessors, what about their social, political, and economic life that is so vital to well-being? And when they retire, will they opt for a lifestyle of leisure and recreation or will they choose to continue working or volunteering their skills? And, for the less fortunate elderly, what will be the choices they can afford?

Responding to these questions is a critical task not only for individual men and women in this age group, but for society worldwide (7–9). As communities across the United States and other nations continue to develop policies and services for their citizens, it becomes increasingly important, given today's demographic realities, that thought be given to society's older members. As in all planning efforts in this arena, establishing a sound policy starts with needs surveys by age group, by gender, and, for the elderly, by health status. Determining the level of health among older members allows each community to evaluate the potential of this age group to continue contributing to society.

Beyond the services typically provided for "seniors"—low-cost clinics, community centers, assistance with taxes and such—policy makers may broaden their vision, finding ways to

- strengthen public health education to include recommendations about hygiene, nutrition, and physical exercise in this population, and
- create opportunities for the elderly to participate more actively in the community (e.g., working part-time on a paid or volunteer basis in outreach programs, in service jobs, as mentors, and as school resource volunteers) not simply to enhance their own lives but to procure vital services that benefit the community at large.

Indeed, if not struggling with disease or poverty, the elderly represent individuals who are variously skilled, literate, energetic, and well informed. Their survival alone suggests a certain resilience.

It is well known among educators that holding low expectations is not conducive to achievement. Yet, do we not hold low expectations of the elderly when we bow down to the myths of aging and arbitrarily reject them as contributing members of society, as buoyed by a sense of purpose and meaning as their counterparts in youth and adulthood? At the societal level, it is foolhardy, in fact, to regard older adults as a "throw-away" population whose usefulness is at an end when, in reality, they represent a largely untapped resource, particularly in the United States (7,8).

It follows, then, that if society is to benefit from the contributions of older people, the agencies and programs set up to provide them with services and programs would do well to

- re-educate themselves about the realities of physiologic aging,
- view retirement as a lifestyle transition, not a termination of employment,
- maintain high expectations of senior citizens, and, of course,
- distinguish between those in need and those able and willing to serve the needs of others.

Few in this age group need or want to take to their rockers; it is well documented that pursuing useful activities enables older adults to stay physically and mentally alert, flexible, and in touch (9) (Chapter 7).

Leo Tolstoy in the nineteenth century went beyond that limited prescription when he wrote in a letter to a friend, "Don't complain about old age. How much good it has brought me that was unexpected and beautiful. . .[and] the end of old age and of life will be just as unexpectedly beautiful".

[a] Sampson A, Sampson S, eds.: The Oxford Book of Ages. Oxford University Press, New York, 1985.

prenatal and postnatal (Table 1). In contemporary Western society, the postnatal period is identified by a chronological timetable that states: "children begin school at age five, young people go to work or to college at 18, old people retire at 60 or 65.... Age is being taken as a criterion for sequencing the multiple roles and responsibilities that individuals assume over a lifetime" (13). In terms of physiology, the most striking changes take place in the embryonal and fetal stages when the developing organism is most "plastic," that is, most susceptible to being modified or modulated by external (e.g., nutrition, physical exercise) and internal (e.g., hormones) influences. Compensatory and regenerative changes continue to occur even in adulthood and old age, although, given their slower pace, these changes do not produce results on a par with those possible at younger ages.

Many animal species are capable of an independent existence at relatively immature ages; other species, including the human species, are not. The human newborn is utterly dependent on adults for food and care. Additionally, throughout infancy, childhood, and adolescence, remolding of body shape continues gradually, together with the acquisition of new functions and the improvement of those already established. The need for nurture of the immature human infant, child, and adolescent has led to the formulation of an evolutionary explanation for the increased life span of the parents and grandparents; that is, even though the older generations either have lost or have experienced diminished capacity to reproduce, they have played, and continue to play an essential role in the preservation of the species by raising and protecting young humans until they have reached maturity and self-support (14).

Developmental changes are complete at about 25 years of age, and the body is stabilized in its adult condition. The mature adult period lasts for approximately another 40 years and encompasses the period of maximal physiologic competence.

As indicated previously, the span from 65 years until death was viewed in the past as a period of progressive decline in normal function and of inevitable increase in disease and disability. The substantial heterogeneity among old persons and observations of positive trajectories of aging without disease, disability, and major physiologic decline have offered a more positive model of successful or healthy aging (Chapter 3). Accordingly, while some of the pathologic processes that may accompany aging will be discussed, in general, the focus throughout this book is an optimistic one for this population.

■ Prenatal and Early Postnatal Life Stages

Both prenatal and postnatal stages may be subdivided into several periods, each distinguished by morphologic, physiologic, biochemical, and psychological features. The main divisions and the approximate time periods of the life span in humans are listed in Table 1.

The prenatal period encompasses three main stages: oval, embryonal, and fetal. The postnatal period begins with birth and continues into neonatal life, infancy, childhood, adolescence, adulthood, and old age. The concept that the life span is divisible into successive stages is not new. Those writing in antiquity established three, four, and seven periods of life for mankind (15). For example, Aristotle divided life into three ages: growth, stasis, and decline. This was modified later in Greek medicine and physiology into four age periods: childhood, youth, maturity, and old age.

While it is necessary in practical terms for researchers to fragment the biologic study of living organisms, such divisions can obscure the dynamic relationships that operate at many levels of organization and cross arbitrarily set age boundaries. We should recall here that human development depends on "a program of genetic switches that turn on in a highly regulated manner, at specific places (in the organism) and times," and that "responses to environmental challenges fostering changes early on may reverberate decades later in the guise of cardiovascular diseases and diabetes" (16). Indeed, there is increasing experimental and epidemiological evidence suggesting that events in earlier ages (and even in utero) can set the stage for disease in adult and old ages (5,16,17) (Chapter 3) . Thus the physiologic profile of a given individual must be assessed with regard to his or her particular life history in all its biosocial complexity. As has been said, "growing old gracefully is the work of a lifetime!"

TABLE 1 Stages of the Life Span

Stage	Duration
Prenatal life	
Ovum	Fertilization through week 1
Embryo	2–8 wk
Fetus	3–10 mo
Birth	
Postnatal life	
Neonatal period	Newborn; birth through week 2
Infancy	Week 3 until end of first year
Childhood	
Early	2–6 yrs
Middle	7–10 yrs
Later	Prepubertal; females ages 9–15; males 12–16
Adolescence	The 6 yrs following puberty
Adulthood	Between 20 and 65 yrs
Senescence	From 65 yrs to death
	65–74—young-old
	75–84—old-old
	85+—oldest-old
Death	

■ Stages of Maturity and Old Age

The mature years are considered a major life stage characterized by stability as manifested in optimal and integrated function of all body systems. Indeed, function in adulthood is taken as a standard against which to measure any degree of physiologic or pathologic deviation that may have occurred at younger ages or becomes apparent with age. In most textbooks of human physiology, the mature 25-year-old, 70 kg, 170 cm male is taken as a point of reference. However, because functional competence is multifaceted, and optimal performance may differ from age to age and from one functional parameter to another, it would be physiologically incorrect to assume that a function is maximally efficient only during adulthood and that differences in one's earlier or later years necessarily represent functional immaturity or deterioration, respectively. Rather, physiologic competence must be viewed as having several levels of integration, depending on the requirements of the organism at any specific age and the type and severity of the challenges to which the organism is exposed. Indeed, adequate function of several selected organs and systems may persist into old age.

Physiology represents the study of function at all levels of biological organization. For this reason, the techniques used by physiologists are often borrowed from other disciplines. Nevertheless, there are certain techniques that are used repeatedly in gerontological studies. *Cross-sectional and longitudinal methods* represent some of the most frequently applied in studies involving humans. Measurements such as *activities of daily living* (ADL) *and instrumental activities of daily living* (IADL) continue to be used to assess physiologic competence and the presence or absence of disease and disability (Chapter 3).

Some of the major difficulties encountered in studying aging in any organism at any specific age is the type and severity of the challenges or stress factors to which the organism is exposed (Chapter 9). In several organs and systems, adequate function often persists into old age. Although the age of 65 years has been accepted as the demarcation between maturity and old age, a person of that age may be quite healthy and a long way from "retiring" from the workplace or, more generally, from the demands of daily life.

If it is difficult to subdivide the adult years into physiologic stages, old age presents even more of a challenge in this regard (Chapter 3). In the elderly, it can be said that:

- There is great heterogeneity of responses among individuals of equal age.
- Changes do not involve all functions to the same degree and at the same time.
- The timetable of functional changes is differentially susceptible to specific intrinsic and extrinsic factors (Chapter 3).

■ Death vs. Immortality

While old age is approached gradually, without any specific physiologic markers of its onset, death is the terminal event that ends life. In broad terms, causes of death may be classified as trauma, accidents, and disease (Chapters 2 and 3). Trauma and accidents (e.g., high-speed vehicle crashes, dangerous occupations, drug abuse, cigarette smoking) are the major causes of

death in young adulthood. Cancer, cardiovascular diseases, and metabolic diseases are the most frequent causes of death in older adults (Chapter 3). Death need not be related exclusively to aging: disease processes that overwhelm the body's defense or repair mechanisms affect persons of all ages, but they are particularly life endangering in the very young and in the very old.

Many diseases, primarily infectious ones, that lead to death in the perinatal and childhood periods—periods of high risk—have been conquered in developed countries. In the United States, the majority of deaths from disease occur in the elderly in whom diminished function makes the accumulation of pathologic events less tolerable than in the young. Indeed, some diseases (e.g., degenerative diseases) occur almost exclusively in the old, and this linkage of pathology with old age has been invoked by some investigators to support the argument, ardently denied by others, that aging itself is a disease. However, old age is not an accepted natural cause of death and is not reported on the death certificate. Although there may be differences of opinion about how long human beings might live, there has never been any doubt about the inevitability of death.

The tacit acceptance of a debilitating old age is now being replaced by one that regards senescence as the "subversion of function," the inevitability of which is open to question. The question now being raised is whether it is simply a "design fault" that we age and die (18,19). Organ or cell transplantation, or cloning, i.e., replacement of defective organs or cells with better functioning ones, represent somewhat clumsy ways to postpone death. Research exploring new pharmacologic, genetic, and bioengineering technologies as well as on improving nutrition and lifestyle habits offers startling potential for ameliorating and prolonging life (Chapters 22–25).

With the elucidation of the human genome, genetic research is attracting increasing support because of its potential use in diagnosing and treating disease. Until now, humankind has considered immortality in the light of extending our life through our offspring; genes, in a sense, are immortal. For many, however, this sense of immortality no longer seems adequate. They are frustrated that in a time when humans have gone into and returned from outer space and manipulated DNA, they have not conquered death. Death, indeed, remains the last "sacred" enemy to be conquered.

The concept of a single causative factor to account for aging pervades many areas of biologic study, and gerontology is no exception. However, it is unlikely that a single "triggering" event is responsible for the aging and death of the human organism; rather, aging and death probably entail numerous and complex interactions at different genetic and environmental levels (18–22). The fact that we cannot answer many questions at this time and that the nature of the aging process remains unclear, need not deter our search. Only by continually posing bold new questions can we hope to accomplish the spirited goals of gerontologists: to "add years to life and life to years." Biologists working in the area of aging have defined three major goals:

1. To prolong human life
2. To significantly enhance physical vigor and vitality at all ages
3. To prevent or treat diseases throughout the life span

New research allows a glimpse into a world in which aging and, perhaps, even death due to age-related diseases, may at least be delayed considerably (19).

TABLE 2 Brief Glossary of Aging-Related Terms

Term	Definition
Aging	Latin "aetas." The lifelong process of growing older at cellular, organ, or whole-body level throughout the life span
Geriatrics	Greek "geron," old man, and "iatros," healer. The branch of medicine specializing in the health and illnesses of old age and the appropriate care and services
Gerontology	Greek "geron," old man, and "logos," knowledge. The multidisciplinary study of all aspects of aging, including health, biological, sociological, psychological, economic, behavioral, and environmental factors
Senescence	Latin "senex," old man. The condition of growing, limited to old years
Life span	The duration of the life of an individual/organism in a particular environment and/or under specific circumstances
Average life span	The average of individual life spans for members of a group (cohort) of the same birth date
Life expectancy	The average amount of time of life remaining for a population whose members all have the same birth date and based on a given set of age specific death rates (generally the mortality conditions existing in the period mentioned)
Active life expectancy	As above, with the additional idea that the years of life be free of a special level of disability
Longevity	Long duration of an individual's life; the condition of being "long-lived," also often used as a synonym for life span
Maximum life span	The length of life of the longest-lived individual member of a species
Biomarkers	Physiologic and anthropomorphic measures (morphological, functional, and behavioral) specific to old age
Physiology	The science that treats the functions and activities of the living organism and its parts
Pathology	The science that deals with the nature of disease (of molecular, cellular, tissue, and organ systems)
Heterogeneity	The quality of being composed or consisting of dissimilar elements or ingredients; with respect to human population, the diversity of function within a specific age group from the molecular to the systemic level
Plasticity	The capacity to be modified or molded by hereditary and environmental influences

Source: Adapted, in part, from Ref. 23.

■ COMMON TERMS RELATED TO AGING

The interpretation of aging as a physiologic process upon which pathology and disease are superimposed has been formalized under the separate disciplines of *gerontology* (the study of aging processes) and *geriatrics* (the prevention and treatment of the disabilities and diseases associated with old age) (Table 2). The terms "aging," "old age," and "senescence" are often used interchangeably despite some substantive differences. "Aging" refers more appropriately to the process of growing old, regardless of chronologic age; for our purposes here, it includes all the physiologic changes that occur with the passage of time, from fertilization of the ovum to death of the individual. The World Health Organization breaks down "old age," classifying persons 60 to 74 years of age as the young-old, those 75 to 84 as the old-old, and those 85 years and older as the oldest-old. Centenarians, of course, are individuals 100 years old and older (Table 2). "Senescence" is generally restricted to the stage of old age characteristic of the later years of the life span (Table 2).

METHODS USED TO STUDY AGING

Human Studies

Physiology represents the study of function at all levels of biological organization. For this reason, methods and protocols to study aging in humans are difficult to implement. Therefore, the use animals or of tissues/cells in vivo or in vitro often are substituted in place of human studies.

Some of the major difficulties encountered in studying aging in humans include:

- The heterogeneity of the aging process in individuals as well as in the total number of individuals in a population
- The heterogeneity of the aging process in systems, organs, cells, and molecules of a single individual
- The considerable duration of the human life span that makes it extremely difficult for one investigator or group of investigators to follow functional changes in the same individual or individuals from conception to death
- The limitation of experimental intervention in humans due to ethical considerations
- The differential influence of genetic and environmental factors in individuals of different age, race, ethnicity, and sex, as well as socioeconomic, health, disease, and disability status.

Animal Studies

Animal studies are often chosen by researchers because of availability, cost, ease of maintenance, and popularity with funding agencies, even though they may not be the best choice for the research in question (22,24,25). Criteria that are important to consider in choosing an animal model are species, sex, genetic properties, and research goals. These criteria must embody the following characteristics:

- Specificity: The model must exhibit the trait (e.g., function) of interest.
- Generality or transferability: The results observed in the chosen model must be applicable to other species.
- Feasibility: The availability, cost, and convenience of the model must be reasonable.

To investigate the applicability of a given study of the aging process and longevity in animals to humans, a number of successful approaches have relied on the use of flies and worms. The costs involved are low, and these invertebrates are readily available, live a short life, and require little space (Chapters 3 and 4). For example, large-scale studies of medflies (*Ceratitis capitata*) have provided an extensive database of longevity and age- and sex-specific mortality for millions of these flies from the early 1990s to the present time (25). In another species of fly, *Drosophila melanogaster*, it was found that physiological performance at later ages and resistance to stress could be extended by selective breeding of the oldest animals in the colony; with the increased longevity, these flies exhibited a higher level of reproduction at later ages but at the cost of reduced fecundity in early life (26,27). In some worms (*Caenorhabditis elegans*), the life span can be increased by as much as six times in some mutants by suppressing the common receptor for the hormones, insulin/insulin-like growth factor (28). In worms, as in flies, while resistance to stress is increased, growth and reproductive functions are reduced (29) (Chapter 3).

Most organisms, from yeast to mammals, suspend reproduction during periods unfavorable for reproduction by entering a different physiologic mode. For example, when food is scarce, yeast enters, a stationary phase during which reproductive activities are interrupted (30,31). Similarly, medflies may change from "waiting" to "reproductive" mode when they improve their diet, from sugar-only to full-protein. In yeast, as well as in worms and flies, an increase in the activity of sirtuins, enzymes associated with life span control, increases longevity. The opposite effect is observed when the sirtuin enzyme is either diluted or reduced; in yeast, the life span shortens by as much as 30%. Sirtuins (e.g., SIRT1) are found in humans, and, although there is reason to suspect that they play a role in the aging of human cells as well, definitive evidence remains to be established.

Another currently popular approach uses mostly mice to create a unique animal model (Chapter 4). With the advent of recombinant DNA technology and the ability to genetically engineer new animals, investigators are now able to alter a specific gene or process. The resulting *transgenic animals*, the most frequently used being *transgenic mice*, carry a fragment of foreign DNA integrated in their genome or have a portion of the genome deleted or mutated (32). In this manner, the role of individual genes may be studied in normal function (as in functional genomics) and in diseases, and potential genetic effects on aging may be clarified (Chapter 4).

Still another approach to the experimental study of the aging process uses single cells or fragments of tissues removed from the body and cultured under in vitro conditions. Such studies establish standardized conditions of cell culture for cells and tissues derived from individuals of different chronological ages and compare specific parameters of cell function such as replication and metabolism. For example, "cell doubling capacity" is taken as one index among several of cell reproductive capability (33) (Chapter 4). Cultured cells and tissues may serve as models for identifying specific cell functions or mimicking aging pathologies characterized by specific types of cell aging. In addition, cells from different tissues, made to de-differentiate in culture, may then be trans-differentiated into cell types of different tissues (34).

Examples of the ways in which comparative studies in animals allow us to better understand aging processes in humans are presented throughout this book.

HEREDITARY AND ENVIRONMENTAL INFLUENCES ON AGING

Developmental processes and their regulation by heredity and the environment have engaged the attention of biologists for many decades. The completion of the Human Genome Project has opened a new and exciting era of studies to identify genes responsible for turning on and off those switches that regulate the ability to adapt to the environment and the length and quality of life. Gene mutants for lengthening or shortening the life span have now been identified in a number of animal species (Chapter 4). While genes dictate the composition of a cell or organism, they also may predispose adult and elderly individuals to a number of complex pathologies caused by environmental risk factors. However, environmental influences may modify both genetic physiologic and pathologic characteristics: the term phenotype (or *phenome*) represents the observable properties that an organism has developed under the combined influences of its genetic constitution and the environmental factors to which it has been exposed. Thus the genes/environment hypothesis of aging states: "the genome proposes but the phenome disposes" (35). At all stages of life, the directing force of heredity and the modifying influences of the

internal and external environment unite in determining physiologic competence and length of the life span (Fig. 1).

Heredity operates through internal factors present in the fertilized egg. *The genes*, or hereditary determiners located in the chromosomes, contain the genetic contribution of each parent. Indeed, it has been said that "the best assurance for a long life remains the thoughtful selection of long-lived parents." It is well known that many of the common disorders that affect humans have a genetic component. The mapping of diseases having a Mendelian genetic transmission mechanism has led to major breakthroughs in our understanding of some of these diseases. In some animal species, one or several genes have been implicated in determining a shorter or longer life (Chapters 4 and 5). With respect to aging, some of the breakthroughs to come from recent human gene mapping

- have led to a better insight into the role of genes as well as into the role of environmental factors on growth, development, and aging,
- have served as a comprehensive guide to a large number of common human disorders,
- may, by controlling for genetic susceptibility, improve our ability to identify and characterize additional genes, risk factors, and gene-to-gene as well as gene-to-environment interactions, and
- may generate successful therapies identifying interventions that can improve the genotype of the susceptible individual.

The environment supplies the external and internal factors that make growth, development, and aging possible and allow inherited potentials to find expression. External factors, such as temperature, humidity, atmospheric gases, drugs, infections, and radiation

- may condition the appearance of and modify the type of genetic expression and alter genetic composition to make possible the creation of new inheritable characters (mutations),
- operate throughout the life span, and
- may generate successful therapies by modifying physical, social, and economic conditions (e.g., better lifestyle, hygienic habits, education, more efficient adaptability).

Internal factors, such as hormones, nutrition, immune reactions, and nervous system signals may modify metabolic and homeostatic conditions leading to better adaptation to stress, for example (Chapter 9).

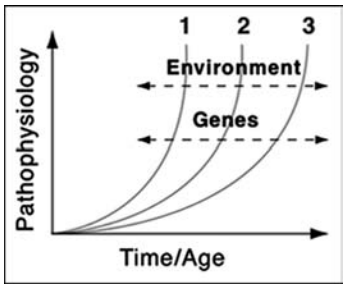

FIGURE 1 Schematic diagram showing that life span is regulated by interactions between genes and the environment. The coordinates represent age from birth to death and degree of physiopathologic change (generic for reduced function and increased illnesses) with progressively older ages. Three different curves illustrate the considerable heterogeneity in life trajectory among old individuals. *Source*: Courtesy of Dr. J. Campisi.

What are the respective influences of genetic and environmental factors in determining the phenotype? *In humans, the best currently available method for estimating the involvement of genetic and environmental factors in determining or influencing the life span is through studies of twins and adopted children*. For example, in a study of a large group of identical or fraternal twins, reared together or separately, heritability was calculated by intrapair differences or similarities in the mortality rates in terms of age at death. From the data reported, genetic factors accounted for one-third of the variance in longevity, and environmental factors for the remaining two-thirds (35). The evidence that genetic factors played, a relatively minor role, especially in twins who died at a young age, merits further examination (36).

One of the main characteristics of animals is their capacity to adapt to an ever-changing environment. Adaptation is attained through a series of physiologic adjustments (regulated by neuroendocrine signals) that serve to restore the normal state after it has been disrupted by altered external conditions that generate "stress" (Chapter 9). Adjustments to stress are grouped under the terms *homeostasis*, *allostasis*, and *hormesis*, and a large part of physiology is concerned with neurologic, endocrine, and immune regulatory mechanisms (e.g., negative and positive feedbacks) that coordinate the responses to stress and, ultimately, are responsible for survival. By the mature stage, many such adjustments are completed in humans. Stress throughout life plays a dual role: it may improve overall functional competence by stimulating and coordinating the various functions of the body to better adapt—a stimulation called hormesis (Chapter 9); or, in cases of repeated and severe stress, homeostasis may fail to occur or may be disrupted. In such cases, the terms "allostasis" and "allostatic load" refer to the sum of all untoward consequences of stress that may contribute to disease and, ultimately, death (Chapter 9).

Largely due to progress in physiotechnology (3), fantastic possibilities are now open of pursuing many avenues of research in humans, ranging from new reproductive technologies to cloning to genetic therapies and to a fuller understanding of the aging process. According to *functional genomics* (37–39), the early image we held of a stable gene is being replaced by one that is dependent on developmental interactions and environmental influences (40,41). In addition, "chance variation," that is, variation due to neither genome nor environment, may play a significant role in development and aging. Many types of wild or inbred laboratory animals, for example, have almost identical genes and environments, and yet they exhibit a wide range of life spans (Chapters 3 and 4). Likewise, human identical twins are not truly identical. The effects of environmental factors are expressed in the heterogeneity of aging processes and may find their origin during development. The remarkable heterogeneity of the elderly population may result from the variability of genetic systems, the exposure to specific environmental conditions, and random variations (42,43). Each should be an object of future studies as, together, they may determine, influence, predispose, and increase susceptibility to aging and disease (Chapter 3).

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2

Human Longevity in Historical Perspective

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INTRODUCTION

Perhaps the greatest of all human achievements has been the enormous increase of human longevity that has occurred over the past few centuries. The average length of life in the early history of our species was probably in the range of 20 to 35 years (Table 1). By 1900, this value had already risen to around 45 to 50 years in industrialized countries. Slightly more than a century later, the world's healthiest countries now have a life expectancy at birth of around 80 years. Thus, roughly half of the historical increase in human life expectancy occurred during the twentieth century. Of course, much of the increase in this average value has been due to the near elimination of infant and childhood deaths. According to the available evidence, in the distant past, around a quarter of all babies died in their first year of life. Today, in the most advantaged countries, less than a half percent of infants meet a similar fate.

The increase of life expectancy at birth for one country, France, depicted in Figure 1, illustrates several key aspects of French demographic history over the past two centuries. First, we see the enormous increase of average human longevity over time, from a life expectancy in the high 30s during the early nineteenth century to values in the high 70s (males) or low 80s (females) at the dawn of the twenty-first century. Second, we witness the differential impact of the various wars on men and women. Two major wars were fought mostly at the front and thus affected male life expectancy much more than female: the Napoleonic wars of the early nineteenth century and World War I during the early twentieth century. Two other conflicts involved a significant occupation of French territory by enemy forces and thus affected men and women in a similar fashion: the Franco-Prussian War of the early 1870s and World War II around the early 1940s. Finally, the graph illustrates the emergence of a large gap in life expectancy between men and women even during peacetime, from a difference of less than two years at the beginning of the interval to around seven to eight years at the end.

TABLE 1 Estimated Levels of Life Expectancy at Birth and Infant Mortality Throughout Human History

	Life expectancy at birth (in years)	Infant mortality rate (per 1000 live births)
Prehistoric	20–35	200–300
Sweden, 1750s	36	212
India, 1880s	25	230
United States, 1900	48	133
France, 1950	66	51
Japan, 2004	82	3

Source: From Refs. 1–4.

The rise of human life expectancy is significant for several reasons. First, it reflects the increasing material comfort of human life over this period as well as the technological and social advances associated with modern systems of public health and medicine. However, changes also come at a certain cost. Thanks to the "longevity revolution," industrialized societies are now faced with a large and growing elderly population (Table 2), which poses a significant challenge in terms of medical care and social support. The rise in the proportion of elderly is balanced to some degree by a decline in the share of young people in the population, as illustrated in Figure 2 for Italy. To some degree, societies must merely reorient themselves toward the care of a large dependent population at the end of life rather than at the beginning. Such adjustments are not without costs, however, as the needs of children and the elderly are quite different. Therefore, careful social planning is required, based on a firm understanding of demographic trends.

This chapter does not provide answers about how to make the needed social and economic adjustments. Rather, it attempts to explain the driving forces behind the increase of human longevity that underlie this momentous shift in population distribution from younger to older ages.

Human Longevity in the Past and the Present

There are two important sets of questions about historical trends. First, how long do people live, why is longevity increasing, and how long will we live in the future? Second, given that we are living longer, are we mostly gaining healthy years of life or are we "living longer but doing worse"? We know a lot more about the first question, partly because death is much easier to define and measure than health or functional status. In the United States, for example, there have been health

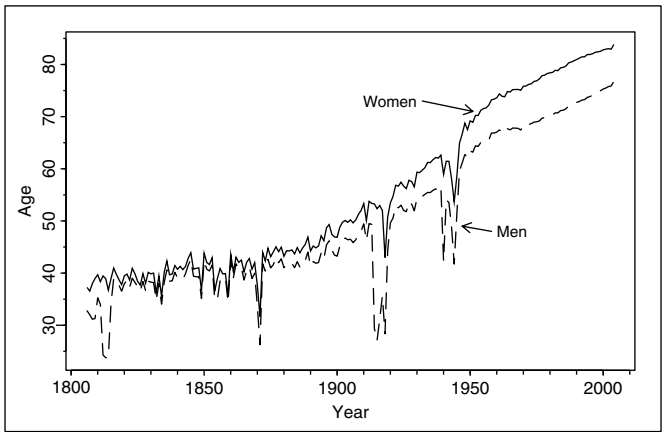

FIGURE 1 Life expectancy at birth by sex, France 1806–2004. *Source*: From Refs. 2,5.

TABLE 2 Estimated and Projected Population Size for Major World Regions,1950–2025, with Percent Under Age 15 and Over Age 65

	1950 population			1975 population			2000 population			2025 population[a]		
Region	Total (millions)	Percent under age 15	Percent over age 65	Total (millions)	Percent under age 15	Percent over age 65	Total (millions)	Percent under age 15	Percent over age 65	Total (millions)	Percent under age 15	Percent over age 65
World	2,519	34.3	5.2	4,074	36.8	5.7	6,086	30.0	6.9	7,905	24.2	10.5
More developed countries	813	27.4	7.9	1,047	24.2	10.7	1,193	18.3	14.3	1,249	15.7	20.8
Less developed countries	1,707	37.6	3.9	3,027	41.1	3.9	4,892	32.9	5.1	6,656	25.7	8.6
Africa	224	42.0	3.2	416	44.9	3.1	812	42.6	3.3	1,344	36.9	4.2
Asia	1,396	36.5	4.1	2,395	39.6	4.2	3,676	30.2	5.9	4,728	22.6	10.2
Europe	547	26.2	8.2	676	23.7	11.4	728	17.5	14.7	707	14.7	21.0
Latin America and the Caribbean	167	40.0	3.7	322	41.3	4.3	523	32.0	5.6	697	23.3	10.1
North America	172	27.2	8.2	243	25.3	10.3	315	21.3	12.4	388	18.3	18.0
Oceania	13	29.9	7.3	21	31.3	7.3	31	26.2	9.7	41	21.1	14.8

[a] Figures for 2025 are medium-variant projections.
Source: From Ref. 6.

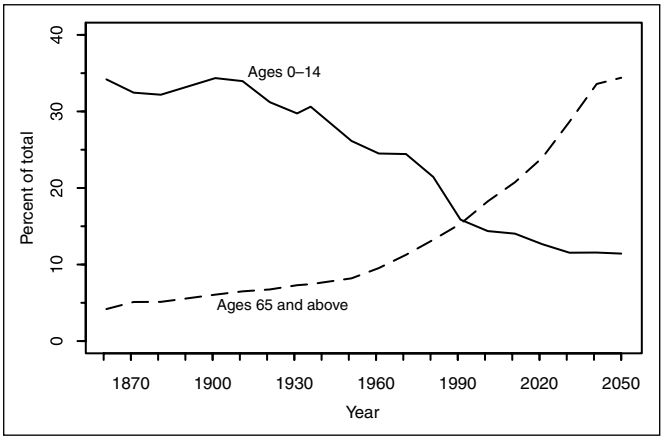

FIGURE 2 Proportion of population aged 0 to 14 versus 65+, Italy 1861–2050. Figures for 2001–2050 are projections. *Source*: From Refs. 7,8.

interview surveys since the late 1950s and a consistent series of direct measurements of health status since the 1970s, in both cases for a representative sample of the national population.[a] These and similar data for other industrialized countries can be used to measure changes in health status, but it is often difficult to compare the results reliably across populations and over time.

On the other hand, we have detailed mortality data from many countries over much longer time periods. These data often include information on the attributed cause of death, although this concept, like health or functional status, is difficult to define and measure in a consistent fashion. Although there have been some attempts to measure early human longevity based on skeletal remains and other information (1), the most useful information on historical mortality trends comes from time series of national data, collected since around 1750 in some parts of Europe. The accuracy of such data is variable, but specialists mostly know which data are reliable or potentially inaccurate. Data on cause of death must always be analyzed with great caution: although some trends are irrefutable (e.g., the historical decline of infectious disease), others appear contaminated by changes in diagnostic procedures and reporting practices (e.g., cancer trends, especially at older ages).

This section describes major trends in human longevity from the past and the present. A later section of this chapter offers some guarded speculations about what the future may hold. We do not address the issue of "healthy life span," although the interested reader may refer to other sources on this topic (9,10) (Chapter 3).

■ Prehistoric and Preindustrial Eras

We do not know much about how long humans lived before 1750. Around that time, the first national population data were collected for Sweden and Finland. For earlier eras, we have some life tables constructed for municipal populations, members of the nobility, and other groups that were probably not representative of the national population at large (11,12). After 1750 and even today, we have extensive and highly reliable mortality information for only a subset of national populations.

For the Middle Ages and earlier, mortality levels have been estimated based on data gleaned from tombstone inscriptions, genealogical records, and skeletal remains (1). The accuracy of such estimates has been a subject of dispute (13–16). In studies based on skeletal remains, a key issue is the attribution of age based on bone fragments. Another problem for estimation based on either skeletal or tombstone data is uncertainty about the age structure of the population, which affects mortality estimates based solely on the distribution of ages at death. The only practical solution is to assume that the population was "stationary," implying a long-term zero growth rate and unchanging levels of fertility and mortality, and even an unchanging age pattern of mortality. Clearly, these assumptions are always violated, but the resulting estimates are useful nonetheless.

For mortality data derived from subpopulations, there is also an issue of whether the data are representative of some larger population. Who gets buried in a society, and who gets a tombstone? Which societies have regular burial practices, as opposed, say, to burning their dead? What kinds of populations have complete genealogical records from a particular time period? Thus, for many reasons, all estimates of mortality or longevity from the preindustrial period (roughly, before 1750) should be viewed with caution. Of the many sources of bias in these estimates, there are both positive and negative factors, which tend to balance each other to some extent (17). They are inaccurate and/or unrepresentative by amounts that cannot be well quantified.

Although these historical estimates may be either too high or too low, they provide us nonetheless with a useful description of the general contours of the history of human longevity. For example, most scholars agree that life expectancy at birth (or $\mathring{e}_0$, in the notation of demographers and actuaries) was probably in the 20s for early human populations. Some very disadvantaged societies might have had life expectancies in the teens, whereas others may have been in the 30s. Since historical levels of life expectancy were in the 20s, compared to around 75 to 80 years today in wealthy countries, the average length of life has roughly tripled.

Most of this increase was due to the reduction of infant and child mortality. It used to be the case, for example, that remaining life expectancy at age 1 was greater than at birth, because the toll of infant mortality was so high. The difference between premodern periods and today is less stark if we consider life expectancy at higher ages. Instead of the tripling of life expectancy at birth, remaining life expectancy at higher ages has roughly doubled over the course of human history. At age 10, for example, life expectancy (i.e., expected years after age 10) may have moved from the around 30 to 33 years to almost 70 years (Thatcher AR. Life tables from the Stone Age to William Farr, unpublished manuscript, 1980). At age 50, it may have gone from around 14 years to more than 30 years (17).

■ Epidemiologic Transition

The epidemiologic transition is the most important historical change affecting the level and pattern of human mortality. The transition refers to the decline of acute infectious disease and the rise of chronic degenerative disease (18). This shift does not imply that degenerative diseases became more common for individuals of a given age. It merely means that infectious disease nearly disappeared, so something else had to take its place as the major cause of death. Increasingly, people survived through infancy and childhood without succumbing to infectious disease (19). Once past these critical early years, survival

[a] The National Health Interview Survey began in 1957 and contains information on health status from individual self-evaluations. The National Health and Nutrition Examination Survey, began in the early 1970s, provides information on the health and nutritional status of individuals based on direct measurement.

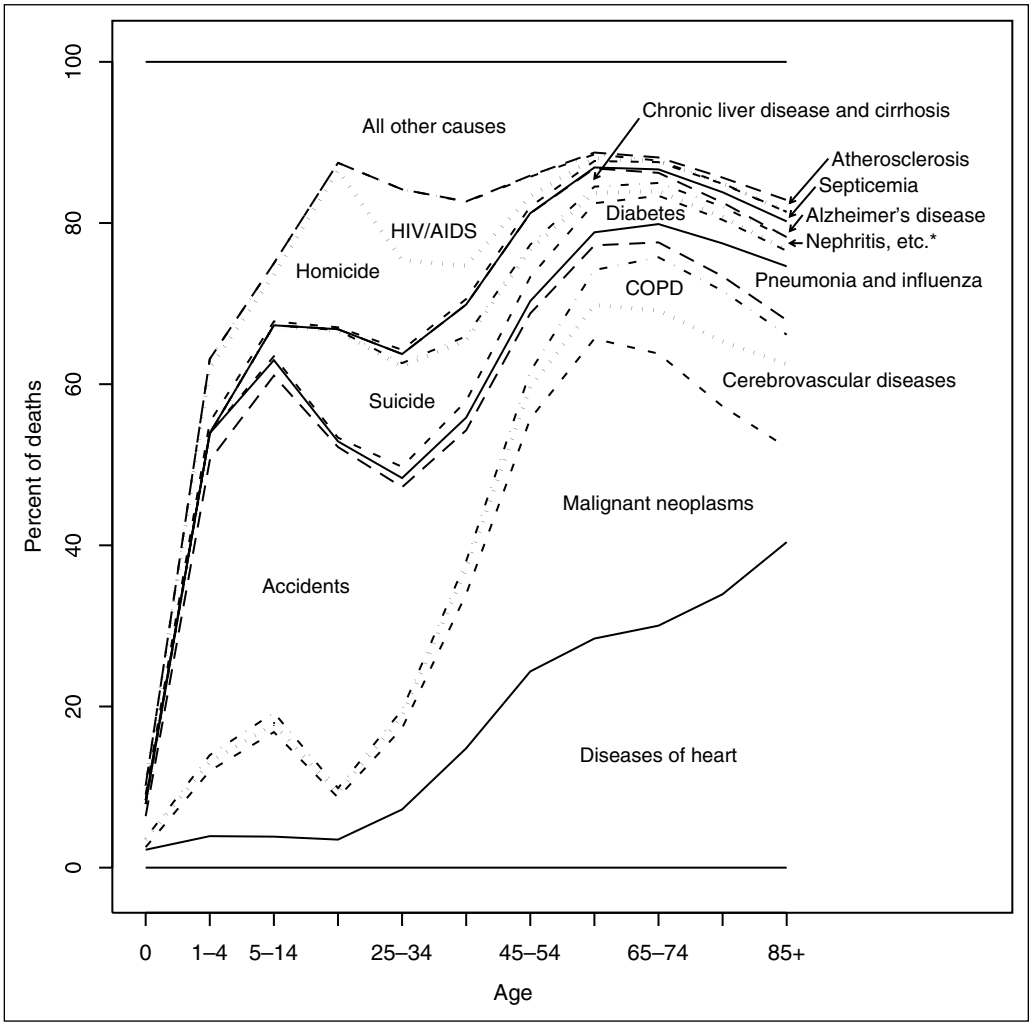

FIGURE 3 Distribution of deaths by cause, United States 1997. *denotes nephritis, nephrotic syndrome, and nephrosis. *Source*: From Ref. 20.

to advanced ages is much more likely, and at older ages, various degenerative diseases present mortality risks even when infection is well controlled.

Thus, heart disease, cancer, and stroke became the most common causes of death in industrialized societies, as the age distribution of deaths shifted to older ages. As seen in Figure 3, which depicts the distribution of deaths by cause in various age groups for the United States in 1997, these three causes account for more than 60% of all deaths at older ages. On the other hand, accidents, homicide, suicide, and HIV/AIDS are the major killers among young adults. Infants and children die mostly from accidents and "other causes"—a residual category that includes, for example, congenital anomalies and childhood diseases.

Trends in Life Expectancy

Life expectancy has been increasing, not just in industrialized societies but also around the world.[b]

The rise in life expectancy at birth probably began before the industrial era—before national mortality statistics were first assembled in Sweden around 1750. As noted earlier, $\mathring{e}_0$ was probably in the 20s during the Middle Ages and earlier. By 1750, Sweden (and probably other parts of northwestern Europe) had attained an $\mathring{e}_0$ of 38, so the upward trend in longevity appears to have begun before the industrial era. Over the next century or more, there was a slow and irregular increase in life expectancy. After about 1870, however, the increase became stable and more rapid. During the first half of the twentieth century, life expectancy in industrialized countries rose quite rapidly. Since 1950, the rise in life expectancy slowed down somewhat, as illustrated in Figure 1 for France.

The cause of the earlier rapid rise in life expectancy and its subsequent deceleration is quite simple: the decline of juvenile mortality to historically very low levels. By around 1950, infant mortality in wealthy countries was in the range of 2% to 3% of births, compared to perhaps 20% to 30% historically. Since then, infant mortality has continued to decline and now stands at around a half percent of all births in the healthiest parts of the world. As babies were saved from infectious disease, their chances of survival to old age improved considerably. Once juvenile mortality was reduced substantially, improvements in life expectancy due to the reduction of mortality in this age range had to slow down, and further gains had to come mostly from mortality reductions at older ages.

The rise in life expectancy during the second half of the twentieth century was slower than during the first half simply because it depended on the reduction of death rates at older

[b] During recent decades the two major exceptions to the worldwide increase in life expectancy have been a stagnation and even reversal of earlier progress in parts of Africa, due to the AIDS epidemic, and in parts of the former Soviet block (especially Russia) due to social disruptions and instability.

ages rather than in infancy and childhood. Put simply, saving an infant or child from infectious disease, who then goes on to live to age 70, contributes more to average life span than saving a 70-year-old from heart disease, who may live another 10 years. Thus, the deceleration in the historical rise of life expectancy is a product of the J-shaped age pattern of human mortality: high in infancy and childhood, low through adolescence and early adulthood, and then rising almost exponentially after age 30. Gains in $\mathring{e}_0$ that come from reducing juvenile mortality are quite large, whereas gains due to a reduction in old-age mortality are inevitably much smaller.

A common mistake is to assert that the deceleration in the rise of $\mathring{e}_0$ reflects a slowdown in progress against mortality. In fact, the reduction of death rates has changed its character in recent decades, but it has not slowed down. At older ages, the decline of mortality has accelerated since around 1970 (as discussed below). So long as the decline of old age mortality continues, life expectancy will continue to increase, driven now by the extension of life at later ages rather than by saving juveniles from premature death.

Rectangularization or Mortality Compression

The age pattern of human mortality can be characterized in various ways. Figure 4 shows the American mortality levels in 1900 and 1995 from three perspectives. The first panel shows death rates by age. These death rates are used to construct a life table, which describes the experience of a hypothetical cohort subject throughout its life to the death rates of a given year. Thus, the middle and last panels show the distribution of deaths and the proportion of survivors at each age among members of such a hypothetical cohort.

Together, these three panels illustrate some major features of the mortality decline that has taken place over this time interval. First, death rates have fallen across the age range, but they have fallen most sharply (in relative terms, since the graph has a semilogarithmic scale) at younger ages. The distribution of ages at death has shifted to the right and become much more compressed. At the same time, the survival curve has shifted to the right and become more "rectangular" in shape. This last change is often referred to as the "rectangularization" of the human survival curve.

It was once asserted that this process of rectangularization reflected the existence of biological limits affecting human longevity (21). This notion of limits to the human life span enjoys little empirical support, as discussed below. Nevertheless, the historical process of rectangularization was both real and extremely significant. It is perhaps best thought of as a "compression of mortality," as documented in the middle panel of Figure 4. As the average level of longevity has increased, so has our certainty about the timing of death.

One measure of this variability is the interquartile range of deaths in the life table or the age span of the middle 50% of deaths over the life course. In the 1750s, in Sweden, the life table interquartile range was about 65 years, so that deaths were spread out widely across the age range. The distribution of age at death became more and more compressed over the next two centuries until the life table interquartile range was around 15 years in industrialized countries by the 1950s. Since 1960, there has been little further reduction in the variability of age at death in the developed world even though the average age at death (as reflected in life expectancy at birth) has continued to increase (22).

Like the historic rise of life expectancy, this compression of mortality was due largely to the reduction of juvenile mortality. Once most juveniles had been saved from premature death, a pattern emerged in which deaths are concentrated in the older age ranges. As mortality falls today among the elderly, the entire distribution of ages at death is rising slowly, but its level of variability seems to have stabilized.

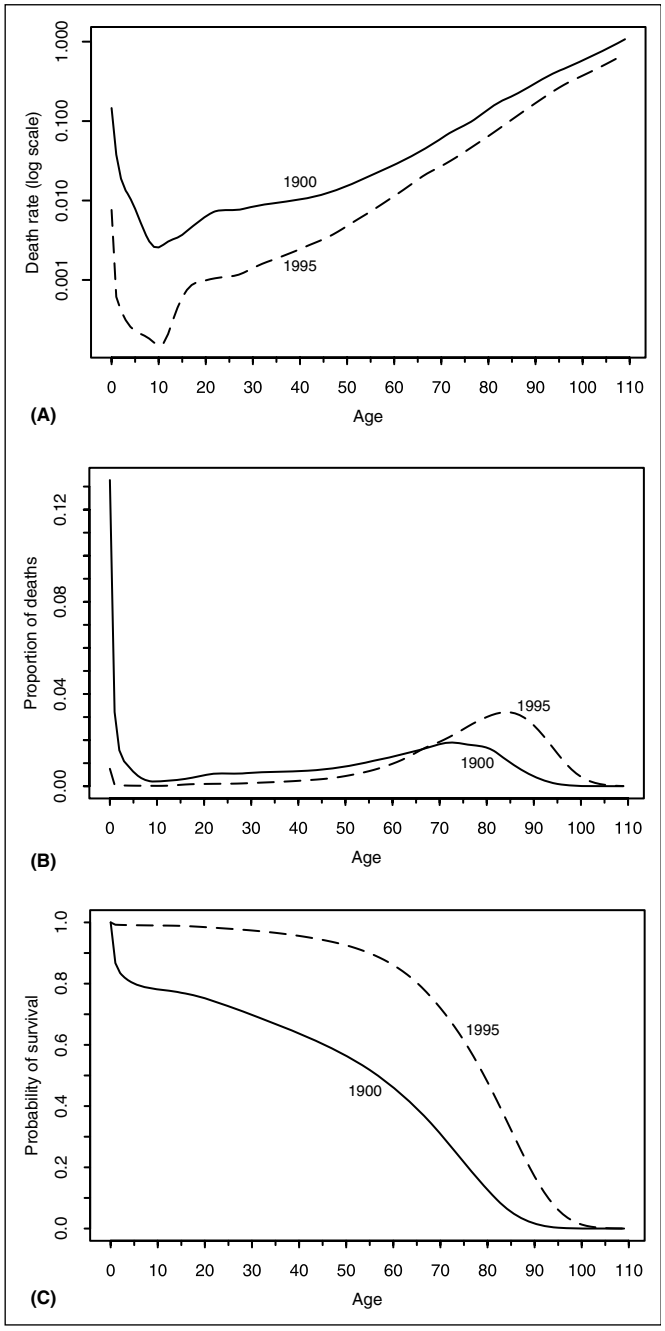

FIGURE 4 Age pattern of mortality from three perspectives, United States, 1900 and 1995. (**A**) Observed death rates by age; (**B**) distribution of deaths by age; (**C**) proportion surviving by age; **B** and **C** present the distribution of deaths and the proportion surviving in a life table for the given year. *Source*: From Ref. 4.

Mortality Decline Among the Elderly

The most significant trend now affecting longevity in industrialized societies is the decline of death rates among the elderly. Until the late 1960s, death rates at older ages had declined slowly, if at all. Traditionally, rates of mortality decline were much higher at younger than at older ages. Since about 1970, however, there has been an "aging of mortality decline," meaning that some of the most rapid declines in death rates are now occurring at older ages (23,24). Thus, the decade of the

1960s marks a turning point, from an earlier era of longevity increase due primarily to the decline of acute infectious disease among juveniles to a more recent era involving the decline of chronic degenerative disease among the elderly.

Cardiovascular Disease

The most significant component of the mortality decline at older ages is the reduction of death rates due to cardiovascular disease (CVD), including both heart disease and stroke. In the United States, heart disease has been the leading cause of death since 1921, and stroke has been the third most common cause since 1938. From 1950 to 1996, age-adjusted death rates for these two causes declined by more than half (by 56% for heart disease and by 70% for stroke). It is estimated that 73% of the decline in total death rates over this time period was due to this reduction in CVD mortality (25).

The exact cause of the decline in CVD mortality is open to debate, although it is surely due to a combination of factors. For the United States, all of the following have been cited as factors contributing to this decline:

1. A decline in cigarette smoking among adults
2. A decrease in mean blood pressure levels
3. Increased control of hypertension through treatment
4. Changes in diet, especially a reduction in the consumption of saturated fat and cholesterol
5. Improvements in medical care, including better diagnosis and treatment of heart disease and stroke
6. The development of effective medications for treatment of hypertension and hypercholesterolemia, and an increase in coronary-care units and emergency medical services for heart disease and stroke (Chapters 3, 15, and 16) (25)

The rapid decline in CVD mortality began around 1968 in the United States and other industrialized nations. Given the precipitous nature of this decline, it has been argued that therapeutic interventions were the most important factor, since changes in diet and lifestyle should have led to a more gradual pattern of change (26). It is worth noting that landmark investigations, such as the Framingham Heart Study, began in the late 1940s and began to provide significant breakthroughs in our scientific understanding of CVD during the 1960s (27).

Cancer

In most developed countries, cancer mortality has begun to decline only within the last 15 to 20 years, although in Japan, death rates from cancer began falling as early as the 1960s (28–30). Of course, cancer takes many different forms, and trends vary greatly by the site of the primary tumor. Lung cancer has become more common due to increased smoking habits, while stomach cancer has been in decline. Among women, mortality due to cervical cancer has fallen dramatically thanks to successful medical intervention (screening and early treatment) while breast cancer has been on the rise apparently due to a number of interrelated factors (lower and later fertility, changes in diet, and possibly other factors as well).

It is sometimes overlooked that some common forms of cancer may be caused by infection. For example, stomach cancer is often brought on by infection with *Helicobacter pylori*. Infection with *H. pylori*, and hence stomach cancer, was especially common in Japan prior to the widespread availability of refrigeration (31,32). Liver cancer is related to hepatitis infection (both B and C strains of the virus), and, thus, reductions in liver cancer hinge on controlling infection as well as curbing excess drinking. A third example is infection by the human papilloma virus, which can cause cervical cancer (33).

These three forms of cancer have tended to decline in recent decades and should decline further as the relevant infectious agents are brought under control (e.g., hepatitis B and C). On the other hand, cancers that have become more common include those strongly influenced by individual behaviors (e.g., lung and pancreatic cancer are linked to smoking, and both have tended to increase over time) and some others whose causes are mysterious or poorly understood (e.g., breast cancer and colorectal cancer, both rising but for unknown or uncertain reasons).

As noted earlier, trends in mortality among the elderly are the main factor behind the continued increase in life expectancy in developed countries. Furthermore, the main components of mortality at these ages are CVD and cancer. These two causes have been in decline during recent decades for reasons that are complex and not entirely understood. It is clear that there are multiple causes involved in bringing down death rates due to CVD and cancer. Medical science has played a part, but so have changes in diet and personal habits as well as community efforts and economic changes that have reduced the spread of infectious agents. It is important to keep this complex causality in mind when speculating about future trends in human mortality.

Summary of Historical Trends

A compact summary of major trends in human longevity in industrialized countries is presented in Tables 3. Amidst the incredible detail available in historical mortality statistics, we cannot help but discern two major epochs: before 1960 and after 1970. The driving trend in the former period was a rapid decline of mortality due to infectious disease, which had an impact across the age range but certainly a much larger effect at younger ages. The sharp reduction in infant and child mortality led to a rapid increase in average life span and a marked reduction in the variability of age at death. It did not, however, have a major impact on maximum life span, which rose very slowly due to the more gradual improvement in death rates at older ages.

TABLE 3 Summary of Major Trends in Human Longevity in Industrialized Countries

	Before 1960	After 1970
Average life span (life expectancy at birth)	Increasing rapidly because most averted deaths are among younger people. Very rapid reduction in infant/child mortality linked mostly to effective control of infectious diseases	Increasing moderately because most averted deaths are among older people. Accelerated reduction in old-age mortality linked mostly to better management of cardiovascular disease
Maximum life span (observed and verified maximum age at death)	Increasing slowly mostly due to gradual reductions in death rates at older ages. (Size of birth cohorts and improved survival at younger ages matter much less)	Increasing moderately due almost entirely to accelerated reduction in death rates at older ages
Variability of life span (standard deviation, interquartile range, etc.)	Decreasing rapidly due to reductions in mortality at younger ages	Stable because death rates at older ages are decreasing as rapidly as at younger ages

From the mid-1950s to the late 1960s, mortality trends in industrialized countries seemed to stabilize. Then, just before 1970, death rates at older ages suddenly entered a period of unprecedented decline. Compared to the earlier era of rapid reductions in infant and child mortality, these changes yielded a slower increase in life expectancy at birth. On the other hand, the rise of maximum life span accelerated, driven by a more rapid decline in death rates at older ages. The variability of life span tended to stabilize during this period, as the entire distribution of ages at death—now concentrated at older ages—moved upward in parallel fashion. The difference between these two distinct eras is illustrated in Table 4 for the country of Sweden.

■ OUTLOOKS FOR THE FUTURE

It is impossible to make a firm scientific statement about what will happen in the future. In truth, scientists can only present the details of well-specified scenarios, which serve as forecasts or projections of the future. They can also help by clearly defining the terms of the debate—for example, by discussing what is meant by the notion of "limits to life span." Limits possibly affecting the increase of human longevity are the first topic of this section, followed by a discussion of extrapolative techniques of mortality projection or forecasting. Our discussion of the future of mortality concludes with a comparison of "optimistic" and "pessimistic" points of view on this topic.

■ Possible Limits to Life Span

If there are limits to the human life span, what do they look like? There are two ways to define such limits: maximum average life span and maximum individual life span (23).

Maximum Average Life Span

Let us consider whether there might be an upper limit to the average life span that could be achieved by a large human population. Average life span, or life expectancy at birth, refers to how long people live on average in a population. In the United States, life expectancy is currently around 75 years for men and 80 years for women (34). Accordingly, these numbers describe the average length of life that can be anticipated, given the mortality conditions of today. For example, baby boys born this year will live an average of 74 years, assuming that age-specific death rates (Fig. 4a) do not change in the future. Just as occurs today, some of these newborns will die in infancy from congenital ailments, some will be killed in car accidents as young adults, and some will succumb in old age to cancer or heart disease.

TABLE 4 Average Change (in Years Per Decade) in Key Mortality Indicators, Sweden

	1861–1960	1970–1999
Average life span (life expectancy at birth)	3.1	1.8
Maximum life span (maximum reported age at death)	0.4	1.5
Interquartile range (of deaths in life table)	−5.3	−0.4

Note: The average change shown here equals the difference between mean values for the last and first 10-year periods (within the indicated time interval) divided by the number of years in between.
Source: From Ref. 2.

As noted earlier, death rates have been falling for several centuries. At every age, the risk or probability of dying is much lower than in the past. Thus, when we talk about life expectancy at birth, we are being conservative and asking what the average life span will be, assuming that death rates do not fall any further in the future. However, it is likely that death rates will continue to decline at least somewhat in future years, so baby boys born today in the United States will probably live longer than 74 years on average.

The question about limits to the average life span can be posed as follows: Can death rates keep falling forever or will they hit some fixed lower bound? Perhaps biological forces impose a certain inevitable risk of mortality at every age. Thus, there might be some age-specific minimum risk of dying that could never be eliminated (23).

Admittedly, it seems implausible that age-specific death rates could ever equal zero in any large population. However, even if the death rate at some age cannot equal zero, can it keep declining toward zero? In other words, zero might be the limit to how far death rates can drop, even if they can never attain zero. Or, is there a higher limit? Perhaps there is some number, such as one in a million, such that it is simply inevitable that one in a million people—say, one in a million 50-year-olds—will succumb to death over the course of a year. If true, then the death rate at age 50 can never fall below one in a million. According to this view, we have a limited capability as a society or as a species: we cannot push the risk of death any lower than some fixed level.

If a nonzero lower limit for death rates exists, how much is it at age 50 or at any age? The answer to this question is quite significant, for if we knew the lower limit of death rates at every age, we could compute the maximum achievable life expectancy at birth. In this way, we would know the upper limit of the average human life span. However, it is quite difficult to identify a nonzero lower bound on death rates that is applicable to all human populations. Yet, if there is no lower limit to death rates except zero, then there is no upper limit to life expectancy except infinity. Nevertheless, the absence of identifiable limits does not mean that large increases in average life span are imminent. It just means that life expectancy can continue to increase as death rates are pushed down further and further.

Why do some people think that an upper limit to life expectancy exists? In fact, there is little empirical support for such a belief. An argument frequently put forward is that the rise in life expectancy at birth slowed down in the second half of the twentieth century. As shown earlier, however, this deceleration resulted merely from a shift in the main source of the historical mortality decline from younger to older ages. Although the rise in life expectancy has decelerated, the decline in death rates at older ages has accelerated in recent decades (35).

Furthermore, if death rates are approaching their lower limit, one might expect a positive correlation between the current level of mortality in a given country and the speed of mortality decline (so that those populations with the lowest level of mortality would also experience the slowest rates of mortality decline). In fact, no such correlation exists for death rates at older ages. In some cases, the fastest reduction in death rates is occurring in those countries with the lowest levels of old-age mortality, just the opposite of what we would expect if death rates were pushing against a fixed lower bound (35).

So long as death rates at older ages keep falling, life expectancy (at birth or at any age) will continue to increase. As discussed below, current forecasts suggest that life expectancy at birth may not rise much above current levels over the next half century. Nevertheless, there is simply no demographic evidence that life expectancy is approaching a fixed upper limit.

Certainly, such a limit may exist, but it is nowhere in sight at the present time (23).

Maximum Individual Life Span

Limits to average life span, or life expectancy at birth, are one issue. When people discuss limits to the human life span, however, they often have another idea in mind: the upper limit to an individual life span. Instead of asking how long we can live on average, we might ask how long one lucky individual can hope to live. This concept is actually much easier to understand than the notion of an upper limit to life expectancy.

Who is the oldest person who has ever lived? Even if we can never have a definitive answer to this question, we can at least imagine the existence of such a person. Maybe he or she (probably she) is still alive today. Or, maybe she lived hundreds of years ago but vanished without leaving a trace—no birth certificate, no census record, and not even a newspaper article about her incredible feat of longevity.

Who is the oldest person alive today? That person might or might not be the oldest person ever. However, identifying the world's oldest person is very difficult even today, because of the widespread practice of what demographers politely call "age misstatement" (36). Putting it less politely, some people lie about their age. Others, if asked, give the wrong age because they do not remember, because they are not numerate, or because they simply never paid attention to such matters. Such age misstatement often occurs in the absence of written records to prove or disprove the reported age.

Should we believe people who claim to be extremely old without proper documentation? Certainly, we should believe them, since there is no point in calling anyone a liar or questioning their memory. In terms of a scientific discussion about longevity, however, experts agree that it is best to ignore undocumented cases of extreme longevity. Thus, when we make statements about who is the oldest person alive today or in the past, we limit ourselves to cases where solid evidence exists (37).

To be accepted as a valid instance of extreme longevity, thorough documentation is required—not just a birth certificate but also a series of documents and a life history that is consistent with the written records. Ideally, if the person is still alive and mentally able, an oral history is obtained and checked against all available evidence—making sure, for example, that this person is not the son or daughter of the person in question.

Indeed, there are numerous examples of supposed extreme longevity that turned out to be cases of mistaken identity (37). Perhaps the most notorious example was a French Canadian named Pierre Joubert, who was supposed to have died at the age of 113 years in 1814. This case was listed for many years by the Guinness Book of World Records (38). When genealogical records were examined closely, however, two men named Pierre Joubert were identified—a father and his son. It was the son who died in 1814, 113 years after the birth of the father (39). Such mistakes are not uncommon, and whether they are the result of deliberate misrepresentation or honest error is irrelevant. In either case, a complete investigation should be required before accepting such reports as factual.

The historical record is still held by a Frenchwoman, Jeanne Calment, who died at age 122 in August of 1997 (40). Madame Calment lived in Arles, a town with very complete civil records (births, deaths, marriages, baptisms, etc.) going back several centuries. Fortunately, since these records were not destroyed in any war, it was possible to trace the life of Jeanne Calment very closely. It was also possible to reconstruct her family genealogy and to document that a disproportionate number of her ancestors were long lived as well. Of course, it is only one example, but the case of Jeanne Calment suggests that extreme longevity may have at least some hereditary component (41).

The oldest man whose age was thoroughly verified was Christian Mortensen, who died in 1998 at the age of 115 (42,43). A Japanese man named Shigechiyo Izumi was reportedly 120 years old when he died in 1986. Recent editions of Guinness World Records still maintain that Izumi is the oldest man on record. However, this case has now been rejected by almost all experts who are familiar with it, including the Japanese man who originally brought it to the attention of Guinness (44–46), and the common belief is that Izumi was in fact "only" 105 years old at the time of his death.

It is reasonable to ask what we have learned in general from these few cases of exceptionally long-lived individuals. Admittedly, the cases of Calment and Mortensen tell us nothing about the trend in maximum longevity. Maybe these are just two cases that we have had the good fortune of documenting in recent years. Maybe there were other individuals who were just as old as Calment or Mortensen who lived years ago, and we missed them. These are valid points, so we must turn to other evidence if we want to know about trends in extreme longevity.

In order to study historical trends in extreme longevity, we need a well-defined population with reliable records over a long period of time. For that purpose, we turn to a small subset of countries that have kept reliable population statistics for many years. The longest series of such data comes from Sweden. These records are thought to be extremely reliable since 1861, even in terms of the age reporting of individuals at very old ages (44). Vital records have a very old history in Sweden, where Lutheran priests were required to start collecting such information at the parish level in 1686. Such records were eventually brought together into a national system in 1749. In 1858, the present-day National Central Bureau of Statistics was formed, which led to further improvements in data quality. Furthermore, by the 1860s, the national system of population statistics was already more than 100 years old, so it was possible to check claims of extreme longevity against birth records from a century before. These historical developments account for the unique quality of the Swedish mortality data.

Figure 5 shows the trend in the maximum age at death for men and women in Sweden during 1861 to 2005. The trend is clearly upward over this time period, and it accelerates after about 1969. The rise in this trend is estimated to be 0.44 years (of age) per decade prior to 1969, and 1.1 years per decade after that

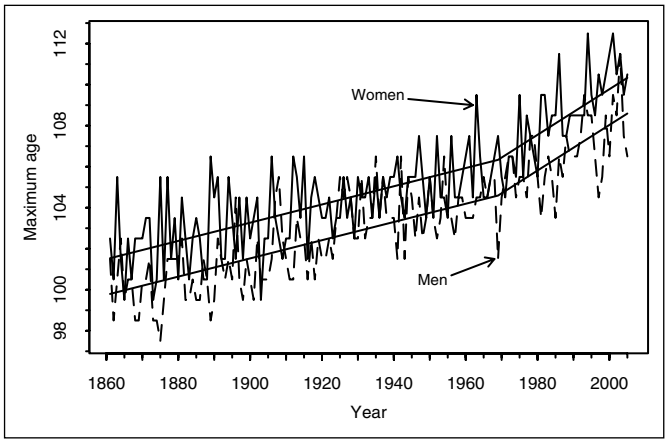

FIGURE 5 Maximum reported age at death, Sweden 1861–1999. *Source*: From Refs. 2,47.

date. More than two-thirds of this increase can be attributed to reductions in death rates above age 70, with the rest due to mortality decline at younger ages and an increase in the size of birth cohorts (47).

These Swedish data provide the best available evidence for the gradual extension of the maximum human life span that has occurred over this time period. Similar trends are evident for other countries as well, although patterns of age misstatement present greater problems of interpretation (44).

Extrapolation of Mortality Trends

Demographers claim some expertise in predicting future mortality levels, and their method of choice is usually a mere extrapolation of past trends. Biologists and others sometimes criticize this approach because it seems to ignore underlying mechanisms. However, this critique is valid only insofar as such mechanisms are understood with sufficient precision to offer a legitimate alternative method of prediction. Although many components of human aging and mortality have been well described, our understanding of the complex interactions of social and biological factors that determine mortality levels is still imprecise. Furthermore, even if we understood these interactions and wanted to predict future mortality on the basis of a theoretical model, we would still need to anticipate trends in each of its components.

The extrapolative approach to prediction is particularly compelling in the case of human mortality:

First, mortality decline is driven by a widespread, perhaps universal, desire for a longer, healthier life.

Second, historical evidence demonstrates that mortality has been falling steadily, and life span has been increasing, for more than 100 years in economically advanced societies.

Third, these gains in longevity are the result of a complex array of changes (improved standards of living, public health, personal hygiene, and medical care), with different factors playing major or minor roles in different time periods.

Fourth, much of this decline can be attributed to the directed actions of individuals and institutions, whose conscious efforts to improve health and reduce mortality will continue in the future.

Even accepting this argument, there is still a question of what to extrapolate. Demographers tend to view death rates as the fundamental unit of analysis in the study of mortality patterns, because these rates are estimates of the underlying "force of mortality" or the risk of death at any moment in a person's lifetime. These risks change over age and time and vary across social groupings (by sex, race, education, income, etc.). Life expectancy and the expected maximum age at death (for a cohort of a given size) can be expressed as a mathematical function of death rates by age. Thus, the usual strategy is to extrapolate age-specific death rates into the future and then to use the results of such an extrapolation to compute forecasts of life expectancy or other parameters of interest.

Predictions of future life expectancy by such methods yield values that are not too different from what is observed today. For example, forecasts made in the late 1990s by the U.S. Social Security Administration put life expectancy in 2050 at 77.5 years for men and 82.9 years for women, compared to 72.6 and 79.0 years in 1995 (48). These forecasts were not true extrapolations, however, because they assumed a slowdown in age-specific rates of mortality decline in the future. Another study using similar data but based on a purely extrapolative technique yielded more optimistic results—a life expectancy at birth in 2050 of around 84 years for both sexes combined (49). Plausible forecasts for Japan yielded slightly higher values of life expectancy at birth: 81.3 years for men and 88.7 years for women in 2050, compared to 76.4 and 82.9 years in 1995 (50).

The life expectancy forecasts of Lee and Tuljapurkar (49) are reproduced here as Figure 6. These projections are based on a clever extrapolative technique pioneered by Lee and Carter (51), which has been very influential in the world of mortality forecasting over the last 15 years. The method yields a range of estimates for each calendar year during the forecast period (in this case, from 1997 to 2096). The inherent uncertainty of future trends is represented in the graph by plotting not only the median forecast, which may be considered the "best estimate," but also by showing two extreme forecasts. The

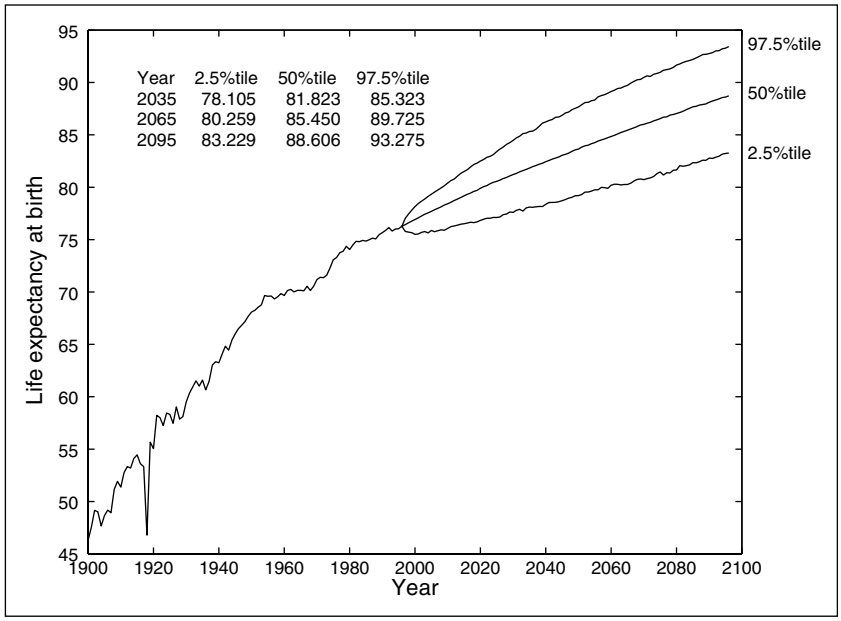

FIGURE 6 Life expectancy at birth, United States, 1900–1996 (actual) and 1997–2096 (forecast). *Source*: From Ref. 49.

median forecast lies at the 50th percentile of the full distribution: half of the estimates lie below or above this value. Figure 6 also presents the fifth and 95th percentiles, thus showing relatively extreme trajectories of future life expectancy.

It is important to remember that these projections are mere extrapolations of the historical experience of one country during a particular time period (the United States from 1900 to 1996). The implicit assumption is that future trends will resemble past ones. This assumption is plausible given the fairly steady pace of mortality decline over the past century. Of course, extrapolation is not without its flaws. It could not, for example, have anticipated the rise of mortality in the former Soviet Union after 1990, the emergence of AIDS in certain populations during the 1980s, or the divergence of mortality trends between Eastern and Western Europe after 1960. However, such observations are less an indictment of extrapolation as a method of mortality forecasting than a demonstration that the greatest uncertainties affecting future mortality trends derive from social and political rather than technological factors.

An important issue for consideration in forecasting mortality is the time frame—both the time frame of the data that form the input to an extrapolation and the time horizon of the projection itself. Although short-term fluctuations have been common, long-term mortality trends in industrialized countries have been remarkably stable. When mortality decline slowed temporarily during the 1950s and 1960s (in the United States and other developed countries), predictions that the rise in human life expectancy had come to an end were commonplace. Similarly, the unusually rapid decline of mortality rates after 1968 fostered expectations of unprecedented gains in longevity that would continue for decades. With the benefit of hindsight, these were both overreactions to rather short-lived episodes in the history of mortality change.

Another common error results from an undue emphasis on trends in life expectancy. Although it continues to increase, the pace of change in life expectancy at birth has been slower in recent decades than during the first half of the twentieth century (Fig.1). As noted earlier, a slower rise in life expectancy at birth was inevitable once juvenile mortality was reduced to historically low levels. However, it does not follow from this observation that gains against mortality in the future will be slower than in the past. Although the increase in life expectancy has slowed down, the decline in death rates at older ages (where most deaths now occur) has quickened (35). An extrapolation of current trends in death rates suggests that life expectancy will continue to increase, though not as quickly as during the first half of the twentieth century. This slow but stable increase in average life span will be driven by the accelerating pace of mortality decline at older ages.

■ Optimism vs. Pessimism

In recent years, the extrapolative approach to mortality prediction has been challenged by assertions that future changes in average human life span may come more or less quickly than in the past. The more optimistic view that life span will increase rapidly in the near future is partly a result of the acceleration in rates of mortality decline among the elderly in developed countries during the past few decades. From a historical perspective, however, this change is relatively recent and should be extrapolated into the future with caution. If the new pattern persists for several more decades, it will then constitute strong evidence that old trends have been replaced by new ones.

Another source of optimism about future mortality rates lies in the potential application of existing technologies (e.g., nutritional supplements, reductions in smoking) or the unusual longevity of certain groups such as Mormons and Seventh Day Adventists (52,53). Such discussions may be a good way to improve health behaviors, but they are not so good at informing predictions, largely because this same sort of advocacy influenced past trends as well. For purposes of prediction, we need to ask whether future positive reforms in lifestyle are likely to be implemented faster or more effectively than were similar reforms in the past.

From time to time, technological breakthroughs provide another source of optimism about future mortality rates. In 1998, the manipulation of a gene that halts the shortening of telomeres during the replication of human cells in vitro was a source of great optimism in the popular media, provoking rather extraordinary claims about the possibility of surviving to unprecedented ages in the near future.[c] Talk of cures for cancer and vaccines against AIDS promotes similar hopes. Such discussions should not be dismissed as mere wishful thinking but should also be seen in historical perspective.

As wondrous as they may be, recent scientific advances should be compared, for example, to Koch's isolation of the tubercle bacillus in 1882, which provided confirmation of the germ theory of disease and led to a great flourishing of public health initiatives around the turn of the century, or to Fleming's discovery of the antibacterial properties of penicillin in 1928, an event that led to the antibiotic drug therapies introduced in the 1940s. Extrapolations of past mortality trends assume, implicitly, a continuation of social and technological advance on a par with these earlier achievements.

More pessimistic scenarios of the future course of human longevity are based on notions of biological determinism or arguments about practicality, yielding the now-familiar claim that life expectancy at birth cannot exceed 85 years (21,55). Sometimes, evolutionary arguments are invoked in support of the notion that further extension of the human life span is impossible, even though existing theories say little about whether and to what degree the level of human mortality is amenable to manipulation (56).

Current patterns of survival indicate that death rates in later life can be altered considerably by environmental influences, and there is little conclusive evidence that further reductions are impossible. Furthermore, as noted before, trends in death rates and in maximal ages at death show no sign of approaching a finite limit. Nevertheless, although claims about fixed limits to human longevity have little scientific basis, a life expectancy at birth of 85 years—the oft-postulated theoretical limit—is within the range of values predicted by extrapolative methods through the end of the twenty-first century (Fig.6). In contrast, more optimistic claims—a life expectancy at birth of 150 or 200 years or even more—are much farther afield and would require a much larger deviation from past trends.

■ Learning from History

The historical rise of human longevity is the result of a complex set of changes beginning several centuries ago. Prior to the 1930s, most of this decline was due to factors other than medical therapy (57), and is generally attributed to improvements in

[c] For example, a segment of ABC's 20/20 (broadcast on 16 January 1998) reported that this discovery would lead to the development of anti-aging drugs within 5 to 10 years, and one researcher interviewed on camera predicted healthy life spans of several hundred years. The finding itself was reported in *Science* magazine (54).

living conditions and public health. With the advent of antibacterial drugs in the 1930s and 1940s, medical treatment began to play an important role in these changes, and this role has expanded in recent decades, thanks to interventions in CVD and cancer, which have contributed to the rapid decline of old-age mortality.

It seems reasonable to expect that future mortality trends in wealthy nations will resemble past changes. Although the focus of our efforts will evolve, the net effect on death rates will probably be similar. For this reason, extrapolation is the preferred means of predicting the future of human mortality. This strategy rides the steady course of past mortality trends, whereas popular and scientific discussions of mortality often buck these historical trends, in either an optimistic or a pessimistic direction. History teaches us to be cautious. Pessimism about the continuation of mortality decline is not new, and earlier arguments about an imminent end to gains in human longevity have often been overturned, sometimes quite soon after they were put forth.[c] On the other hand, dubious claims about the road to immortality are probably as old as human culture itself, although they have not influenced official mortality forecasts as much as their more pessimistic counterparts.

Although imperfect, the appeal of extrapolation lies in the long-term stability of the historical mortality decline, which can be attributed to the complex character of the underlying process. This combination of stability and complexity should discourage us from believing that singular interventions or barriers will substantially alter the course of mortality decline in the future. In this situation, the burden of proof lies with those who predict sharp deviations from past trends. Such predictions should be based on theoretical results that are firmly established and widely accepted by the scientific community. Certainly, history can be overruled by a genuine consensus within the scientific community but not by unproven theories, intuition, or speculation.

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[c]A French demographer asserted in 1978 that the biological limit to human life expectancy at birth was 73.8 years for men and 80.3 years for women. Life expectancy in Japan exceeded these values in 1982 for men and in 1985 for women. Similarly, the United Nations predicted in 1973 that life expectancy at birth for developed countries would equal 72.6 years in 1985–1990, though later estimates showed that the life expectancy actually attained was 74.0 years. Corresponding figures are 58.7 and 60.5 years for developing countries, and 60.7 and 63.0 years for the world (2,58–60).

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3

Comparative Aging, Geriatric Functional Assessment, Aging and Disease

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INTRODUCTION

The diversity of topics examined in this chapter reflects the inherent complexity of the aging process. Despite the rapid progress of research in the last few decades, studies of aging and longevity still remain fragmented and incomplete. This first section compares longevity among animal species to identify common traits that may correlate with longevity. Senescence is assumed to occur in all living organisms and to lead to death. *Hence, senescence is considered as being universal, progressive, deleterious, and irreversible* (1). However, in certain plants and invertebrates, aging may progress very slowly or may not occur at all.

In the sections entitled "Comparative Physiology of Aging" and "Differential Aging in Humans," functional competence is assessed in old humans; one classic characteristic of the elderly population is its heterogeneity, not only among species but also among individuals of the same species, and, within the same individual, among the various constituents of the organism. A more recent recognition of this heterogeneity is the potential for different paths of aging, including "successful or healthy" aging. Senescence has been viewed in evolutionary terms as an inevitable product of natural selection (Chapter 5). The survival of the species would demand the aging and death of its members once reproductive vigor is no longer optimal. Survival beyond the period of reproductive activity would represent a luxury that few species are able to afford. "Evolution coddles you when young and forsakes you when old" (2). However, according to the evolutionary "grandparents hypothesis," a growing number of humans survive well beyond the reproductive age (Chapter 2) to protect and educate the young during the long period necessary for them to achieve maturity and adult fitness (see below). In the section entitled Functional Assessment in the Elderly, the respective roles of genetic and environmental factors are discussed with respect to their involvement in the increased susceptibility to disease, the high risk of comorbidity, and the possibility of expansion or compression of morbidity in the elderly population.

The section entitled Aging and Disease presents sporadic cases of syndromes that occur in humans. These syndromes are grouped under the general term of progeria and share multiple characteristics of premature (early onset) or accelerated (rapid progression) aging. Although these (and other similar syndromes such as Down syndrome) are rare and duplicate only a few, but not all, characteristics of aging, their study may provide useful information on normal aging. For example, stimulated by evolutionary ideas, a stock of flies has been developed that live twice as long as normal. Indeed, data of mortality and longevity dynamics of flies (as well as worms and rodents) suggest new strategies for longer life applicable to other species (Chapters 4 and 5) (3).

COMPARATIVE PHYSIOLOGY OF AGING

In humans, increases in life expectancy in the past century have been ascribed overwhelmingly to reductions in environmental causes of mortality that are extrinsic to the aging process. Longevity in evolutionary terms is difficult to reconcile with the findings, in recent decades, that, in humans, both mortality and disability rates have dropped for all older men and women, across virtually all social classes and ethnic groups, and in most developed and developing countries. It is possible that other factors, in addition to "physical fitness and reproductive capability," may be subject to positive selection such as the "grandparents hypothesis," that is, the important contribution of the grandparents in raising the young (Chapters 2 and 5). Comparison of life spans of several species (selective longevity) may show some differences in longevity and allow us to draw some relationships between the life span and the selected physiologic characteristics (physiological correlates of longevity).

Selective Longevity

The duration of life (longevity), the onset of aging, and the rate of mortality significantly differ among animal and plant species. In eukaryotes, the life span varies from a few days' duration in yeast cells (in Saccharomyces, mean chronological life span is 6 to 30–40 days, depending on environment and nutrients) to a duration of 5000 years in the California bristlecone pine (*Pinus longaeva*). In vertebrates, aging and death, assumed to occur in all species, were originally attributed to species-specific genetic programs. However, the early view of a rigid, genetically preprogrammed life span is gradually being replaced by one of greater plasticity in response to environmental modifications (Chapters 1 and 5). It is now possible to envision that aging may be retarded by a combination of appropriate genetic and environmental modifications. *Accordingly, the life spans of sexually reproducing species may be categorized according to rapid, gradual, or negligible rates of senescence* (4,5) (Fig. 1).

Rapid senescence is usually observed in organisms that have a short period of optimal function in adult life. In addition to the ones listed in Figure 1, the Pacific salmon (Oncorhynkus) represents a prime example of an organism characteristic of this group. The salmon undergoes severe stress (with high blood cortisol levels, Chapter 9) at the time of spawning and dies shortly thereafter at about one year of age (6). However, if reproduction and the associated stress are blocked, the salmon will continue to live for some (six to seven) years (6). Other species fitting into this category, and listed in Figure 1, include yeast, some nematodes (Caenorhabditis), the housefly (Drosophila), and some longer-lived (10–100 years) species that

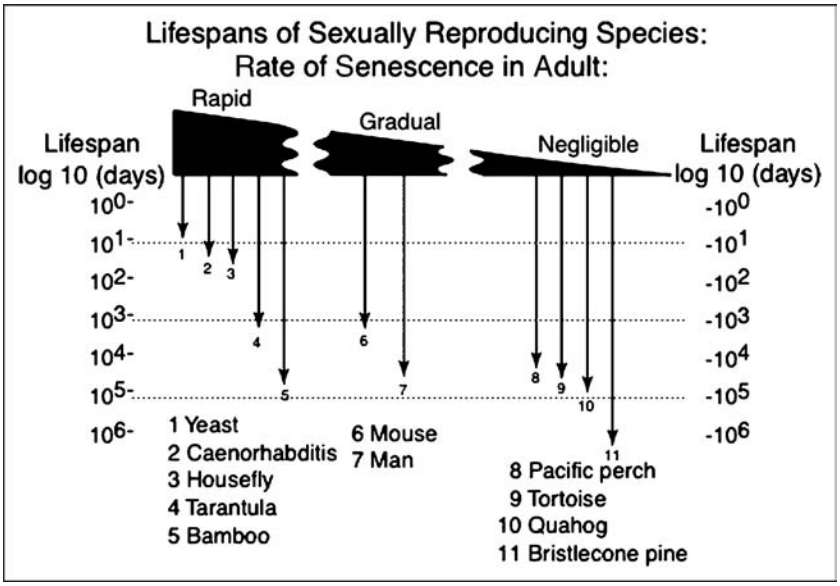

FIGURE 1 Comparison of maximum life span in different animal species. Species differ widely in longevity (total life span from zygote to oldest adult in years) and in the rate of senescence in adult (semiquantitative scale ranging from rapid to gradual to negligible senescence). Data on maximum life spans depend on husbandry conditions (temperature, nutrition). *Rapid senescence*: Yeast (*Saccharomyces cerevisae*) two to four days during asexual budding; nematode (*Caenorhabditis elegans*) 30 days; housefly (*Drosophila melanogaster*) 60 days; Pacific salmon (Onchorynchus) three to six years; male tarantula (*Eurypelma californica*) 10 years; vascular plants: thick-stemmed bamboo (*Phyllostachys bambusoides*, *P. henonis*) 120 years; *Puya raimondii* (related to the pineapple) 150 years. *Gradual senescence*. Mouse (*Mus musculus*) 4.2 years; human (Jeanne Calment) 122.4 years. *Negligible senescence*. Fish (Pacific perch), rockfish (*Sebastes aleutianus*) 140 years; orange roughy (*Hoplostethus atlanticus*) 140 years; warty oreo (*Allocyttus verrucosus*) >130 years; sturgeon (*Acipenser fulvescens*) 152 years; tortoise (*Geochelone gigantea*) 150 years; bivalve mollusc: ocean quahog (*Artica islandica*) 220 years; Great Basin bristlecone pine (*Pinus longaeva*) 4862 years. Ring dating often underestimates, and the true ages are probably greater than 5000 years. Inclusion of clonal, asexual, reproducing species, e.g., clones of the crosote bush, an asexually reproducing species, would extend the upper range of postzygotic life spans to >10,000 years. *Source*: Courtesy of Dr. C. E. Finch. From Refs. 4,5.

die after a very short senescence period, such as the tarantula (*Eurypelma californica*) and some species of bamboo (such as in the genus Phillostachys).

Gradual senescence characterizes the majority of animals and plants with life spans ranging from one year to more than 100 years. Humans (and many animals used in the laboratory for the study of aging) fall into this category. In these animals, midlife is marked by a progressive decline in function and a progressive increase in the number of diseases affecting the same individual simultaneously (see comorbidity below).

Negligible senescence occurs in species that, at older ages, do not show evidence of physiologic dysfunctions, acceleration of mortality, or limit to the life span. Examples of species in this category include some fishes (e.g., rockfish, orange roughy), turtles (e.g., tortoises such as the *Testudo sumerii* that may live to 150 years), and some trees [e.g., bristlecone pine (7)]. Other life forms—particularly plants and invertebrates—in which aging may not occur at all have been considered immortal. Examples of selective immortality include some protozoa (e.g., Paramecium) and metazoa (e.g., sea anemones such as *Cereas pedunculatus* in which the individual "jellies" have a fixed life span, but the larval "stub" produces a constant supply of new blanks as old ones are removed, and the specimens remain vigorous indefinitely) (8). The absence of signs of aging may not be equated with immortality. Rather, these organisms may better fit the negligible senescence category in which they die from a cause that might kill them at any age.

The molecular pathways involved in the regulation of chronologic life span appear conserved in yeasts, worms, and flies and, possibly, in rodents and humans (9). Survival would depend on the activity of partially conserving glucose (in yeast) or insulin/insulin-like growth factor-1 (IGF-1) (in worms, flies) or growth hormone (GH)/IGF-1 (in mice, and perhaps, also in humans) signals. This signaling downregulates antioxidant enzymes, heat shock proteins, and glycogen or fat accumulation while promoting growth, fecundity, and, eventually, mortality (Table 1). When mutations are introduced in the genes that regulate the life span, such as downregulation or inactivation of the receptors for the insulin/IGF-1 ligand or of *AKT* (protein kinase and transcription factor or kinase B), the life span is significantly prolonged in yeast (10), worms (11), flies (12), and mice (13) (Fig. 2). In some of these mutants, energy metabolism shifts from aerobic to anaerobic, free radical accumulation is reduced, and resistance to stress is increased (Table 2). Increased resistance to stress may be mediated in part by the activation of antioxidant enzymes and heat shock proteins that are synthesized in response to stress. An example of such proteins are the chaperone proteins that help other proteins to fold, thereby avoiding the production of inactive proteins or protein aggregates (14,15). However, the increased longevity is also associated with several unwanted side effects such as reduced growth, delayed maturation, and reduced fecundity. In humans, GH or IGF-1 deficiencies or excesses may cause several diseases (e.g., dwarfism, gigantism, and obesity). The effect of such diseases on the life span is usually negative (16,17). Further discussion of the use of mutants as animal models of aging is presented in Chapters 4 and 5.

■ Physiologic Correlates of Longevity

Data from several orders of placental mammals (i.e., whose embryos are nourished through a placenta) show a *highly significant relationship between life span and body weight: the bigger the animal, the longer the life span* (Table 3). For example, the

TABLE 1 Regulation of Longevity from Yeast to Humans

Organisms	Yeast	Worms	Flies	Rodents	Humans
Ligands	Glucose	Insulin/IGF-1	Insulin/IGF-1	GH/IGF-1	GH/IGF-1
Receptors	Gpr1	DAF-2	INR	IGF-1 receptor	IGF-1 receptor?
Stress resistance	SOD, catalase	SOD, catalase	SOD	SOD, catalase	↓ Fat accumulation?
Proteins	HSPs ↓ Glycogen accumulation?	HSPs ↓ Fat and glycogen accumulation?	↓ Fat accumulation?	HSPs ↓ Fat accumulation?	
Development	Growth Fecundity	Growth Fecundity	Growth Fecundity	Growth Fecundity	Growth Fecundity?
Longevity	← Shorter than in mutants →				

Note: Conserved regulation of longevity in wild-type eukaryotes (yeast, worms, flies, mice, and possibly humans) would depend on the ability of conserving glucose (yeast), Insulin/IGF-1 (worms and flies), or GH/IGF-1 (rodents, humans?) pathways. These pathways would upregulate aerobic metabolism, facilitate glycogen and fat utilization by reducing their accumulation, promote growth and fecundity, decrease antioxidant enzymes and heat-shock proteins, and would determine length of life span and mortality. The sign (?) indicates, in humans, that the evidence of a definitive association of longevity with the GH/IGF-1 proteins is not yet conclusively established.
Abbreviations: IGF-1, insulin-like growth factor-1; GH, growth hormone; Gpr1, G protein–coupled receptor 1; DAF2, insulin receptor; INR, insulin-like receptor; SOD, superoxide dismutase; HSPs, heat shock proteins.

elephant may reach or exceed 70 years in captivity, whereas the rat seldom lives more than three years. There are, however, many exceptions to this generalization. A human female has reached 122 years of age, whereas other larger mammals show a shorter potential longevity (horse, approximately 60 years; hippopotamus and rhinoceros, approximately 50 years; bear, 30 years; camel, 25 years). Among domestic carnivores, cats, although generally smaller in size than dogs, live longer than dogs.

The same data show an even more significant relationship between life span and brain weight (Fig. 3). For example, insectivores with a smaller, simpler brain have a shorter life span than ungulates, and the latter have a simpler brain and shorter life span than humans (Fig. 3). *Among mammals, humans with the longest life span have the heaviest brain in relation to body weight and also the most structurally complex (18)*. Among the major brain regions, the neocortex (the largest and, ontogenetically, most recently developed part of the cerebral cortex) shows the strongest correlation with the life span, with potentially important physiologic implications (19). *The most obvious is that the higher the ratio between brain weight and body weight ratio is, particularly, the greater the degree of cerebral cortical expansion, the more precise the physiologic regulations; hence, the greater the chance for longer survival*. Such an interpretation seems well justified in view of the essential role the nervous system plays in regulating vital physiologic adjustments, especially responses to environmental demands. Maintenance of the physiologic optimum and reduction of the magnitude of the fluctuations, occurring over time, diminish the probability of irreversible changes per unit time and, thus, slow the rate of aging and reduce the incidence of death.

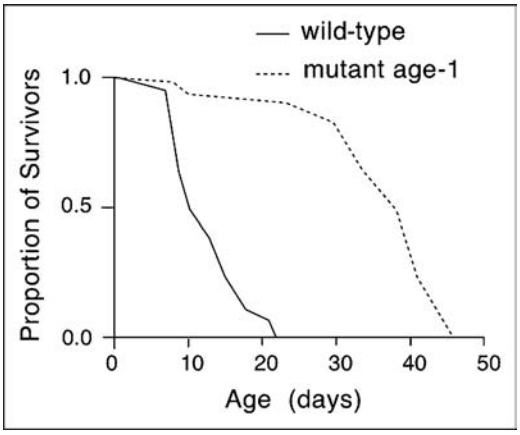

FIGURE 2 Inactivation of insulin/IGF-1R increases the longevity of *Caenorhabditis elegans*. Worms (hermaphrodites) were cultured on agar at 25°C. The dead worms were identified by the absence of movement in response to tactile stimulation and by the presence of tissue degeneration. Mutation of the gene *age-1* in the insulin/IGF-1 pathway prolongs the mean and maximal duration of life. *Abbreviations*: IGF-1R, insulin-like growth factor-1 receptor; IGF-1, insulin-like growth factor-1. *Source*: Courtesy of Dr. G. J. Lithgow.

Some caution, however, is advisable when directly correlating body or brain weight to life span. Correlations are not always appropriate when the terms of comparison represent different entities, one essentially anatomical (stature, brain weight) and the other essentially evolutionary, functional, and biochemical (duration of life). Another limitation is the paucity of accurate data on the maximum life span of most animal species that live in the wild and are subject to predation and premature death. While the brain is functionally a most important organ, a similarly positive relationship exists between size of several organs (e.g., adrenal, liver, spleen) and life span (4).

Another example of the relationship between a physiologic parameter and the length of the life span is basal metabolic rate (BMR): the higher the metabolic rate, the shorter the life span (Table 3) (20). BMR represents the amount of energy liberated per unit time by the catabolism of food and physiologic processes in the body. BMR is measured with the subject at rest, in a comfortable ambient temperature, and at least 12 hours after the last meal. BMR is expressed in kilocalories per unit (m^2) of body surface area and can be compared among different

TABLE 2 Mutations That Prolong Longevity in Invertebrates and Mammals

Mutants	Action on longevity	Physiological alterations	Mechanisms of action
Yeast ↓ *SCH9* (*AKT*)/*RAS**	↑ Longevity	↓ Growth ↓ Growth rate	↑ Resistance to oxidative stress and heat shock
Worms ↓ Insulin/*IGF-1R*/*AKT**	↑ Longevity	↑ Resistance to stress ↓ Fertility, growth ↓ Development Metabolism	Energy readjustment from aerobic to anaerobic metabolism ↓ Free radical accumulation ↑ Chaperone expression
Flies ↓ Insulin/*IGF-1R*/*AKT*	↑ Longevity	Growth Development ↓ Metabolism	Energy readjustment from aerobic to anaerobic metabolism ↑ Chaperone expression
Rodents ↓ *IGF-1R*	↑ Longevity	Normal or slightly decreased: Growth Development	Slight energy readjustment from aerobic to anaerobic metabolism ↑ Resistance to oxidative stress Insulin sensitivity ↓ Free radical accumulation
Humans	Presence of six gene polymorphisms involved in insulin/IGF-1 signaling was identified in 122 Japanese centenarians (older than 105 years)(94)		

Note: Note the association of increased longevity with shifts from high (aerobic) to low (anaerobic) energy consumption. Such shifts occur easily in invertebrates but are limited in mammals, hence the greater increase in longevity observed in invertebrates. Data in humans are very limited; six gene polymorphisms involved in insulin/IGF-1 signaling were identified in 122 Japanese centenarians (older than 105 years)(94).
Abbreviations: IGF-1R, insulin-like growth factor-1 receptor; IGF-1, insulin-like growth factor-1.

individuals. The energy thus liberated appears as external work, heat, and energy storage.

Some have argued that the life span of a species is limited by a fixed total metabolic potential that is consumed over a lifetime. Comparison of shrews and bats shows that shrews, with the highest metabolic rate of all mammals, have a life span of one year compared to bats, with a much lower metabolism and a life span of over 15 years (21). The higher metabolic rate could accelerate the accumulation of nuclear errors (DNA damage) or of cytoplasmic damage (accumulation of free radicals) and thereby shorten the life span (Chapters 5 and 12). An example of environmental manipulation that reliably extends the life span, at least in rodents, is caloric restriction that, perhaps, slows the oxidative damage that may underlie aging (Chapter 23) (22). Another example, in fruit flies, shows that mutation of the gene that encodes a protein, necessary for transport and recycling of metabolic by-products, would double the life span (23). In fishes, exposure to temperatures lower than those optimal for the species may increase longevity by decreasing immune responsiveness and, thereby, preventing autoimmune disorders. In *Caenorhabditis elegans*, exposure to low temperature induces mutations of several temperature-sensitive genes responsible for the formation of larvae, which are both developmentally arrested and long lived; when the so-called "dauer" larvae are allowed to grow, the life span of the adult mutant *C. elegans* is markedly longer than that of the wild type (Fig. 2) (13). Cumulative damage deriving from metabolism may result in chromosomal damage (24), such as the mutation of a DNA-putative helicase that occurs in Werner's syndrome (WS), a premature aging disease in humans (25).

Fecundity, as an expression of reproductive function and measured by the number of young born per year of mature life, appears also to be inversely related to longevity (Table 3). Shrews, with a short life span, have a large litter size and produce two litters per year, whereas the longer-lived bats have only one young per year. *Duration of growth (during the developmental period preceding adulthood) has also been related to the life span*. For example, comparison of the chimpanzees to humans shows that growth periods last approximately 10 years in the chimpanzee

TABLE 3 Physiologic Correlates with Longevity

Index studied	Correlation
Body weight	Direct
Brain/body weight	Direct
Basal metabolic rate	Inverse
Stress	Inverse
Reproductive function/fecundity	Inverse
Length of growth period	Direct
Evolution	Uncertain

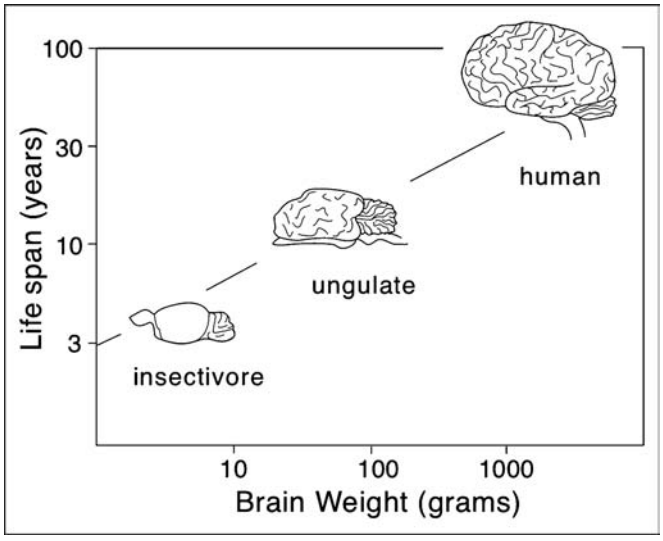

FIGURE 3 Comparison of the relationship of brain weight to life span in vertebrates. Humans have the heaviest brain in relation to body weight among all vertebrates and also the longest life span.

and 20 years in humans, and their respective average life spans are approximately 40 and 80 years.

Duration of the growth period may be prolonged in experimental animals; *the onset of developmental maturation can be delayed by restricting food intake in terms of total calories or of some specific dietary components* (26). With these dietary manipulations, not only is the life span prolonged but some specific functions, such as reproduction and thermoregulation, are maintained until advanced age, the onset of aging-related pathology is delayed, and its severity is reduced (Chapter 23).

The factors described above, including body and brain size, metabolic rate, fecundity, duration of growth, as well as stress and conditions of life, superimposed on the genetic makeup, undoubtedly contribute to the difference in longevity among species. As discussed already, natural selection requires that individual members of a group survive through the reproductive period to ensure continuation of the species; thereafter, survival of the postreproductive individual may very well become indifferent or even detrimental (e.g., food competition) to the group. In this sense, a gene that ensured a maximum number of offspring in youth but also produced disease at later ages might be positively selected (21). Currently, in humans, not only is life expectancy increased, but people also live well beyond the reproductive years, women some 40 years beyond the childbearing period. Longevity of a species beyond the reproductive years (see above) may be explained, in evolutionary terms, by older members contributing to survival and nurture of the new generation throughout the maturing years, thereby preserving the entire population structure and protecting the development and progress of our society (27).

■ DIFFERENTIAL AGING IN HUMANS

■ Successful or Healthy Aging: Functional Plasticity Persists in Old Age

Chronologic age (age in number of years) and physiologic age (age in terms of functional capacity) do not always coincide, and physical appearance and health status often do not always correspond to what is typical at a particular chronological age. Although specialized knowledge is not required to estimate age, in many cases, people may look younger or older than their chronologic age. Early attempts were made to standardize functional profiles of old persons, similar to the well-established diagnostic charts of growing children. Yet, these attempts have failed given *the characteristic and substantive heterogeneity of aging processes in human populations*. Indeed, some individuals show signs of old age at a much slower or faster rate than others and the variability among individuals of the same age in response to a variety of physiologic or psychologic tests increases with old age (Fig. 4).

As already mentioned (Chapter 1), in humans, the physiologic "norm" is represented by the sum of all functions in a 25-year-old man free of any disease, with a weight of 70 kg and a height of 170 cm. Comparison of old individuals with this "ideal health" inevitably discloses a range of functional decrements with advancing age. Early studies were conducted in selected samples of "representative" elderly (28). Because the prevalence of chronic disease increases in old age, a large part of the data relating to functional loss with aging in early studies may have been due to the effects of disease rather than to aging. In those earlier studies, comparison of several functions from young to old age revealed a gradation of decrements with old age. More recent approaches have challenged the inevitability

FIGURE 4 The heterogeneity of the elderly population as illustrated by scores on a hypothetical test. Results from a large number of tests show that the mean of two scores (as represented by the barograms) is the same for both young and old individuals. However, the much greater standard error of the mean (bracketed lines) in older than in younger individuals indicates a greater variability among individuals from the old population.

of functional impairment and of disease in the elderly by grouping aging processes into three possible trajectories (29):

1. Aging, with disease and disability
2. Usual aging, with the absence of overt pathology, but with the presence of some declines in function
3. Successful (or healthy) aging, with little or no pathology and little or no functional loss

Such a grouping of aging processes:

- De-emphasizes the view that aging is exclusively characterized by declines in functional competence and health
- Re-focuses on the substantial heterogeneity among old persons
- Underscores the existence of positive trajectories (i.e., without disability, disease, and major physiological decline)
- Highlights the possible avoidance of many, if not all, the diseases and disabilities usually associated with old age

Conditions for successful old age include interactions among several factors, such as higher level of formal education and financial income, strong social support, consistent participation in educational and intellectual activities and adherence to moderate exercise, good diet, and hygienic habits (e.g., no drugs, no smoking, avoidance of excessive alcohol intake) (Fig. 5).

Functional plasticity, that is, the capacity of the individual to adapt to environmental demands without loss of physiologic competence, is most effective at young developmental ages. However, as discussed throughout this book, functional plasticity persists into old age, albeit at less intensity. As we consider the many cross-cultural differences that greatly influence aging, factors such as diet, exercise, drugs, and psychosocial environment should not be underestimated as potential moderators of the aging process. Taking these elements into account, the prospects for avoidance, or eventual reversal, of functional loss with age are vastly improved, and the risks of disability and disease are reduced. Successful or healthy aging in certain functions is a demonstration that aging may occur with little loss of function.

■ Differential Timetables of Aging in Organs and Systems

Changes with aging lack uniformity, not only among individuals of the same species but also within the same individual: onset,

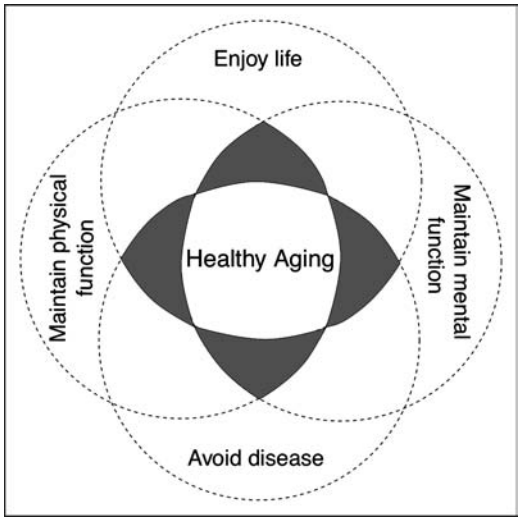

FIGURE 5 Successful or healthy aging. Successful aging is based on maintenance of mental and physical function, avoidance of diseases, and enjoyment of life.

rate, and magnitude of changes vary depending on the cell, tissue, organ, system, or laboratory value considered (28). A number of laboratory values, many of which often may remain unchanged with aging, or may decrease or increase, depending on the individual examined, are illustrative of the heterogeneity of changes with aging (Table 4). Laboratory values are usually presented as having a normal range. Older persons often have values in the extremes (high or low) of normal range (30).

In whichever organ and system considered, timetables of aging represent an approximation, for the onset of aging cannot be pinpointed precisely by any specific physiologic sign (such as menarche for ovarian maturation). A classic example of a unique timetable involving an organ that develops and ceases to function during a specific period of the life span is the ovary; its function begins at adolescence (in humans, approximately 10–12 years) and stops at menopause when ovulation ceases (in humans, approximately 50 years) (Chapter 10).

Aging is often a slow and continuous process, and, therefore, some of its effects are observed only when they have progressed sufficiently to induce changes that can be identified and validated by available testing methods. *An illustrative example is atherosclerosis.* Atherosclerotic lesions begin in childhood, when they remain functionally silent. The lesions become manifest in middle and old age, when the lesions have progressed sufficiently to induce pathological consequences that affect the entire cardiovascular function (Chapters 15 and 16).

Fasting blood sugar values are minimally affected by aging, with the exception of late-onset diabetes (insulin-resistant diabetes), where blood sugar levels are elevated (Chapter 13). However, even in the nondiabetic elderly, when blood sugar levels are tested under increased physiologic demand (e.g., a sugar load, as in the sugar tolerance test), the efficiency with which the organism is capable of maintaining levels within normal limits and the rapidity with which these levels return to normal are significantly reduced in old as compared to adults (Chapter 13). Other examples such as nerve conduction velocity, cardiac index (cardiac output/min/m^2 of body), renal function (filtration rate, blood flow), and respiratory function (vital capacity, maximum breathing capacity) are less capable of withstanding stress in old compared to young individuals. An important general point, highly illustrated by the above examples, is *that exposure to stress reveals age differences not otherwise detectable under steady-state conditions;* it clearly demonstrates the declining ability of the old organism to withstand or respond adequately to stress (Chapter 9).

Other body functions begin to age relatively early in adult life. For example, deterioration of both vision (especially accommodation) and hearing begins in the teens and continues to progress until around 60 years of age (Chapter 8). Auditory deterioration may also be hastened by the environmental noise to which, in our civilization, individuals are often continuously exposed from young age. Some comparative studies in isolated populations living in a quiet environment (e.g., forest-dwelling African tribes) and who have maintained good auditory function into old age seem to support the view that continuous exposure to noise is harmful to hearing (Chapter 8).

Differential aging also includes declines in stature (or standing height), sitting height, breadth of shoulder, and depth of chest—all of which show a progressive reduction with aging in contrast to head diameters, which remain practically unchanged. The considerable and progressive diminution in stature that occurs with aging in all humans may be ascribed, at least in part, to alterations in bone structure (e.g., osteoporosis) (Chapter 20).

Body weight usually increases at the initial stages of senescence as a consequence of increased fat deposition

TABLE 4 Some Laboratory Values in Old Age

Unchanged		Decreased	Increased
Hepatic function tests	Arterial blood test	Serum albumin	Alkaline phosphatase
Serum bilirubin	pH	HDL cholesterol (women)	Uric acid
AST	PCO_2	Serum B_{12}	Total cholesterol
ALT	Renal function tests	Serum magnesium	HDL cholesterol (men)
GGTP	Serum creatinine	PO_2	Triglycerides
Coagulation tests	Thyroid function tests	Creatinine clearance	Plasma TSH (?)
Biochemical tests	Plasma T4	Plasma T_3 (?)	Plasma glucose tolerance tests
Serum electrolytes	Complete blood count	White blood count	Fasting blood sugar (may be within normal range)
Total protein	Hematocrit		Postprandial blood sugar
Calcium	Hemoglobin		
Phosphorus	Red blood cells		
Serum folate	Platelets		

Note: The sign (?) indicates that changes in values with aging may show great individual variability.
Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGTP, γ-glutamyltransferase; HDL, high-density lipoprotein; PO_2, partial pressure of oxygen; PCO_2, partial pressure of carbon dioxide; T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone.

(especially in the subcutaneous layer), which reaches a peak at approximately 50 years in men and 60 years in women (31). Both body weight and fat deposition progressively decrease at later ages (between the fifth and the seventh decades) when morbidity and mortality increase (32). With advancing age (often 85+ years of age), some "frail" elderly loose considerable amount of weight as the consequence of a "cascade" of comorbidities and physiologic instability. Indeed, "frailty," is considered to be a specifically geriatric "wasting syndrome" that elevates the risk for a variety of health conditions (e.g., falls, functional decline, morbidity, and premature mortality) (33).

Sudden or progressive changes in body weight, abnormal in severity and timing, may also predict onset and course of disease. Thus, body weight represents a relatively simple measure of the physiologic and, eventually, pathologic assessment of the health status of the elderly (as it is an indicator of the growth progress during development).

A more complete discussion of changes with aging in various functions is presented in the corresponding chapters of this book.

■ FUNCTIONAL ASSESSMENT IN THE ELDERLY

Assessment of physiologic competence, critical at all ages to determine the health status of the individual, may serve also as a basis for the diagnosis and prognosis of disease. In this section, parameters of physiologic status are evaluated first and then incorporated into geriatric assessment.

■ Assessment of Physiologic Age in Humans

Assessment of physiologic competence and health status in humans, at any age, is a multifactorial process that requires quantitative measurements of numerous parameters at progressive ages (Fig. 6) (34). Assessment must reflect the different variables that influence the age-related timetables for key bodily systems, combine them to represent a cohesive health profile of the individual, and satisfy a number of criteria:

- The variables must be indicative of a function important to the competence or general health of the individual and capable of influencing the rate of aging.

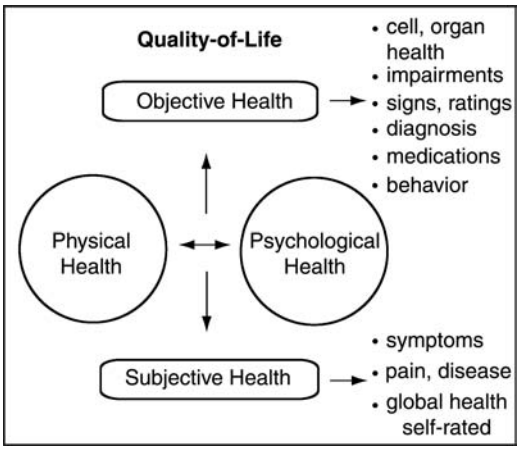

FIGURE 6 Assessment of the health status and quality-of-life in humans depends on multiple measures. These include several parameters of objective and subjective physical health together with parameters of psychological health.

- They must correlate with chronological age.
- They must change sufficiently and with discernible regularity over time to reveal significant differences over a three- to five-year interval between tests.
- They must be easily measurable in an individual or cohort of individuals without hazard, discomfort, or expense to the participant, or excessive labor or expense for the investigator.

The validity of any assessment lies in the choice of the proper test or battery of tests best qualified to provide an overall picture of current health and to eventually serve as a basis for prediction of future health and length of the life span. Such a choice is complicated by the need to also take into account the financial feasibility and the facilities available for the testing. The relatively large number of tests for assessing physiologic competence and health status in the elderly reflects the current failure to reach a consensus on the best checklist. A global measure of physiologic status may be derived from many different combinations of tests. Selection will depend on the purpose of the assessment and who will use these data. With respect to purpose of assessment, measurements may be expected to

- describe the functional status of an individual at progressive chronologic ages,
- screen a selected population for overall physiologic competence or competence of specific functions using cross-sectional or longitudinal sampling methods (Chapter 1),
- monitor the efficacy of specific treatments, drugs, exercise, and diet, and
- predict persistence or loss of physiologic competence to determine incidence of disease and to evaluate life expectancy.

Approximately 10% of nondisabled community-dwelling adults aged 75 and older lose independence each year. A number of simple qualitative and timed performance tests may be useful in identifying subgroups of older persons who are at increased risk for functional dependence, for example, are unable to perform activities of daily living (ADLs) and instrumental activities of daily living (IADLs) (see below) (35,36).

The choice of tests will depend on whether the user is a health provider, specialist, researcher, or case manager. Even with a precise identification of the nature of the assessment needed and of the user who needs it, it still remains difficult to choose the most significant and feasible tests. Some tests, although relatively innocuous in young and healthy individuals, may be troublesome for the elderly. In addition, some of the tests depend on the self-awareness of the individual tested, and her/his responses often overevaluate or underevaluate the symptoms (Box 1). Yet, it may be contrary to the interest of the elderly to assert that they represent a vulnerable group needing special protection. Rather, benefits may accrue for the elderly from their participation in medical and psychosocial survey research. Not only may such tests lead to the discovery of an unsuspected illness and its eventual treatment, but they may also provide altruistic commitment and mental satisfaction.

■ Geriatric Assessment

Geriatric assessment involves a multidimensional diagnostic process (including physiologic assessment) designed to establish functional capabilities or disabilities as well as medical and psychosocial characteristics (Fig. 6) (Table 5). The assessment is usually conducted by a professional multidisciplinary team with the

BOX 1 *Self-Awareness of Disease in the Elderly*

It has been claimed that the elderly complain excessively about poor health, aches, pains, and a variety of symptoms. In fact, considering their multiple pathologies, they do not complain enough. Overall, they view their health status positively, as is the case of 75% of the 55- to 64-year-old group; it is only in 13% of the older age group (85+) that this optimistic view is replaced by a poor health outlook. In addition to age, self-evaluation of health status depends on several variables, including income, race, and geographic residence (affluent, white, suburbanites have a more positive evaluation of health than low-income, black, central-city or rural-area-dwelling group). Sex differences are not evident despite the greater degree of disability in older women.

It is possible that this optimistic outlook results from self-overevaluation and may be responsible for the reticence of some old people to seek help from health care providers. Such reticence may be due to several factors:

- The mistaken assumption that nothing can be done about the problem
- The fear of a dreaded disease, that is, cancer, dementia, others
- The lack of knowledge of what normal aging is
- The failure to discriminate among a variety of deficits and disturbances, those due to old age from those due to disease

To remedy some of these misconceptions, a vigorous educational campaign, geared to the level of the particular audience, should stimulate awareness of the many interventions available to support a healthful life span. It should not be limited to geriatric centers—education of aging should start in secondary schools in parallel with other programs (e.g., sex education) related to health and social issues.

goal of formulating a comprehensive plan for therapy and long-term follow-up for ambulatory, noninstitutionalized individuals or institutionalized geriatric patients (37), including centenarians (38). Major purposes of the assessment are as follows:

1. To improve diagnosis (medical and psychosocial)
2. To plan appropriate rehabilitation and other therapy
3. To determine optimal living conditions, arrange for high-quality follow-up care and case management
4. To establish baseline information useful for future comparison

Most of these assessment programs include several tests, which have been grouped into three categories:

TABLE 5 "Simple" Functional Assessment of Ambulatory Elderly

History
Physical examination, including neurologic and musculoskeletal evaluation of arm and leg, evaluation of vision, hearing, and speech
Urinary incontinence (eventually fecal incontinence), presence and degree of severity
Nutrition
Dental evaluation
Body weight
Laboratory tests, depending on nutritional status and diet
Mental status
Folstein Mini Mental, Status Score, if <24, search for causes of cognitive impairment
Depression
If Geriatric Depression Scale is positive:
Check for adverse medications
Initiate appropriate treatment
ADL and IADL
Home environment and social support
Evaluation of home safety and family and community resources

Abbreviations: ADL, activities of daily living; IADL, instrumental activities of daily living.
Source: Adapted from Ref. 36.

1. Tests that examine overall physical health (i.e., degree of physiologic competence) and absence of disease
2. Tests that measure the ability to perform basic self-care activities, the so-called ADLs (Table 6)
3. Tests that measure the ability to perform IADLs (Table 6), which reflect the ability to manage personal affairs

ADLs and IADLs, or the lack of them, are taken as reflecting the ability to live independently in the community. Despite the current wide use of these tests in geriatric assessments for identification of health status and eventual disabilities, these tests must be assessed with caution inasmuch as their evaluation is carried out by health professionals rather than by the patients and the opinions of the two do not always coincide (39).

Quality of life in later years may be diminished if illness, chronic conditions, or injuries limit the ability of some to care for themselves without assistance. ADLs and IADLs are widely utilized as representative measures useful in home dwelling populations and as representative of the capability for independent living or, vice versa, as indicators of disability (Fig. 7). Disability is the inability to perform a specific function because of health or age and results from impaired functional performance. In most testing, the degree of wellness, that is, the absence of disability or disease, is recorded and, reciprocally, the presence and severity of disability or disease is recorded (40–42). The severity of the disability may be measured in terms of whether a person

1. does not perform the activity at all,
2. can only perform the activity with the help of another person or if a person is available (but does not actually give aid), and
3. can perform the activity with the help of special equipment.

Disability is, frequently, coded according to five degrees of severity: (*i*) no disability, (*ii*) at least one IADL disability but no ADL disability, (*iii*) one or two ADL disabilities, (*iv*) three to four ADL disabilities, and (*v*) five to six ADL disabilities (43,44). For example, in 1995, among noninstitutionalized

TABLE 6 Categories of Physical-Health Index Measuring Physical Competence[a]

Physical health	Activities of daily living (ADL)	Instrumental activities of daily living (IADL)
Bed days	Feeding	Cooking
Restricted-activity days	Bathing	Cleaning
Hospitalization	Toileting	Using telephone
Physician visits	Dressing	Writing
Pain and discomfort symptoms	Ambulation	Reading
Signs on physical exam	Transfer from bed	Shopping
Physiologic indicators (e.g., lab tests, X rays, pulmonary and cardiac functions)	Transfer from toilet	Laundry
	Bowel and bladder control	Managing medications
	Grooming	Using public transportation
Permanent impairments (e.g., vision, hearing, speech, paralysis, amputations, dental)	Communication	Walking outdoors
	Visual acuity	Climbing stairs
	Upper extremities (e.g., grasping and picking up objects)	Outside work (e.g., gardening, snow shoveling)
Diseases/diagnosis	Range of motion of limbs	Ability to perform in paid employment
Self-rating of health		Managing money
Physician's ratings of health		Traveling out of town

[a] Many of the items presented are components of several measures of physical and functional health as discussed in a number of geriatric screening and assessment programs.

persons 70 years of age and older, 32% had difficulty performing and 25% were unable to perform at least one of nine physical activities (Table 6).

With advancing age, disability intensity increases in men and women, with the highest level at 85 years and older (85+) (Fig. 7). Disability is usually associated with the incidence of chronic conditions and diseases. These include in decreasing frequency arthritis (especially in women), heart disease, stroke, respiratory disease, and diabetes (Table 7). It is to be noted that the greater intensity of disability for women, becomes manifest at the later ages of 74 to 85+ years. *Thus, women, who have a longer average life span than men (Chapter 2), live longer with disability.* The causes of this sex difference are not entirely known. Probably, females are at higher risk for a number of chronic degenerative conditions (e.g., osteoporosis, diabetes, arthritis) that interfere with those functions (e.g., walking, doing housework) necessary for independent living but not causing death. Men, on the other hand, are more subject to lethal diseases and die before developing debilitating disabilities.

The passage from independence to disability may be gradual or abrupt. Disability may be accelerated by the onset or worsening of disease (45–47) or it may be delayed by a high level of education associated with high income and good lifestyle habits (48–51). However, disease prevention and improvement of environmental conditions cannot forestall the progressive loss of function and corresponding increase of disability that occur with advancing age (52,53). Indeed, in some populations of socioeconomic-advantaged elderly, although death has been postponed, the prevalence of disease and disability has not, especially in women (54).

In parallel with differences in socioeconomic, educational, and hygienic habits, older populations differ in physical functioning and disability by race. For example, Afro-Americans report higher levels of disability than white persons. In 1995, among noninstitutionalized persons 70 years of age and

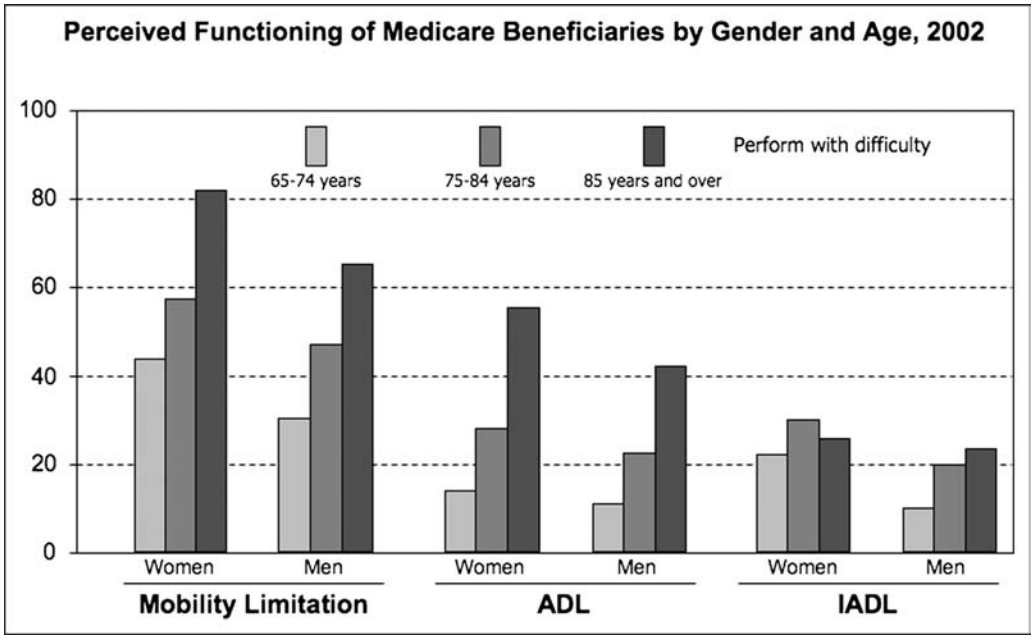

FIGURE 7 Percentage of persons 65 years of age and older who have difficulty or inability to perform physical activity, ADL, and IADL. Examples of activities include, for physical activities, walking for a quarter mile; for ADL, bathing, dressing, getting in and out of bed; for IADLs, preparation of one's own meals, shopping, heavy housework. *Abbreviations*: ADL, activities of daily living; IADL, instrumental activities of daily living. *Source*: Courtesy of Dr. J. M. Guralnik. Data from Ref. 40.

TABLE 7 Percent of Persons 70 Years of Age and Older Who Report Specific Conditions as a Cause of Limitation in Activities of Daily Living: United States, 1995

Type of condition	Percent
Arthritis	10.6
Heart disease	4.0
Stroke	2.6
Respiratory	2.5
Diabetes	1.5

Source: From Ref. 44.

older, Afro-Americans were 1.3 times as likely as white persons to be unable to do certain physical activities and 1.5 times as likely as white persons to be unable to perform one or more ADLs. Similar conclusions may be drawn for other ethnic (e.g., Hispanic) groups, although additional research is needed to further identify the social and health factors that contribute to these ethnic differences (45,46,55).

In the last 25 years, the proportion of noninstitutionalized old women and men unable to do one or more ADLs or IADLs has declined, the decline being particularly marked in women. This trend may be viewed as evidence of progressively healthier (and even successful) aging relative to previous decades (56–58). Unlike the proportion of noninstitutionalized elderly, the overall (including persons in nursing homes and other similar institutions) proportion of elderly persons unable to perform ADLs continues to increase with the lengthening of the life span, even though the level remains quite low (33).

■ Disuse and Aging

Many changes that accompany aging coincide with those associated with physical inactivity and they are generally recognized as effects of disuse. Thus, bed rest as a consequence of disease may be superimposed on aging changes and further accelerates the aging process. Studies of long-term space travel have revealed that weightlessness in space also induces changes resembling those of aging and physical inactivity. The relationship between disuse (due to bed rest, insufficient exercise, or lack of gravity) and aging has some practical implications because the same practices of prevention and rehabilitation may apply to both.

Association of disuse and aging is apparent at all functional levels. A brief list of the functions affected is presented in Table 8. At the top of the list is a reduction in maximum oxygen consumption (VO_2 max), an indirect measure of the ability of the organism to transport oxygen from the atmosphere to the tissues. This transport is significantly reduced with old age (at the rate of about 1% per year) and with bed rest; in both cases, a program of physical activity slows the decline. VO_2 max depends on cardiac output, which also decreases with both advancing age and bed rest. Cardiac output (5.0 L/min) represents the amount of blood pumped by each ventricle (stroke volume) per unit of time (cardiac rate) (Chapter 20). Simultaneously, blood pressure increases with age and weightlessness, probably due to increased peripheral circulatory resistance.

In all cases, both younger and older subjects are able to benefit from exercise (Chapter 24). Studies of exercise and movement programs for elders (including nonagenarians) have shown significant increase in lean body (muscle) mass, improvement in several joint movements, and subjective perception of improved mobility and well-being. The capacity for improvement in muscle mass and power upon exercise is quite striking in humans as well as in experimental animals. At all ages, "practice makes perfect" and "use or loose it." However, for the elderly more than for other age groups, activity programs must be tailored to the individual for optimal benefits (Chapter 24).

TABLE 8 Physiologic Parameters in Aging, Physical Inactivity Weightlessness (In Space)[a]

Reduced
Maximum oxygen consumption (VO_2 max)
Resting and maximum cardiac output
Stroke volume
Sense of balance
Body water and sodium
Blood cell mass
Lean body mass
Glucose tolerance test
Sympathetic activity and neurotransmission
Thermoregulation
Immune responses
Increased
Systolic blood pressure and peripheral resistance
Vestibular sensitivity
Serum total cholesterol
Urinary nitrogen and creatinine
Bone calcium loss
Variable
Endocrine changes
Altered EEG
Altered sleep
Changes in specific senses

[a] Possibly responsive to physical activity.
Abbreviation: EEG, electroencephalogram.

■ AGING AND DISEASE

Aging is associated with increased incidence and severity of diseases, accidents, and stress. Deleterious factors, not themselves lethal, may, from an early age, gradually predispose the individual to functional losses or to specific diseases in later life. The epidemiology of aging is that branch of medical sciences that deals with the incidence, distribution, and control of disease in a population. The major objectives of epidemiological aging-related studies are to identify diseases at their onset, or even before, they become manifest, and to develop public health and medical strategies to prevent, postpone, or moderate them (59). The choice and outcome of these strategies have been, for many years now, a subject of debate. Although the increasing longevity of the elderly would reasonably predict an "expansion" of morbidity, several investigators have proposed an opposed and more optimistic view of "compression" of morbidity. According to the hypothesis of "compression of morbidity," morbidity and disability would be compressed into the final years or even months of life (60). Accordingly, the age of onset of functional decline and major degenerative diseases (e.g., heart disease, dementia, and cancer) would be postponed. *This postponement of the onset of disease would lead to the rectangularization of the survivorship curve (Chapter 2)*. Although the hypothesis itself remains controversial, there is consensus regarding the goal of "compressing morbidity" into the later years of life. Research on modifications of the environment and of behavioral factors known to increase mortality risks from fatal diseases have already uncovered valuable clues concerning functional failure in old age and provided a better understanding of the aging process itself.

The effects of aging are difficult to isolate from those caused by disease or from gradual degenerative changes that develop fully with the passage of time. Any demarcation among these effects can be only tentatively drawn at the present state of our knowledge. For example, it is reasonable to question whether the atheroma—the characteristic lesion of atherosclerosis—represents a degenerative process or a disease. In other words, does atherosclerosis result from age-related cellular and molecular changes in one or several of the arterial wall constituents or from mechanical injury or infection of the vascular wall? (Chapter 15). Dementia (contrary to the opinion of some pessimists!) is not a normal and unavoidable consequence of aging and should be investigated like any other disease process (Chapter 7). Likewise, anemia is not a normal correlate of aging, and, when present, a cause for this condition should be investigated (Chapter 17). Indeed, one of the tasks of the geriatrician is to be able to distinguish aging from disease and to treat both as independent but related entities. In geriatrics, it is necessary to differentiate the aging process from disease and to correlate the physical state with the psychosocial environment (59).

One of the challenges of geriatrics arises from the multiplicity of problems confronting the elderly (58). One cannot adequately treat disease without considering the psychologic, economic, and social situation of each individual (Table 9). This global, "holistic" view of the individual should apply to all ages, of course, but it becomes crucial for the elderly, for whom loneliness, social instability, and often, financial hardship have enormous impacts on health and well-being.

Although the study of a specific disease projects the much more concrete image of a task achievable within a limited time, both clinical and basic research orientations have merits and should be pursued simultaneously.

■ Complexity of the Pathology of Aging

"Old age is almost always combined with, or masked by, morbid processes." Late life is a period of increasing and multiple pathology and morbidity (incidence of disease is also referred to as morbidity) during which it becomes progressively more difficult to distinguish between "normal" aging and specific diseases that affect old people. As indicated at the beginning of this chapter, aging, among living organisms, has been qualified as almost entirely universal, intrinsic, progressive, deleterious, and irreversible (1). Disease may be viewed as a process that is:

- Selective (i.e., varies with the species, tissue, organ, cell, and molecule)
- Intrinsic and extrinsic (i.e., may depend on environmental and genetic factors)
- Discontinuous (may progress, regress, or be arrested)
- Occasionally deleterious (damage is often variable, reversible)
- Often treatable (with known etiopathology, cure may be available)

In humans, the probability of dying from disease or injury increases with the passage of time. In every organ, tissue, and in many cells, a time-dependent loss of structure, function, and chemistry proceeds slowly as the consequence of accumulating injuries. For example, deterioration of skin elastin may result from bombardment by ultraviolet photons (Chapter 21); degeneration of articular cartilage, from countless mechanical insults (Chapter 20); and opacity of the crystalline lens, from molecular injuries (Chapter 8). Indeed, it is when these injuries act at the molecular level that the most significant consequences for aging may occur, such as failure of DNA repair or oxidative damage or progressive cross-linking of collagen (the major structural protein of the body) (Chapters 5 and 21). The implication of the increased number of older persons in the population and the greater susceptibility to diseases and disability in old persons compared to young and adults has not only biomedical but also socioeconomic *implications (Box 2)*.

One of the main characteristics of old age pathology is comorbidity, that is, the multiplicity of diseases simultaneously affecting the same individual (33,45,52,59,60,66–68). The prevalence of two or more diseases, each capable of limiting ADL (Table 7), increases with age for men and, with a greater severity, for women (Fig. 7). Autopsy often reveals numerous lesions involving so many organs of the body that it is difficult to know which disease was responsible for death. The pathologist is often perplexed as to how the patient managed to live so long with such a "load" of diseases. This pathologic multiplicity is particularly evident in the 70- to 90-year-old age group in contrast to the 60- to 70-year-old group with a limited number of lesions at autopsy.

The multifactorial pathology of the elderly poses many problems in diagnosis and treatment. As children are not just "little or young adults," the elderly are not just "old adults." Of course, the wide range of individuality and variability makes many generalizations inappropriate; however, certain principles should be kept in mind when considering the clinical manifestations of disease in the elderly (Table 10). Disease presentation is often atypical. Sepsis (a feverish toxic condition resulting from the spread of bacteria or their products from a focus of infection throughout blood or tissues) without fever is common. Myocardial infarction may occur without chest pain and present either without symptoms or with fainting or congestive heart failure. Even for such well-characterized diseases as thyrotoxicosis, appendicitis, peptic ulcer, and pneumonia, the elderly patient often has atypical symptoms. For example, a patient with pneumonia may present with a chief complaint of confusion but lack any history of cough.

Another characteristic of the elderly is that diseases tend to be chronic and debilitating rather than acute and self-limiting; symptoms tend to be more subtle and vague. Thus, recognition and diagnosis of disease in the elderly require a high degree of alertness on the part of health-care providers.

A consequence of multiple pathology is the need for multiple therapies. This treatment is, in turn, associated with the danger of "*polypharmacy*" (i.e., the administration of too many drugs and medications together) (Chapter 22). Due to their impaired homeostatic mechanisms, the elderly do not tolerate therapeutic mistakes as well as younger patients. Considerations of risk versus benefit, while important in all medical decisions, become crucial when dealing with elderly patients. Altered drug reactions and interactions to commonly used drugs should be considered potential dangers lest they lead, together with the decreased physiologic competence and multiple pathology, to the most serious of complications of medical treatment of the elderly, iatrogenic disease (i.e., medically induced disease) (Chapter 22).

TABLE 9 Holistic View of the Elderly

In geriatrics, it is necessary
to differentiate the aging process from disease
to correlate physical state with psychosocial environment

BOX 2 *Setting or Not Setting Limits on Health Care for the Elderly and the Quest for a Good Death*

Quality-of-life in later years and the cost to be paid to achieve it continues to elicit heated debate and controversy. In the 1980s, the concept was first publicly formulated that health care should be denied or "rationed" to older persons in the United States (61). It was argued that "In the name of medical progress, we have carried out a relentless war against death and (health) decline, failing to ask. . .if that will give us a better society. Neither a longer lifetime nor more life-extending technology is the way to that goal" (62).

This and similar statements represent a backlash against a stereotyped group of old individuals who are in need of assistance and ready to reap the benefits of a "welfare state for the elderly." This negative view was compounded by the rapidly increasing costs of medical care, by the tremendous growth of federal (e.g., Medicare) spending for the elderly and the fear of bankruptcy of the Medicare programs, and by the realization that not all elderly are poor and in need of public aid but, on the contrary, many represent a well-off elite.

Arguments against the "setting of limits" based exclusively on age, include (*i*) the heterogeneity of the elderly, many of whom age "successfully" and require little or no public support; (*ii*) the realization that denying access to high-technology medicine to the elderly may not substantially reduce overall health costs (primarily incurred in neonatal care) and save money; and (*iii*) the difficulty of managing ethical choices and legal consequences. Setting limits would ". . .burden the elderly, undermine our (United States) democratic freedoms and would not guarantee any significant reductions in expenditures."

Within this debate of the relationship between advances in technology and care of the elderly, we must include also "the quest for a good death" (63). The best decision to achieve it may be to decline the use of the most advanced tools for prolonging life of very old and frail individuals (for a few days or sometimes a few hours) in favor of the choice "to die in one's own bed with a minimum of intrusive therapy." The essential feature of this cultural discourse is the notion of patient- and family–centered care and respect, in conflict with "inhumane life-prolonging treatments" for those who are dying. The choice between "heroic interventions" and "humanistic medical care" for the dying elderly should be based not only on medical decisions but on a number of factors, including the responsibility of the dying person for his/her own choice and the continuing communication with his/her family.

The increase in the proportion of the elderly in the population and, within this group, that of the very old, dictates that policies for health and medical care (including care at the time of death) be reorganized to meet the changing demographic demands (64,65). Their reorganization should follow those guidelines of medicine, law, ethics, public policy, religion, and economics that are acceptable to a civilized and caring society. Americans should decide for themselves just what is a "natural life course" and a "natural death" and whether any of us is "too old" for health care. "Be careful! Your decisions about someone else's life might affect you sooner than you think!"

■ Diseases of Old Age

Of the many diseases that afflict the elderly, certain medical problems are clearly restricted to the older population, while some overlap with those found in younger adults. Diseases that are primarily limited to the elderly are listed in Table 11. Diseases associated with aging with known and unknown etiologies are also listed in Table 11.

A list of common diseases responsible for death, compiled from hospital records in the United States (Fig. 8) and other developed countries, shows cardiovascular diseases (including hypertension and myocardial and cerebral vascular accidents) and cancer to be the diseases most related to old age (Table 12). It should be noted that when the information is secured not from general hospitals but specifically from geriatric units, the distribution of diseases is somewhat different, with atherosclerosis (and its cardiovascular consequences) being the major cause of hospitalization and deaths, while cancer is a lesser cause, especially after 80 years of age (Table 12).

TABLE 10 General Characteristics of Disease in the Elderly

Symptoms
Vague and subtle
Atypical
Unreported
Chronic vs. acute
Multisystem disease
Altered response to treatment
Increased danger of iatrogenicity (medically induced morbidity and/or mortality)

Together with the higher morbidity, after 60 years of age, hospitalization days per person per year increase dramatically from two days to two weeks (Fig. 8). Such an increase has a significant impact on the cost of medical care.

As the characteristics of disease are different in the elderly, likewise, the goal of treatment is modified compared to treatment at young and adult ages. Frequently, a cure is not the main objective; rather efforts are shifted toward prevention and relief (Chapter 22). Often, the priority is to maximize the ability of the elderly to function. When cure is not possible, rehabilitation can help in some cases; in other cases, the provision of proper care can assist in preventing the development of further complicating illnesses. Quality of life versus prolongation of life becomes a significant issue that creates medicolegal and ethical dilemmas that are hotly debated but remain largely unresolved (Box 2).

■ Causes of Death

The relationship between the aging process and disease is supported by demographic studies of diseases of old age and

TABLE 11 Diseases of the Elderly

Limited to aging	Associated with aging
Osteoporosis	Known etiology
Osteoarthritis	Septicemia
Prostatic adenocarcinoma	Pneumonia
Polymyalgia rheumatica	Cirrhosis
Temporal arteritis	Nephritis
	Cerebrovascular disease
	Myocardial infarction
	Unknown etiology
	Adult-onset, Type 2 diabetes
	Neoplasm
	Hypertension
	Alzheimer's disease
	Parkinson's disease
	Emphysema

causes of death. Major causes of death in the U.S. population are obtained from vital statistics (69, 70). Patterns of death have varied considerably from the first half of the twentieth century until now. In the early years of the twentieth century, the three major causes of death in this country were tuberculosis, pneumonia, and diarrhea-enteritis. By 1950, the major causes of death at ages 65 to 84 years and older were cardiovascular diseases (including stroke) and cancer (neoplasms) (Fig. 9), which have persisted up to today. A comparison of the causes of death from 1996 to 2003 for those aged 85 years and older shows, in the span of seven years, a marked decrease in the number of deaths due to heart disease, although it still remains the leading cause of death. Percentage of deaths due to cancer and stroke remain unchanged but deaths due to Alzheimer's disease (AD) increase markedly. The percentage of deaths due to pneumonia and influenza slightly decreases, whereas that of diabetes and chronic obstructive pulmonary disease slightly increases. Other values are practically similar in 1996 and 2003 (Table 13).

For the year 2003, when we compare the causes of death at ages 65 to 84 with those of over 85, heart disease still remains the leading cause of death, although its percentage is lower in the 65 to 84 age group than in the 85+ age group. Cancer remains the second highest cause of death with a percentage more than double in the 65 to 84 than in of 85+ age group; pneumonia/influenza and Alzheimer's are slightly lower and the other causes of death are similar (Table 13).

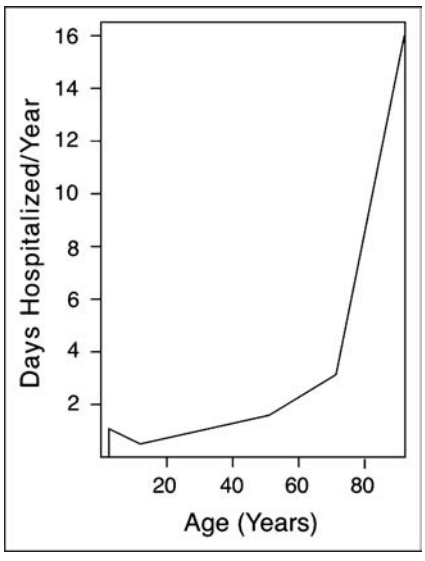

FIGURE 8 Days spent in hospital according to age. Number of days hospitalized per year according to age increases after 55 years of age and increases precipitously after age 70.

It has been claimed that the life expectancy of adults would be extended by one to three years if malignant neoplasms were cured, by five to seven years if atherosclerosis were prevented, and by approximately 10 years if both diseases were abolished. It is true that the spectacular advances in medicine in the last 100 years have been partly responsible for the dramatic increase in the average life span and, especially, the reduction of mortality due to cardiovascular disease (Chapter 2). However, it appears unlikely that a gain of such magnitude may be attributed to a decrease in cardiovascular disease alone. Efforts, therefore, should be expended to elucidate the basic mechanisms of disease, including the physiology of the aging process per se. An appreciation of these mechanisms is indispensable for a better understanding of the aging process. Without such an understanding, the etiopathology of the diseases of old age and their rational treatment cannot be achieved and the life span cannot be much further improved or prolonged.

■ Genetic Epidemiology

Many of the common diseases that affect humans have been known for many years to have a genetic component. More than 7000 rare human allele diseases are inherited in a Mendelian fashion (one gene–one disease), and many of the genes responsible for the phenotype have now been mapped, leading to major breakthroughs in our understanding of these conditions (71). It is only in recent years that a comprehensive guide to human disease gene mapping in complex diseases has become available. In these diseases, genetic variation interacts with environmental influences to modify the risk of disease (72,73). *Today, genetics is at the core of research on cancers, coronary heart disease, high blood pressure, neurological and psychiatric disorders, and a host of other clinical conditions (74–76)*.

Advances in genetics, molecular biology, and biotechnology have facilitated the discovery of new biomarkers of susceptibility to specific diseases and to environmental exposure that are being evaluated as part of molecular epidemiological research. A 1987 report of the National Research Council noted the dynamic nature of the various types of biomarkers underlining the continuum of cause-to-effect relationships (Fig. 10). Precise measures of potential etiologic determinants and phenotypes have become available to epidemiological research and are providing new links between genetics, environment, and disease. A few aspects of these interrelations are briefly presented, and more information may be found in some comprehensive textbooks (72,73).

It is apparent that monogenic (one gene) or polygenic (multiple genes) alterations may increase or decrease the risk of developing a trait. For example, the "risk" of late-onset AD, but not the disease phenotype, is increased (or decreased) depending on the type of lipoprotein mutation (Chapters 7 and 16). Lipoproteins are specific lipid-containing macromolecules in the plasma that have an important (predisposing or protective) role in the development of the atherosclerotic lesion (Chapter 16). The risk of AD is increased up to 12-fold for individuals carrying two copies of the APOE-4 allele, while the risk may be halved for those carrying one copy of the APOE-2 allele. The frequency of the APOE-4 allele in the general population is 16%,

TABLE 12 Common Fatal Diseases in Old Age

General hospital (% affected)					Geriatric unit (% affected)		
	Age					Age	
Disease	65–69	70–74	75–79	80+		80–89	90+
Cancer	29	27	27	24	Atherosclerosis	21	30
Cardiovascular	25	25	32	36	Myocardial infarct	19	10
Respiratory	14	12	13	10	Bronchopneumonia	17	25
Digestive	12	9	13	16	Cancer	10	7
Nervous system	11	9	8	6	Cerebral thrombosis	9	–
Renal tract	4	7	5	3	Chronic bronchitis	7	–
Other	5	1	2	5	Other[a]	16	28

[a] Senile dementia frequent in old age. Due to chronicity, patients are placed in long-term care facilities.

while that of the APOE-2 allele is 7% (77–79). As with other complex genetic traits, there is an "incomplete" correlation between the APOE-4 genotype and the AD phenotype: APOE-4 may contribute to the risk of developing AD but may not, by its presence alone, cause AD (77–79).

In examining the role of genes in the etiology of diseases, we must distinguish (*i*) causal genes, single-gene mutations leading directly to a disease phenotype (as in Mendelian disorders, e.g., Huntington disease) from (*ii*) susceptibility genes that are associated with a disease but by themselves are not sufficient to cause the disease (80). This would be the case of the APOE-4 mutation mentioned above with reference to AD.

Determination of the genetic component of a disease depends on three major and sequential steps:

1. Determination of familial aggregation (e.g., capturing information about disease in specific relative sets, siblings, cousins, etc., with twins being a special case, or entire pedigrees, i.e., nuclear families or extended kindred)
2. Determination of evidence of familial aggregation and, further, discrimination among environmental, cultural, and genetic factors that may contribute to the mutation clustering
3. Determination of evidence of genetic factors and their identification. Complex disease genes express traits that:
   - Show no clear Mendelian inheritance (one gene/one phenotype)
   - Have moderate-to-high evidence of genetic inheritance
   - Exhibit familial aggregation cases
   - Are polygenic (involve multiple genes) or are multifactorial (involve multiple genes interacting with the environment)

Thus, there are several ways in which genetic susceptibility may influence a disease:

- By itself
- By making the carrier more susceptible to the disease

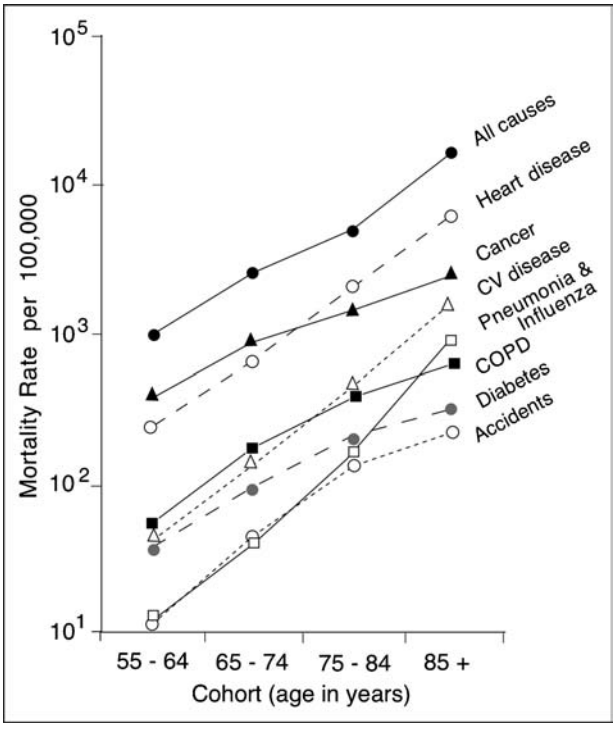

FIGURE 9 Age-specific death rates for leading causes of death in the older U.S. population, 2002. *Abbreviations*: COPD, chronic obstructive pulmonary disease; CV disease, cardiovascular disease. *Source:* From Ref. 69.

TABLE 13 Ten Leading Cause of Death. United States, All Races, Both Sexes

Ages: 65–84	Ages: 85+	
2003	2003	1996
1. Heart disease (28.2%)	1. Heart disease (36.2%)	1. Heart disease (41.2%)
2. Cancer (27.7%)	2. Cancer (11.6%)	2. Cancer (11.7%)
3. COPD (7.1%)	3. Stroke (9.4%)	3. Stroke (10.5%)
4. Stroke (6.6%)	4. Alzheimer's disease (5.5%)	4. Pneumonia/ influenza (6.6%)
5. Diabetes mellitus (3.6%)	5. Pneumonia/influenza (4.6%)	5. COPD (3.5%)
6. Pneumonia/influenza (2.4%)	6. COPD (4.4%)	6. Diabetes mellitus (1.9%)
7. Alzheimer's disease (2.2%)	7. Diabetes mellitus (2.2%)	7. Alzheimer's disease (1.9%)
8. Nephritis (1.9%)	8. Nephritis (2%)	8. Accidents (1.8%)
9. Accidents (1.9%)	9. Accidents (1.9%)	9. Atherosclerosis (1.6%)
10. Septicemia (1.5%)	10. Septicemia (1.4%)	10. Nephritis (1.4%)
All others (17%)	All others (20.9%)	All others (17.9%)

Abbreviation: COPD, chronic obstructive pulmonary disease.
Source: Data from Ref. 70.

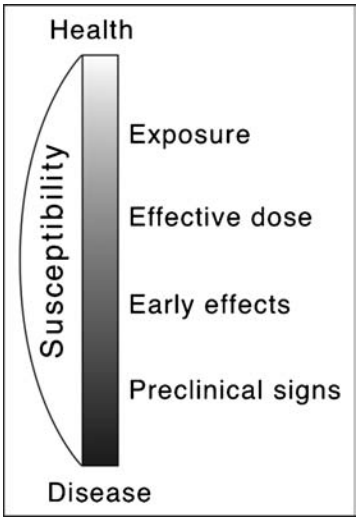

FIGURE 10 Dynamic nature of various types of biomarkers. Note the continuum of cause-to-effect relationships from health to disease, and, superimposed, a continuum of genetic effects with a corresponding increased susceptibility to environmental factors.

- By exacerbating the expression of a risk factor or the risk factor may exacerbate the genetic effects

As already mentioned in Chapter 1, mapping genes involved in diseases helps us to better understand the respective role of genetic and environmental factors in the etiology of human diseases. Mapping provides new practical screening protocols for those who are genetically predisposed and it also improves our ability to find therapeutic interventions for prevention or cure of the disease, once genetic susceptibility to specific environmental conditions is identified. However, findings of molecular medical genetics, when incorporated into public health initiatives, may lead to long-term and serious social consequences. Therefore, educational programs to target the public at large as well as the health agencies must be carefully constructed and integrated to avoid stigmatizing persons susceptible to specific diseases (81–84).

■ DISEASE AS A TOOL FOR THE STUDY OF AGING

Sporadic cases of syndromes having multiple characteristics of premature (early-onset) or accelerated (rapid-progression) aging occur in humans (85,86). It is unclear how far any of these syndromes may be regarded as a genuine acceleration of timing mechanisms that determine senescence. They are apparently pleoitropic genetic defects, and when one of the major features is accelerated aging, they are designated as progeria. One such example is Wermer's Syndrome (WS). When accelerated aging is associated with other prevalent defects, such as mental retardation and short stature, they are called progeria-like or progeroid or segmental syndromes. One example is Down syndrome (85).

Progeria syndromes do not quite duplicate all the pathophysiology of aging. Each syndrome presents an acceleration of only some of the characteristics associated with normal aging. Progeria is described as being of two main types, infantile and adult. The infantile form (Hutchinson Gilford syndrome) becomes apparent at a very early age and is associated with stunted growth, failure of sex maturation, and early signs of aging, such as skin atrophy, hypertension, and severe atherosclerosis. Death in Hutchinson–Gilford syndrome occurs in the twenties, usually consequent to coronary heart disease. The adult form of WS resembles more closely the changes associated with aging, with respect to both the affected individual's physical appearance and the disease pattern. The onset of this premature aging syndrome occurs between the ages of 20 and 30 years, and death ensues a few years from the onset, usually due to cardiovascular disease. Tissue culture studies of fibroblasts in infantile and adult syndromes reveal that the period during which cells replicate shortens, which is interpreted as being supportive of accelerated aging (Chapter 4).

■ Similarities and Differences Between WS and Aging

Werner's Syndrome is an inherited disease with clinical symptoms (forming a syndrome) that resemble premature aging (87). Early susceptibility to a number of major age-related diseases is a key feature of this disorder. Principal WS features include shortness of stature; senile appearance; cataracts and graying of the hair beginning at 20 to 30 years; skin changes (i.e., tautness, atrophy or thickening, ulceration) designated as scleroderma; joint deformities, soft-tissue calcifications and osteoporosis; atrophy of muscles and connective tissue; early cessation of menstruation; and increased incidence of neoplasms. Most of these conditions occur in aging as well but at a later age and with more or less severity (85).

The gene responsible for WS (known as *WRN*) is located on the short arm of chromosome 8. The predicted protein (1432 amino acids in length) shows significant similarities to DNA helicases (the enzymes capable of unwinding the DNA double helix). Four mutations in WS patients have been identified, one of the four was found in the homozygous state in 60% of Japanese WS patients examined. The identification of a mutated putative helicase as the gene product of the WS gene suggests that defective DNA metabolism is involved in the precociously aging WS patients (25).

Among some of the major differences between WS and aging is the type of inheritance—universal, multifactorial in aging, and autosomal-recessive in Werner's. Further, there is a high incidence of hypertension in aging but not in WS; the presence of dementia and other degenerative disorders of the central nervous system occur in aging but not in WS. The occurrence of soft-tissue calcifications is uncommon in aging but common in WS.

These differences are sufficient to justify the statement that WS is not merely a process of premature or accelerated aging. Rather, WS may be viewed as a "caricature" of aging. Both WS and aging may represent the result of generalized metabolic processes or aberrations thereof. Indeed, the overlap between the two entities is not surprising inasmuch as the various tissues of the human organism have only a limited repertoire of reactions to genetic abnormalities and environmental insults. Irrespective of similarities or differences, a study of the features of WS and aging will conceivably be useful in achieving an understanding of both.

The etiology of WS remains obscure, but among the several causes proposed, neuroendocrinologic dysfunction is supported by the occurrence of stunted growth, failure of gonadal maturation, and diabetes, either singly or in combination. However, the phenotypes observed in the affected individuals may result from mechanisms related to aging processes. If this is so, it could be inferred that cell autonomous functions dictate the pace of aging, at least in some organs and tissues. Further investigations of the WRN protein may reveal why particular systems and organs are differentially affected with aging (87).

■ Down Syndrome

Down syndrome (mongolism) is another example characterized by several symptoms, including accelerated aging and premature death, and is due to trisomy at chromosome 21. The incidence of the syndrome is greatest among children born from mothers 40 years of age and older, and the genetic abnormality has, therefore, been related to aging processes involving the oocytes (Chapter 10). Although in 20% to 30% of cases, the extra chromosome is contributed by the father, paternal age does not seem to have any significant effect on the incidence of the syndrome.

Individuals affected by Down syndrome may present somatic malformations, but the major deficit is represented by severe mental disability. Affected subjects who live to reach 30 years of age and longer present many signs of accelerated aging, including Alzheimer disease (AD) superimposed on the mental retardation (88) (Chapter 7). Animal models have also been proposed for the study of Down syndrome. The mouse is the animal of choice, for it is possible to introduce trisomy of chromosome 16 and produce individuals with some phenotypic characteristics of Down syndrome, such as cardiovascular defects, neurologic alterations, and retardation of brain maturation as well as early aging (89). Proposed as a good in vitro model to experimentally induce the premature aging of neurons is the transfer of the trisomy 21 from the fibroblast donor cells to neuroblastoma cells.

■ Experimentally Induced Aging and Disease in Animals

The use of animals, isolated organs, tissues, and cells, has led, and continues to lead, to a better understanding of the aging process. This use is regulated by specific rules governing the choice of the most appropriate model to answer each specific question (Chapter 1). Within this context, cause/effect relationships between disease and aging have been variously explored, depending on the hypotheses entertained by different investigators. Most often, intentionally induced disease has been used to accelerate the onset and course of aging processes to test therapeutic measures that might prevent or slow disease and aging. Some attempts have focused on "segmental" aging, that is, induction of aging in a selected organ, tissue, or cell type to mimic specific aspects of aging. Examples of these experimental approaches include genetic manipulation, increased free radical accumulation, inoculation of slow viruses, interference with nervous and endocrine functions, induction of wear-and-tear and stress, administration of mutagens/carcinogens, and others. Principles and methodology for the choice of animal models as "biomarkers" of aging and for the in vivo and/or in vitro induction of accelerated or delayed aging and aging-associated disease are reviewed in specialized textbooks (90–92). A few examples are briefly indicated below.

■ Examples of Induction of Pathology as a Tool to Study Aging

Premature aging has been induced in a number of laboratory animals as well as in captive wild animals by various methods. One of the favorite methods is to expose the animal to stress, that is, to excessive environmental (including physical, emotional, and social) demands. Stress will activate or interfere with all major regulatory systems, that is, it will disturb neuroendocrine balance (Chapter 9), alter immunologic competence (Chapter 14), and increase the production of free radicals (93). Stress or injection of cortisol to mimic adrenocortical stimulation, in association with a high-lipid or high-cholesterol diet, accelerates atheroma formation in rabbits (Chapter 15). High doses of vitamin D given along with calcium to rats precipitate calcification of the skin, heart, blood vessels, and other tissues usually susceptible to calcium deposits in old age (Chapters 20 and 21). Still other interventions utilize inoculation of animals with viruses or viral particles (prions) to mimic some of the degenerative diseases of old age. Slow viruses or prions induce "scrapie" in sheep and "mad cow disease" in cows, both conditions that resemble symptomatically and pathologically (e.g., accumulation of amyloid) to the human AD. Hippocampal slices from transgenic mice or infusion of amyloid β-protein intraventricularly in the brain of rats induces amyloid plaque deposits (Chapter 7).

Despite the availability of currently promising animal models, none exhibits all the characteristics and symptoms of old age in humans. Current advances, especially in molecular biology and genetics, promise faster progress in our knowledge of this area (Chapter 4).

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4

Cellular Senescence, Cell Death, and Transgenic Mouse Models of Aging

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INTRODUCTION

Cellular senescence and apoptosis (or programmed cell death) are evolutionarily conserved processes that occur throughout the life span of complex organisms such as mammals (Chapter 5). Apoptosis is essential for embryonic development, whereas it is likely (although not certain) that cellular senescence does not contribute to embryogenesis. In addition, apoptosis is important for tissue homeostasis in adult organisms—for example, during the development of immunity to certain infections (Chapter 14). Both apoptosis and cellular senescence are crucial for preventing the development of cancer (malignant transformation or tumorigenesis). Thus, both processes are beneficial. On the other hand, cellular senescence and apoptosis are thought to contribute to aging and/or certain age-related pathologies. At first glance, it may seem paradoxical that the same processes can be both beneficial (by preventing cancer) and detrimental (by promoting aging). However, the idea that genes or processes can equally promote early-life fitness and drive aging phenotypes is consistent with the evolutionary theory of antagonistic pleiotropy (discussed in Chapter 5).

Cellular senescence arrests the proliferation (used here interchangeably with "growth") of cells that are damaged or at risk for malignant transformation. Apoptosis completely eliminates such cells. Complex and partially overlapping networks of molecular interactions control these processes, and—together with the level of damage and cell type—determine whether cells undergo senescence or apoptosis in the face of stress or damage. This chapter describes the characteristics and causes of cellular senescence and apoptosis (see sections entitled Cellular Senescence—Causes and Characteristics, and Cell Death—Causes and Characteristics). It then discusses the roles these processes play in suppressing tumorigenesis and their potential roles in organismal aging (see section entitled Consequences of Cellular Senescence and Cell Death). Finally, it discusses how mouse models have been used to understand how apoptosis and cellular senescence contribute to tumor suppression and aging in vivo in mammals (see section entitled Mouse Models).

CELLULAR SENESCENCE—CAUSES AND CHARACTERISTICS

Cellular senescence arrests the growth of mitotically competent cells in response to a variety of stimuli, ranging from chromosomal damage to supraphysiological mitogenic signals. Mitotically competent cells are cells that retain the ability to divide. Examples of such cells include the basal keratinocytes of the skin, the epithelial cells that comprise much of the gastrointestinal tract, liver, and other epithelial organs, the endothelial and smooth muscle cells of the vasculature, the lymphocytes of the hematopoietic system, and the fibroblasts that produce and maintain the stroma that supports many organ structures. Postmitotic cells, by contrast, have lost their ability to proliferate as a consequence of differentiation. Examples of postmitotic cells are mature neurons and differentiated skeletal and heart muscle cells. The senescence response is limited to mitotically competent cells, causing them to lose their capacity for proliferation—essentially irreversibly—such that they now resemble postmitotic cells.

Mitotically competent cells can, and often do, exist in a reversible, growth-arrested state termed quiescence. Quiescent cells can be stimulated to proliferate by appropriate physiological signals. For example, hepatocytes (epithelial cells in the liver) are generally quiescent in vivo, but are readily stimulated to proliferate when the liver is injured or damaged (Chapter 19). The important feature that distinguishes quiescent from senescent (and postmitotic) cells is the reversibility of the growth-arrested state. There are no known physiological signals that can induce senescent or postmitotic cells to resume proliferation.

With regard to pathology, a fundamental difference between mitotically competent and postmitotic cells is their potential to give rise to cancer. Cell proliferation is essential for malignant transformation (1). Because cellular senescence irreversibly arrests cell proliferation, the senescence response is a potent tumor-suppressive mechanism.

Replicative Senescence

Cellular senescence was first formally described as the process that limits the proliferation of human fibroblasts in culture (2). Such cells often grow well when first cultured, but gradually lose the ability to divide after 40 or more population doublings (Fig. 1).

Nonetheless, the cells remain viable. They continue to metabolize RNA and protein, but arrest growth with a G1 DNA content and do not initiate DNA replication in response to physiological mitogens (3,4). This phenomenon is often termed "replicative senescence," and cells that undergo replicative senescence are said to have a finite replicative life span. Replicative senescence is now considered a specific example of a more widespread response termed "cellular senescence" (discussed below).

Many cell types from many species undergo replicative senescence—both in culture and in vivo. However, the mechanisms responsible for replicative senescence vary, depending on the cell type and species (5–10). This chapter focuses on mammalian cells, principally from rodents and humans. Nonetheless, a finite replicative life span may be an ancestral

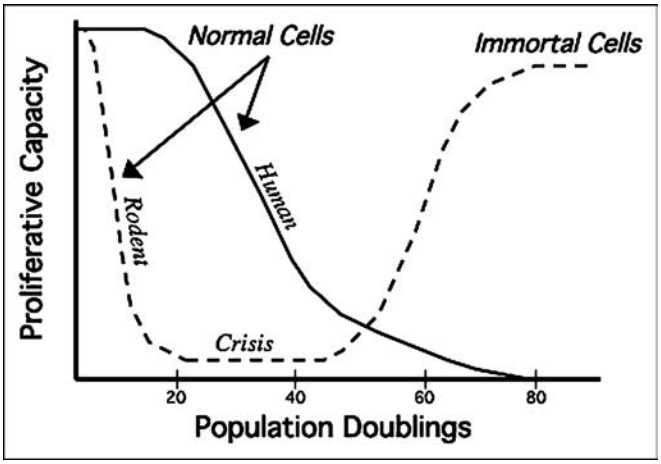

FIGURE 1 Replicative senescence and immortalization. Most normal mitotically competent cells proliferate for only a finite number of population doublings, a process known as replicative senescence. For any pool of cells, cell type, genotype, and species determine the number of population doublings at which complete replicative senescence is achieved (no remaining proliferative capacity). For example, mouse or rat fibroblast populations generally reach complete senescence after 10 to 15 doublings, whereas many human fibroblast populations proliferate for >50 population doublings. Cells from mice or rats often spontaneously immortalize (achieve replicative immortality) after a period of genomic instability termed crisis. Human cells, by contrast, rarely spontaneously immortalize.

phenotype. Even simple organisms such as single-celled yeast have only a finite capacity for cell division (11).

Replicative senescence has been studied most extensively using mammalian cells in culture. These studies have led to several general conclusions (Fig. 1) (12–16):

1. Most normal somatic cells do not divide indefinitely, although the mechanisms that limit replicative life span vary.
2. The germ line and early embryonic cells have an unlimited cell division potential (replicative immortality).
3. Most cancer cells have an unlimited cell division potential.
4. Replicative (and cellular senescence) is controlled by tumor-suppressor genes, which are inactivated by mutations or epigenetic changes in most cancer cells.
5. Replicative senescence is exceedingly stringent in human cells, which only rarely spontaneously immortalize.
6. Replicative senescence is less stringent in rodent cells, which, after a period of genomic instability termed "crisis," often become replicatively immortal.
7. Immortalization greatly increases the probability of malignant transformation.

Figure 1 illustrates the dynamic changes in proliferative capacity that cell populations undergo during replicative senescence and immortalization.

Telomere Shortening

A major cause of replicative senescence in human cells is progressive telomere shortening and eventual telomere dysfunction. Telomeres are the repetitive DNA sequences and specialized proteins at the ends of linear chromosomes (17). Vertebrate telomeres comprise the double-stranded sequence 5′TTAGGG3′, repeated a few hundred to many thousand times (depending on the species), a 3′ single stranded overhang of a few hundred bases, and a host of associated proteins that facilitate the formation of large loops, termed t-loops, that cap the chromosome ends (18,19). Telomeres protect chromosome ends from degradation or fusion by cellular DNA repair processes. Thus, they prevent loss of genetic information and genomic instability, which can result in cell death or malignant transformation.

Owing to the biochemistry of DNA replication, the 3′ ends of duplex DNA molecules cannot be completely replicated. This end-replication problem causes the loss of 50 to 200 bp from each chromosome 3′ end during every S phase (20). Thus, telomeres shorten progressively with each cell cycle (Fig. 2).

When critically short, telomeres may fail to form a proper t-loop and become uncapped. Dysfunctional, or perhaps near-dysfunctional, telomeres trigger the senescence response. Thus, telomere shortening limits the number of cell divisions—that is, causes replicative senescence. The senescence response prevents further cell division, thereby avoiding the risk of genomic instability and malignant transformation.

How do short dysfunctional telomeres trigger a senescence response? Although there are many gaps in our knowledge, recent results show that dysfunctional telomeres trigger senescence because they induce a classic DNA damage response (21,22). The responses to both dysfunctional telomeres and nontelomeric DNA damage require activation of the p53 tumor-suppressor protein, which arrests cell proliferation either transiently or permanently (senescence), depending on the level of damage. Uncapped, dysfunctional telomeres are thought to resemble DNA double-strand breaks.

Telomeres shorten as many human cell types proliferate in culture (23–26). Telomeres also shorten with age and as a function of proliferative history in vivo (26–28). These findings have led to the hypothesis that telomeres are determinants of longevity. However, there is no correlation between longevity and telomere length per se. For example, laboratory mice have long telomeres (30 to >50 kb) compared to humans (15–20 kb) (29). Yet, mouse cells generally have a shorter replicative life span in culture, and, of course, mice are shorter lived than humans. Mouse cells undergo replicative senescence in culture due to mechanisms other than telomere shortening. These mechanisms are incompletely understood, but include stressful culture conditions, such as the ambient and supraphysiological oxygen in which mammalian cells are typically cultured (5,8,16). Thus, replicative and cellular senescence can be caused by many stimuli, of which dysfunctional telomeres are but one.

Germ Line and Stem Cells

How do the germ line and early embryonic cells avoid the end-replication problem? These cells express the enzyme telomerase, a specialized reverse transcriptase that can add the repetitive telomeric sequence to telomeres de novo (17). In the presence of optimal telomerase activity, telomere lengths remain stable during cell proliferation. Thus, the germ line and early embryonic cells can proliferate indefinitely without triggering telomere-dependent replicative senescence (Fig. 2).

During embryonic and postnatal development, telomerase expression is maintained in the testes, where sperm are continually produced; however, most cells of other tissues repress the enzyme by midgestation (17,29,30). Telomerase may be completely repressed in these somatic tissues, or expressed at very low levels (31). In addition, some adult tissues contain telomerase-positive stem cells (32–34).

Many adult tissues contain unipotent or pluripotent stem cells, which can differentiate into one or multiple cell types respectively (33,35,36). Several lines of evidence suggest that either the pool of adult stem cells or their differentiation

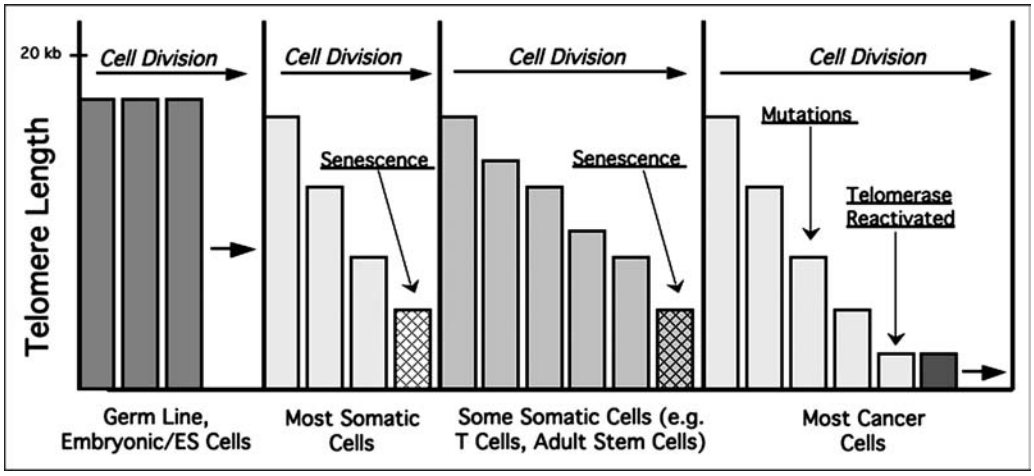

FIGURE 2 Telomerase and telomere-length dynamics. The germ line and early embryonic cells express telomerase. Thus, telomeres are maintained at stable lengths (generally 10–20 kb in humans) as these cells divide. By contrast, most somatic cells do not express telomerase, and telomeres therefore shorten with each cell division. When telomeres shorten sufficiently to malfunction, cells undergo a senescence arrest (replicative senescence). Some somatic cells (e.g., T-cells, some adult stem cells) express telomerase, but the activity is insufficient to completely prevent telomere shortening. In these cells, telomeres shorten, but more gradually than in telomerase-negative cells, and eventually the cells senesce. Most cancer cells harbor mutations that allow them to ignore senescence-causing signals. As their telomeres shorten, there is strong selection for mutations that reactivate telomerase or stabilize chromosome ends by other means.

capacity declines with age, and that this decline contributes to the loss of function and repair seen in many aging tissues (36). Adult stem cells may account for the low telomerase activity seen in some adult tissues (34,37). If so, why does telomerase not permit unlimited renewal of stem cells during the adult life span?

First, telomerase may indicate the presence of more differentiated cells that undergo limited expansion in vivo and express suboptimal levels of telomerase. For example, human T-cells transiently express telomerase when activated by antigen, but telomerase activity is apparently suboptimal because telomeres shorten, albeit slowly, and the cells eventually undergo replicative senescence (Fig. 2) (38–40). In other tissues, short telomeres, even in the presence of telomerase, can limit renewal capacity, presumably because telomerase is not expressed at levels high enough to completely prevent telomere dysfunction (41).

Second, the low levels of telomerase detected in some adult human tissues might derive from premalignant or malignant cancer cells (34), which, as discussed below, frequently express telomerase.

Third, in at least some tissues, stem cell function, not number, limits regeneration and repair (42) (Chapter 25). This is apparent even in mice, which, compared to humans, have longer telomeres and less stringent repression of telomerase in somatic tissues (29).

Cancer Cells

Cancer cells must overcome the end-replication problem in order to develop a malignant tumor (1). They do so most often by expressing telomerase (43). Although some cancer cells use an alternative, recombination-based mechanism (44), most malignant tumors are telomerase positive. Many tumor cells harbor mutations that inactivate genes required for the senescence response (senescence bypass mutations) (13,14). Thus, many tumor cells proliferate despite short dysfunctional telomeres, eventually turning on telomerase to stabilize them (Fig. 2). If telomerase is inhibited, either by drugs or by genetic manipulation, many cancer cells lose telomeric DNA as they divide, and eventually undergo senescence or, more often, apoptosis (45). Thus, long-lived organisms may repress telomerase as a strategy to prevent or retard the development of cancer.

■ Cellular Senescence

Dysfunctional telomeres are among a growing list of signals that induce a senescence response. These signals include non-telomeric damage DNA, chromatin perturbations, the expression of certain oncogenes, and strong mitogenic signals (Fig. 3).

Cells arrest growth with a phenotype essentially indistinguishable from replicatively senescent cells when they experience moderately high levels of DNA damage, caused, for example, by ionizing radiation or oxidants (46,47) (Chapter 5). Likewise, they become senescent when treated with agents, or suffer mutations, that disturb normal chromatin organization (48–52). Cells also become senescent when they experience strong mitogenic or stress signals, especially those delivered by certain oncogenes. Examples include the overexpression or activation of growth factor–signaling molecules such as RAS or RAF or growth-promoting transcription factors such as E2F (53–56).

All these inducers of cellular senescence have the potential to cause or facilitate the development of cancer. Thus, DNA damage, chromatin perturbations, and the expression of oncogenes do not transform normal cells; rather, they cause cells to arrest growth with a senescent phenotype. Thus, cellular senescence very likely evolved as a failsafe mechanism to ensure that potentially oncogenic cells irreversibly withdraw from the cell cycle, and thus become incapable of tumorigenesis.

■ Role of the p53 and p16/pRB Tumor-Suppressor Pathways

Agents such as ionizing radiation or activated oncogenes can, of course, transform cells. However, they do so only after cells acquire mutations that allow them to bypass the senescence response. Senescence-bypassing mutations tend to inactivate the

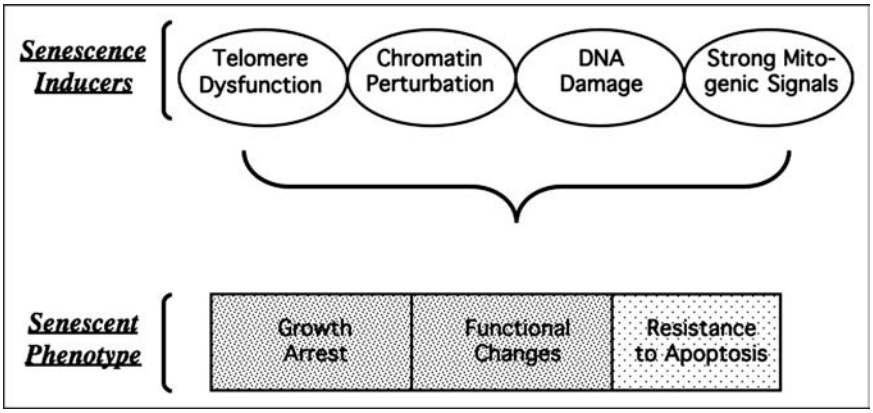

FIGURE 3 Inducers and characteristics of cellular senescence. Many stimuli, all of which have the potential to cause or contribute to cancer, induce cellular senescence. These inducers fall into the broad categories of disrupters of telomere function, disrupters of chromatin structure, DNA-damaging agents, and agents that deliver strong mitogenic or stress signals. The resulting senescent phenotype has two invariant features: an essentially irreversible growth arrest and functional changes, often the result of increased expression of genes that encode secreted factors that can alter the tissue microenvironment. In addition, some cells acquire resistance to signals that cause apoptotic cell death.

functions of genes encoding components of the p53 or pRB tumor-suppressor pathways (13,14). p53 and pRB are multifunctional nuclear proteins that affect the expression of many cellular genes, and the pathways they govern comprise numerous upstream regulators and downstream effectors. Indeed, overexpression of p21, a downstream effector of p53, or p16, a tumor suppressor in its own right and positive regulator of pRB, is sufficient to cause a senescence arrest growth (57). p21 and p16 inhibit cyclin-dependent kinases, among other activities, and thus ultimately halt cell cycle progression by preventing the inactivation of pRB by phosphorylation (58).

Despite converging on pRB phosphorylation, there are differences in how the p53 and pRB pathways induce senescence. The p53 pathway responds primarily to DNA damage, although it also responds to nongenotoxic stress, and halts cell cycle progression primarily by directly inducing p21 expression (59,60). p16 and activation of the pRB pathway, however, are induced primarily by nongenotoxic stress, the nature of which remains poorly understood (61). This pathway halts cell cycle progression by repressing growth-promoting genes.

Some cells express little or no p16, regardless of the stress (9,62). Such cells senesce via the p53 pathway only. Remarkably, although they do not respond to physiological mitogens, they resume proliferation if p53 is subsequently inactivated (9). Because these cells senesced owing to severe or irreparable DNA damage (e.g., dysfunctional telomeres), they harbor mutations and become genomically unstable upon resuming growth. In contrast, other cells readily express p16 (9,62), including as a delayed response to genotoxic stress (63). However, such cells do not resume proliferation even if p16, pRB, or p53 are subsequently inactivated (9). This stringent p16-mediated growth arrest likely derives from the ability of active (unphosphorylated) pRB to establish repressive chromatin, which does not require pRB for maintenance (64). Thus, the p53 and p16/pRB pathways both establish and maintain the senescence growth arrest, but the p16/pRB pathway provides a dominant barrier to growth resumption. Spontaneous p16 expression in the absence of DNA damage or dysfunctional telomeres can contribute to the loss of proliferative capacity both in culture and in vivo (9,62,65–68).

■ The Senescent Phenotype

What happens when cells become senescent? Cellular senescence entails many changes in gene expression, only some of which are important for the growth arrest. In general, senescent cells appear to acquire two, in some cases three, characteristics that, together, define the senescent phenotype (Fig. 3):

1. An essentially irreversible arrest of cell proliferation
2. In some cell types, resistance to signals that cause apoptotic cell death
3. Changes in differentiated cell functions

Growth Arrest

The most striking feature of senescent cells is their essentially permanent withdrawal from the cell cycle. This growth arrest is due to the repression of genes that are essential for cell cycle progression, and the overexpression of genes that inhibit cell cycle progression (69,70). The latter (overexpression of cell cycle inhibitors) is responsible for the fact that the senescence growth arrest is dominant—that is, when a senescent cell is fused to a proliferating cell, the proliferating cell arrests growth (71).

Resistance to Apoptosis

Some cell types—for example, human fibroblasts, T-cells, and mammary epithelial cells—become resistant to signals that trigger apoptosis when induced to undergo cellular senescence (72,73). As discussed below, apoptosis is a response to physiological signals, stress, and damage, which eliminates superfluous, dysfunctional, or potentially malignant cells in a controlled manner. Thus, some cells, upon becoming senescent, also become resistant to stimuli that would normally eliminate them. This attribute of senescent cells may explain why they persist in vivo and accumulate with age (74–80).

Functional Changes

Cellular senescence entails a host of selected changes in differentiated functions. Some of these changes are general and common to most, if not all, cell types. These include

1. an enlarged cell size,
2. increased lysosome biogenesis,
3. decreased rates of protein synthesis and degradation,
4. frequent lobulated nuclear morphology (3,81,82), and
5. expression of a senescence-associated β-galactosidase (74).

This activity derives from the acidic, lysosomal β-galactosidase, but can be detected at neutral pH in situ in cultured cells and in vivo (74,83).

Senescent cells also show changes in the expression of cell type–specific genes. For example, senescent human adrenocortical epithelial cells selectively lose the ability to induce 17α-hydroxylase, a key enzyme in cortisol biosynthesis (84), whereas senescent dermal fibroblasts increase the expression of collagenase [matrix metalloproteinase-1 (MMP-1)] and stromelysin (MMP-3), which degrade extracellular matrix proteins (85,86). Likewise, senescent human endothelial cells upregulate interleukin-1a, a proinflammatory cytokine (87), and downregulate expression of thymosin-b-10, which sequesters cytoplasmic G-actin (77). Interestingly, endothelial cells that simultaneously express senescence-associated β-galactosidase and lack thymosin-b-10 are found at the base of atherosclerotic lesions (Chapter 15), implicating senescent endothelial cells in atherogenesis (77).

Many genes that senescent cells overexpress encode secreted proteins (88–90). Further, senescence-associated secreted factors can have marked effects on neighboring cells and the local tissue microenvironment. For example, senescent fibroblasts inhibit both alveolar and ductal differentiation of mammary epithelial cells at least in part due to their secretion of MMP-3 (91,92). Thus, senescent cells can disrupt local tissue integrity and function, which is consistent with the idea that the senescence response is antagonistically pleiotropic (88).

Senescent cells accumulate with age and at sites of age-related pathology. It is therefore possible that the presence of senescence cells, and particularly their secretory phenotype, might cause or facilitate the development of aging phenotypes and age-related disease.

■ CELL DEATH—CAUSES AND CHARACTERISTICS

Cells can die by either of two fundamentally different mechanisms: One is apoptosis or programmed cell death, which entails controlled reactions that prevent the dispersion of cellular contents into the surrounding tissue. The other is necrosis, which entails cell lysis into the surrounding tissue. Both modes of cell death may contribute to age-related pathologies.

■ Apoptosis

Apoptosis is a highly orchestrated, genetically programmed process that allows cells to die in a controlled fashion (93,94). All cells have the intracellular machinery necessary for programmed cell death. Whether they use that machinery depends on the cell type, the tissue context, the presence or absence of physiological signals, and the extent to which a cell is damaged or dysfunctional. Like cellular senescence, apoptosis is a double-edged sword. Too little can cause damaged or dysfunctional cells to accumulate, whereas too much can cause tissue atrophy and degeneration.

Functions

During embryogenesis, apoptosis eliminates damaged or dysfunctional germ cells, superficial cells during tissue morphogenesis, and cells that fail to make the proper functional connections with neighboring cells (95). The key features of apoptosis, and its major regulatory and effector molecules, have been conserved throughout metazoan evolution. However, the number and complexity of proteins and reactions that regulate and execute apoptosis have increased greatly during vertebrate evolution (96). Further, in complex organisms, apoptosis is important for maintaining tissue homeostasis, and for eliminating dysfunctional, damaged, and/or potentially cancerous cells throughout life (93,97).

An important function of apoptosis is to provide a mechanism for eliminating unwanted cells without cell lysis. This is important because cell lysis frequently results in local tissue destruction, owing to the release of degradative enzymes from the lysed cells and the inflammation reactions that it frequently elicits. Thus, apoptosis allows organisms to remove damaged or dysfunctional cells with minimal collateral damage to the tissue. In postmitotic tissues, where cell proliferation cannot replace lost cells, apoptosis minimizes the cell loss. In renewable tissues, where cell proliferation can replace cells that are lost, apoptosis is an important mechanism for maintaining the size, integrity, and health of the tissue.

Salient Features

Apoptosis can be initiated by physiological signals acting through specific receptors, or by damage to the nucleus, mitochondria, or other organelles (Fig. 4) (94).

Once initiated, apoptosis entails a series of biochemical steps that culminate in the controlled destruction of nuclear DNA and cross-linking of cellular constituents (98,99). These steps proceed in distinct phases: activation, commitment, and execution. Once cells enter the commitment phase, the execution phase and demise of the cell is inevitable. A prominent feature of all phases is a cascade in which a series of proteases, termed caspases, are sequentially activated by site-specific proteolysis (100). Caspases activated in the last steps of the cascade then specifically cleave cellular proteins. Most proteins that are cleaved by caspases are inactivated. Some proteins, however, are activated by caspase cleavage. A prominent feature of apoptosis is the intimate involvement of mitochondria (101,102). Mitochondria sense apoptotic signals by mechanisms that are only partially understood, and respond by releasing cytochrome c into the cytosol. These mitochondrially derived molecules then initiate or reinforce signals that activate caspase cascades. The net result includes the activation of an endonuclease, inhibition of DNA repair, and activation of a cross-linking transglutaminase. During the last stages, cells shrink and disintegrate into small, cross-linked fragments, which are engulfed and degraded by macrophages or neighboring cells (Fig. 4).

Apoptosis is controlled by more than 100 proteins, which act in complex and interrelated pathways that are linked to other cellular process, such as proliferation and differentiation. It is beyond the scope of this chapter to provide a comprehensive description of the regulation and execution of apoptosis. However, the salient features of apoptosis are shown in Figure 4.

Physiological Signals

Apoptosis can be induced by a variety of physiological (as opposed to damage) signals (94). In healthy tissues and organisms, these signals serve to remove unnecessary or dysfunctional cells. They also serve to attenuate or reverse proliferative responses, such as the activation-induced cell death of T-lymphocytes, which terminates immune responses.

Apoptosis can be initiated by ligand–receptor interactions at the plasma membrane (94). These receptors then transduce the signal via specialized proteins at the cytoplasmic surface of the plasma membrane (Fig. 4). Although several apoptotic

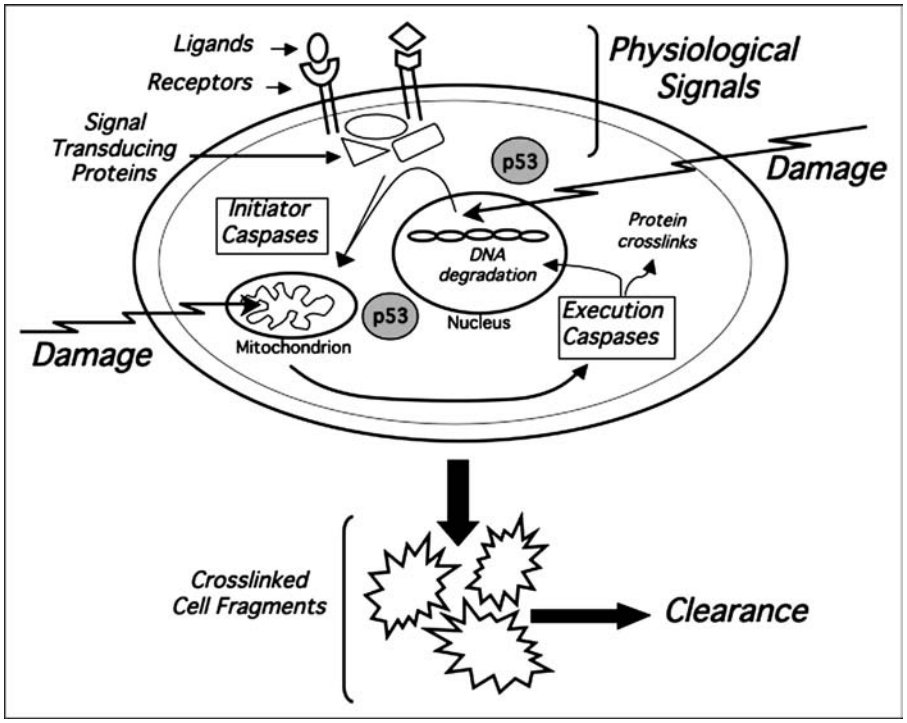

FIGURE 4 Major pathways and steps in apoptosis. Apoptosis can be initiated by physiological signals, such as the binding of ligands to their death-promoting receptors, or by endogenously or exogenously induced damage to the nucleus, mitochondria, or other cellular components. The initial stages of apoptosis entail the sequential activation of initiator caspases, which results in the release of mitochondrial signals such as cytochrome c. Mitochondrial signals and upstream caspases activate execution caspases, which cleave cellular proteins, destroying some and activating others. Subsequently, an endonuclease degrades the nuclear DNA, and a transglutaminase cross links cellular proteins. Ultimately, the cell disintegrates into small cross-linked fragments that are cleared from the tissues by macrophages or neighboring cells, which engulf and degrade them.

ligands are active in the immune system (e.g., FAS ligand, tumor necrosis factor, interferons) (93), many hormones, cytokines, and other physiological regulators can induce apoptosis outside the immune system, depending on the cell type, tissue, and physiological context (99,103). For example, transforming growth factor-β, a multifunctional cytokine, causes growth inhibition, extracellular matrix production, or apoptosis, depending on the cell type and microenvironment. In addition, some cytokines, hormones, and growth factors deliver antiapoptotic signals and are required to prevent cell death. For example, nerve growth factor deficiency causes certain neurons to die by apoptosis. Similarly, T-lymphocytes deprived of certain interleukins (cytokines that act in the immune system) die by apoptosis.

Physiological mediators of apoptosis are not limited to factors that interact with plasma membrane receptors. Some hormones that act via nuclear receptors also stimulate or protect cells from apoptosis (103). For example, glucocorticoids promote apoptosis in lymphocytes (104), whereas testosterone protects germ cells in the testis from undergoing apoptosis (105) and estrogen protects certain neurons from dying by apoptosis (106).

Aging entails many changes in the levels or availability of hormones, cytokines, and growth factors—the physiological mediators of apoptosis in adult tissues. It is therefore not surprising that aging also entails changes in the regulation of apoptosis in certain tissues.

Damage Signals

Apoptosis can also be induced by cellular damage, particularly to DNA. Apoptosis-inducing damage can originate from endogenous sources (e.g., reactive oxygen species produced by mitochondria), or exogenous sources (e.g., ionizing radiation or chemicals). Upon damage, cells mount an appropriate damage response, which entails four options:

1. A transient arrest of proliferation (generally in the G1 or G2 phase of the cell cycle) in order to repair the damage
2. A permanent growth arrest (cellular senescence)
3. Death by apoptosis
4. Death by other processes such as necrosis (discussed below)

What determines which options a cell chooses? Important considerations are the cell type and physiological context, as well as the level and type of damage. Thus, a given dose of ionizing radiation typically causes senescence in human fibroblasts, but apoptosis in T-lymphocytes. Likewise, a higher dose of radiation may cause human fibroblasts to undergo apoptosis, and an even higher dose may cause the same cells to die by necrosis.

Damage that causes apoptosis eventually triggers the release of cytochrome c from mitochondria. It is still not clear how damage, for example to nuclear DNA, signals to the mitochondria. One possible mediator is the p53 tumor suppressor. p53 is rapidly activated by phosphorylation after DNA damage (60). Activated p53, in turn, can stimulate the transcription of genes such as *PUMA* and *NOXA*, which act at the mitochondria to increase permeability (107). In addition, damage-activated p53 can itself translocate to the mitochondria where it interacts with other proteins to facilitate mitochondrial permeability (108,109).

Role of p53

p53 is crucial for three of the four damage-response options: the transient growth arrest, senescent growth arrest, and apoptosis (59,93,108,109). p53-dependent apoptosis is interesting because it is not crucial for embryonic development. Genetically engineered mice that completely lack p53 develop normally (110). However, these mice die prematurely of cancer. Thus, p53 function is more important in adult, compared to developing, mammals. One of its most important functions is to promote apoptosis, which suppresses the development of cancer (111). p53 may be more important for damage-induced apoptosis, compared to apoptosis induced by physiological signals. Consistent with this idea, cells can also undergo p53-independent apoptosis (96,99).

■ Other Cell Death Mechanisms

Necrosis is an alternate mode of cell death, which differs from apoptosis in significant ways. Necrosis is characterized by swelling of the cell and its organelles, including the mitochondria. Subsequently, cell membranes lose their integrity, and cellular constituents, including many degradative enzymes, leak into the surrounding tissue. This leakage often damages contiguous cells, and causes local exudative inflammation. Thus, unlike apoptosis, necrotic cell death is chaotic, and generally detrimental to the tissues in which it occurs (94,101).

Necrosis is generally induced by highly noxious stimuli, such as cytotoxins, hyperthermia, hypoxia, metabolic poisons, and direct cell or tissue trauma. In some pathological conditions—ischemic injury, for example—both apoptotic and necrotic cell death may occur. Cells are thought to die by necrosis, rather than apoptosis, when they experience more severe insults. In addition, cells may die by necrosis when one or more components of an apoptotic pathway are inactivated, which can occur as a consequence of mutation or physiological processes. For example, senescent human fibroblasts may become resistant to apoptosis-inducing signals because they cannot stabilize p53 in response to damage (73). As a result, senescent cells die by necrosis, rather than apoptosis. Necrosis, and the accompanying inflammation reactions, may increase during aging owing to age-related defects in the cellular machinery that controls and/or carries out apoptosis.

Recently, cell death pathways with both unique characteristics and characteristics of both apoptosis and necrosis have been described (112). These alternative cell death modes are generally distinguishable by their morphological characteristics, differential sensitivities to pharmacologic agents, and/or biochemical reactions. At present, little is known about how these alternative pathways impact normal or pathological physiology. However, some of them are prominent in age-related neurodegenerative diseases (112).

■ CONSEQUENCES OF CELLULAR SENESCENCE AND CELL DEATH

Cellular senescence and cell death may play a role in aging or age-related pathology by two distinct means. First, because both processes are important for suppressing cancer, they are longevity-assurance mechanisms: mechanisms that postpone the onset of aging phenotypes or age-related disease; in this case, cancer. On the other hand, as dysfunctional senescent cells accumulate in renewable tissues, and apoptosis depletes cells from postmitotic tissues, both processes may contribute to aging phenotypes. This apparent paradox—the ability to be beneficial early in life, but detrimental late in life—is consistent with the evolutionary theory of antagonistic pleiotropy, as discussed earlier in Chapter 5. What is the evidence that cellular senescence and cell death both suppress cancer and contribute to aging?

■ Cancer

There is now very persuasive evidence that both cellular senescence and apoptosis are crucial for preventing the development of cancer (14,94,97,113–115), which is a major age-related disease (111,116).

First, most mammalian cancers harbor mutations that blunt or inactivate the senescence response and/or render them resistant to cell death (1). In addition, several well-recognized oncogenes, such as those encoded by oncogenic papillomaviruses, act primarily by blocking senescence and/or apoptotic responses (117). Conversely, well-recognized tumor suppressors, such as p53, control cellular senescence and apoptosis. These tumor suppressors, or one of their regulators or effectors, are inactivated in the vast majority of cancers (118,119).

Second, genetically engineered mouse models have been developed in which cells fail to undergo cellular senescence or apoptosis in response to specific stimuli (111,120–122). These mice inevitably develop tumors at an early age, and generally die of cancer. Human clinical data likewise suggest that apoptosis and/or senescence restrains malignant tumorigenesis (93,113,118). For example, human naevi are benign tumors of melanocytes that frequently harbor oncogenic mutations. Naevi, but not malignant melanomas, also frequently contain high levels of senescent cells (123). In addition, DNA-damaging chemotherapy can cause tumor cells to undergo apoptotic or senescence responses that, like the response of normal cells, are controlled by the p53 or p16/pRB pathways. Tumors that fail to respond are much more likely to be lethal (124).

Together, these findings strongly suggest that cellular senescence and apoptosis are powerful tumor-suppressive mechanisms. Nonetheless, mammals do develop cancer, although generally late in life. Thus, senescence and apoptosis are effective at preventing cancer in relatively young organisms, but less effective in older organisms. Why might this be the case?

Mutations are essential for the development of cancer (1), and it takes time for cells to accumulate the mutations that allow them to avoid senescence and/or apoptosis. Mutations accumulate throughout the mammalian life span (125,126). However, the exponential rise in cancer that occurs during the last half of the life span (111,116) cannot be explained by mutations alone. Cancer also requires a permissive tissue in which to develop; in many cases, a healthy tissue environment can suppress the development of cancer from even highly mutated cells (111,127–129). Aging entails changes in tissue structure and integrity, which may create a permissive environment for the growth and malignant transformation of mutant cells.

■ Aging

How do cellular senescence and cell death contribute to aging? At present, there is no precise answer to this question. There is, however, mounting indirect evidence that both processes can and do contribute to aging phenotypes, both directly and indirectly.

Cellular Senescence

As discussed above, senescent cells acquire altered functions, including increased secretion of molecules that can erode the structure and integrity of tissues if chronically present. In the case of stromal fibroblasts, for example, these secreted

molecules include proteases, inflammatory cytokines, and growth factors (88,130). Thus, senescent cells secrete molecules that can directly (by protease action) and indirectly (by stimulating inflammation) disrupt tissue structure. They also secrete growth factors that can disrupt proliferative homeostasis, and even stimulate the growth of mutant preneoplastic or neoplastic cells (69,131,132).

There is some evidence for this scenario. Cells that express senescent markers increase with age in several mammalian tissues (74–76,080,133,134). Moreover, such cells have been found at sites of age-related pathologies, such as benign prostatic hyperplasia, atherosclerosis, osteoarthritis, and kidney fibrosis (77–79,135). It is possible, then, that the factors secreted by senescent cells can cause or contribute to age-related pathologies. Because senescent cells not only disrupt tissue architecture but also secrete growth factors, it is possible that they also contribute to the development of late-life cancer (69,131,136,137)—an irony, but one that is consistent with antagonistic pleiotropy.

Finally, recent evidence suggests that adult stem cells in brain, bone marrow, and pancreas of mice undergo a p16-mediated growth arrest with increasing frequency as the animals age (65–67). This growth arrest, which has hallmarks of senescence, appears to be responsible for the age-associated declines in neurogenesis and hematopoiesis and development of diabetes. Thus, cellular senescence may also contribute to aging by preventing the proliferation of stem or progenitor cells. These findings further suggest that there has been an evolutionary trade-off between tissue regeneration and protection from cancer (138,139).

Cell Death

The most obvious potentially detrimental effects of cell death are in postmitotic tissues, where cells are not readily replaced. Some postmitotic tissues such as certain regions of the brain or skeletal muscle can be replenished by recruiting stem or progenitor cells. However, such cells seem to have a finite replicative life span that eventually is exhausted, whether due to telomere dysfunction, stress-induced p16 expression, or other mechanisms (65–67, 140–142). Moreover, the tissue or systemic milieu of aged organisms compromises the recruitment and/or function of adult stem cells (42). Thus, eventually, cell death depletes postmitotic tissues of cells. Apoptosis, necrosis, and other forms of cell death have been observed in several age-related pathologies, such as sarcopenia and Alzheimer's disease (99,112,143).

Both apoptosis and necrosis may increase with age, owing to increased levels of oxidative or other forms of damage. Apoptosis may benefit young organisms by efficiently eliminating dysfunctional or damaged cells. As organisms age, however, cellular reserves may become exhausted, such that the loss of cells leads to tissue atrophy or degeneration.

In contrast to postmitotic tissues, there is evidence that apoptosis may become less efficient with age in some mitotically competent mammalian tissues (144). The mechanism responsible for this change is unknown. Nonetheless, it is likely to increase the level of damaged or dysfunctional cells during aging. Interestingly, caloric restriction, which extends the life span of many species (145), increases the basal rate of apoptosis in some rodent tissues. This increase may in part explain why caloric restriction delays most age-related pathologies, including cancer (144).

■ MOUSE MODELS

A crucial test for the proposed roles of cellular senescence and apoptosis in aging and age-related disease is to manipulate these processes in intact organisms. At present, this approach is practical only in mice. Mice are now fairly easy to manipulate genetically, and their small size and relatively short life span make them the mammalian model of choice for aging studies. There is, however, always the caveat that mice are not humans, and there are significant differences between mouse and human cells in processes that are likely important in aging (e.g., telomere biology and oxidative stress resistance) (8,16). With that caveat in mind, what do mouse models tell us about the roles of cellular senescence and cell death in aging? There are numerous mouse models of accelerated or retarded aging, and it is beyond the scope of this chapter to review them all. Therefore the discussion below focuses on a sampling of informative models, particularly those that elucidate the relationship between cell fate and organismal aging and life span. Two broad categories of mouse models for studying aging are discussed here: those that accelerate aging and those that retard aging.

■ Accelerated Aging

Mouse models of accelerated aging are more abundant than models of retarded aging. However, they are also more prone to artifacts—it is relatively easy to generate sick mice, some of which may only coincidentally show symptoms that resemble aging phenotypes (146). In addition, some mouse models have a shortened life span because they develop a single age-related disease—for example, the cancer-prone mouse models discussed earlier (111,120–122). Single disease models are not discussed here. Rather, a few models are discussed in which multiple aging phenotypes and diseases develop at an accelerated rate. None of these models precisely phenocopy normal aging, but they do show segmental premature aging—that is, the accelerated appearance of some, albeit not all, features of normal aging. These models have validated the importance of cellular processes in organismal aging, and provided insights into the physiology of aging and certain age-related disease.

Telomeres and Premature Aging

Among the first mouse models to show multiple signs of accelerated aging was the telomerase knockout mouse. These mice completely lack telomerase activity owing to a germ-line disruption in the gene encoding the essential telomerase RNA component, *mTR* (147). Interestingly, *mTR*-/- mice are asymptomatic for the first three to four generations. Subsequent generations, however, are shorter lived and prematurely develop hair loss and graying, ulcerative skin lesions, delayed wound healing, reduced fertility, reduced stress resistance, and increased cancer. As noted earlier, mice have telomeres that are much longer than humans. It is notable, then, that the reduced longevity and fitness of *mTR*-/- mice were not manifest until the mouse telomeres had substantially shortened to lengths more typical of those found in aged human tissues. Thus, this model demonstrates a critical role for telomere function not only in suppressing cancer, but also in ensuring many other aspects of organismal fitness.

The reduced-longevity and premature-aging phenotypes of *mTR*-/- mice are even more pronounced when combined with a deficiency in *WRN* (Chapter 3), the gene that is defective in the human premature-aging disorder, Werner syndrome (WS) (148). *WRN* encodes a DNA exonuclease/helicase that participates in several processes important for genomic stability, including telomere maintenance (149). Humans with WS develop multiple aging phenotypes (e.g., wrinkled skin, thin gray hair, cataracts, osteoporosis, type II diabetes, cancer)

shortly after puberty, and generally die in the fifth or sixth decade of life. By contrast, *WRN-/-* mice are largely asymptomatic. However, when crossed to *mTR-/-* mice and allowed to propagate for three to four generations, *WRN-/-mTR-/-* double mutants rapidly develop many of the symptoms seen in WS (150). In addition, cells from the double-mutant mice, like cells from WS patients, undergo rapid senescence in culture. This mouse model, then, suggests that many of the progeroid symptoms in WS are a consequence of improper telomere maintenance. Further, it supports the idea that cellular senescence can cause or contribute to some aging phenotypes and diseases.

p53 Mutant Mice

As discussed earlier, p53 is a potent tumor suppressor and important for the senescence and apoptotic responses to damage. It might be expected, then, that hyperactive p53 would confer superior protection from cancer. Further, since cancer is a major age-related pathology in mice, as it is in humans, hyperactive p53 might also increase longevity. Several mouse models have been developed to test these ideas, all with surprising results.

Two transgenic mouse models were made in which a truncated form of p53 was constitutively overexpressed. One model expressed an artificially truncated p53 protein (151), whereas the other expressed a naturally occurring isoform (152). p53 forms a tetramer, and both short p53 proteins appeared to integrate into tetrameric p53 and increase its activity. Not surprisingly, both mouse models were remarkably tumor free. Surprisingly, though, both were short lived and prematurely developed aging phenotypes including gray hair, thin skin, osteoporosis, cataracts, and diabetes. Cells from these mice were also more prone to apoptosis (151) and senescence (152). These models suggest that tumor suppression by p53, presumably acting through cellular senescence and apoptosis, can drive aging and hence is antagonistically pleiotropic.

Two additional mouse models suggest that tumor suppression by p53 might be linked to aging, albeit more subtly. One model contained an extra copy of the p53 locus (153). In contrast to models expressing short p53 isoforms, the protein encoded by the transgenic locus was not constitutively active, although it was activated normally by damage or stress. The second model expressed a hypomorphic allele of *MDM2*, which degrades p53 after it responds to damage or stress. Thus, in both mouse models, p53 was not constitutively active, but rather was hyperactive only after damage or stress. Not surprisingly, these mice also were remarkably tumor free. However, they did not age prematurely and had life spans comparable to control mice, suggesting that tumor suppression need not always come at the cost of aging. On the other hand, these mice were not long lived. Given that cancer is a major cause of death in mice, it is therefore also possible that noncancer pathologies were enhanced.

Reduced Genome Maintenance

There are now numerous mouse models in which DNA repair or genome maintenance systems have been compromised by genetic manipulation. These models include disruptions to virtually all the major DNA repair systems, as well as disruptions to genes that regulate the DNA damage response. Given the importance of mutations for cancer development, it is not surprising that many of these mice are cancer prone. However, a number of these mice also show segmental premature aging, and cells from such mice also show an increased propensity to undergo cellular senescence or apoptosis (154,155). Collectively, these mouse models suggest that genome maintenance systems are important longevity-assurance mechanisms, and that the cellular responses to damage (senescence and cell death) can contribute to aging phenotypes. But have these mouse models taught us anything beyond the general importance of genome maintenance for longevity assurance? A recent mouse model has provided an important insight.

The XPF–ERCC1 complex is a structure-specific endonuclease that is essential for repairing helix-distorting DNA lesions and interstrand cross-links. Mice carrying a severe mutation in *XPF*, similar to that found in a human patient, are extremely short lived. They die with several symptoms of accelerated aging and a high level of apoptosis in many tissues (156,157).

Gene expression profiles from the liver of these mice showed that the severe genotoxic stress caused by *XPF* deficiency increased the expression of genes that provide antioxidant defenses and decreased the expression of genes important for insulin/insulin-like growth factor-1 (IGF-1) signaling (156). The insulin/IGF-1 signaling pathway is now known to be an evolutionarily conserved pathway that regulates aging; modest decreases in the output of this pathway have been shown to increase the life span of nematodes, fruit flies, and mice (11,158). Notably, this pattern of gene expression was also seen in control mice subjected to chronic low-level genotoxic stress, calorie restriction, and normal aging (156). These findings suggest that damage and aging elicit a similar set of conserved metabolic responses: an increase in antioxidant protection and a decrease in insulin/IGF-1 signaling. These responses are similar to the effects of caloric restriction and presumably act to preserve, or attempt to preserve, somatic tissues (Chapter 23). This study also illustrates an important consideration in evaluating gene expression changes that occur during aging; although in some cases, changes in gene expression might reflect decrements in function or pathology, in other cases, as in the case of *XPF* mutant mice, they may indicate beneficial compensatory changes.

■ Delayed Aging

A large number of mutations have now been identified that extend the life span of simple model organisms such as yeast, nematodes, and fruit flies. Many cause a doubling or more of mean and maximum life span. Most can be organized into a few broad categories: mutations that blunt the insulin/IGF-1 signaling pathway, reduce mitochondrial activity and/or oxidative byproducts of energy production, increase stress resistance, and optimize genome maintenance (11,15,159,160).

Because many of these mutations act in conserved pathways, analogous mutations have been studied in mammals, principally mice. A consistent finding in mice is that, whereas some mutations do indeed extend longevity, for the most part, the life span extensions are much more modest and rarely exceed a 40% increase. It is beyond the scope of this chapter to review all the mouse models of extended longevity that have been created and studies, but a few are discussed below.

Insulin/IGF-1 Signaling

Among the most extensively studied mouse models of delayed aging are the Ames and Snell dwarf mice, which harbor mutations in transcription factors that regulate pituitary development. Such mice are among the longest lived of all the delayed-aging mouse models, showing life span increases of 50% to 70% and postponement of all major age-related pathologies. Both models are deficient from birth in several

hormones, but the most important deficiency is growth hormone, because very similar effects are achieved by germline disruption in the growth hormone receptor (161). In turn, the most important consequence of growth hormone deficiency for life-span extension is the marked reduction in IGF-1 production (158). These mice also show a reduction in insulin levels (158). Moreover, cells from these animals are resistant to multiple exogenous stresses, although it is not known whether this stress resistance is due to reduced senescence or reduced apoptosis (162).

Mice that are constitutively deficient in growth hormones and IGF-1 suffer from dwarfism, compromised fertility, and other problems (163). However, mouse models in which there is a more modest reduction in IGF-1 signaling, for example, mice in which only one copy of the IGF-1 receptor was genetically inactivated, are phenotypically normal yet long lived (164). In this case, however, life span extension was also modest (16–33%). Likewise, genetic manipulations that selectively eliminate the insulin receptor in adipose tissue increase longevity, but again only modestly (<20%) (165). Thus, blunting the insulin/IGF-1 pathway increases longevity in mice, as it does in simpler organisms, although to a significantly lesser extent.

Mitochondrial and Energy Production

A surprising number of mutations or partial gene disruptions that extend the longevity of simple organisms, especially nematodes, compromise or reduce mitochondrial function (166,167). In mammals, however, compromised mitochondrial function generally shortens life span (168,169). Moreover, in both in vivo and cell culture studies, it also increases the susceptibility to cellular senescence and apoptosis (170,171).

However, recent findings suggest that subtle perturbations to mitochondrial function and energy production can modestly extend life span in mouse models. For example, within a cohort of outbred mice, animals with relatively higher mitochondrial uncoupling produce lower levels of reactive oxygen species and are relatively longer lived (172). Likewise, a partial reduction in CKL-1, a mitochondrial protein that is important for the synthesis of the mobile electron carrier ubiquinone, modestly increases the life span of both nematodes and mice (173). Recently, a transgenic mouse model with reduced core body temperature was created by overexpressing a mitochondrial uncoupling protein in neurons that control body temperature (174). These animals presumably expended less energy, and showed a modest increase in life span (12–20%). These and other studies support the idea that mitochondrially generated oxidative stress is an important cause of aging, and that manipulations that reduce this stress promote longevity. Nonetheless, the life span extensions achieved by reducing mitochondrial oxidation reactions or energy production are small, almost certainly because mitochondria are also crucial for life in complex organisms and therefore can tolerate only minor manipulations before vital functions are compromised.

Stress Resistance

A common correlate of delayed aging is increased stress resistance (160,175). This is true for both simple and complex animals. For example, as noted earlier, cells from long-lived mice are resistant to multiple stresses (162). In addition, animals engineered to be stress resistant tend to be long lived. There are several examples of this phenomenon in simple animals, but fewer examples in mammals (160,175).

One important example in mammals is the germ-line disruption of $p66^{SHC}$ in mice (176). $p66^{SHC}$, an isoform of the signaling adaptor protein SHC, has been shown to participate in the regulation of mitochondrial membrane potential, thereby indirectly controlling the level of cellular oxidative stress (177). Cells from mice that lack $p66^{SHC}$ expression are resistant to oxidative stress, evident as a marked reduction in the level of apoptosis. Moreover, the mice themselves live about 30% longer than wild-type control mice (176). Future research will very likely create additional mouse models in which stress-protective proteins such as chaperones are overexpressed to test the idea that stress resistance and longevity are as directly linked in mammals as they are in simple model organisms.

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5

Theories of Life Span and Aging

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INTRODUCTION

The gerontologist Sacher (1) noted that there are different theoretical frameworks within which biological research on aging can be considered, each of which is associated with what he termed a "primitive" question. The best known is the conventional aging-oriented approach designed to address the question "Why do we age?" Sacher notes that this is an ontogenetic issue and the attack on it is guided by a research paradigm concerned with molecular, genetic, and physiological processes. The basic experimental approach to this question involves the comparison of specific functions or structures in old and young animals such as mice. However, he observed that this far-reaching correspondence between laboratory rodents and humans concealed a paradox: if these two species are so similar in molecular, cellular, and physiological makeup, the data on the ontogeny of aging in rodents brings us no closer to understanding why a rodent grows as old in two years as humans do in 70 years. Thus, the second approach to aging research flows from this paradox and is designed to address the longevity-oriented question: "Why do we live as long as we do?" This question cannot be answered within the framework of ontogenetic research on aging but rather requires the development of an evolutionary-comparative paradigm. But then, a third approach to aging research is death oriented and is designed to answer the question "Why do we die?" which is a problem separate from aging and longevity; there is no necessary relation between aging and dying. None of these questions by themselves adequately frames the field of aging research because, although there is obvious overlap, they each possess different conceptual centers—evolutionary (life span), physiological (aging), and death (stochastic).

Distinguishing between longevity- and aging-oriented paradigms is important for several reasons. First, longevity and aging are fundamentally different concepts and, like the mouse aging and life span example, neither concept can be completely understood without also considering it in the context of the other. Second, unlike the evolutionary theory of senescence that is based solely on individual natural selection, these theories include processes of sexual selection and kin selection, bringing life history theory more fully to bear on questions concerned with the latter portion of the life cycle. Third, extending the scope of aging-related theory allows consideration of behaviors that are characteristic of both younger and older individuals including divisions of labor and intergenerational transfers. Because mortality factors unrelated to aging (accidents, acute diseases, and socioeconomic factors) can be included in a more general conceptual framework, approaches that extend beyond conventional research strategies can be employed to understand how and why people live as long as they do, why they age, and why they die.

We have organized this chapter around two of the concepts that constitute "life's finitude" as originally described by Sacher (1). The first section is on the theory of longevity and includes both conceptual and empirical background information. The second section is on the theories of aging; it includes the theory on why all eukaryotes senesce and die followed by an overview of the main theories of aging at molecular, cellular, systemic, and evolutionary levels. We end with a brief conclusion on the importance of theory in aging science.

THEORY OF LIFE SPAN

Life span is an evolved life history characteristic of an organism that refers to the duration of its entire life course (Chapter 1). Application of the concept is straightforward at both individual and cohort levels and specifies the period between birth and death for the former (individual) and the average length of life or life expectancy at birth for the latter (including both real and synthetic cohorts) (Chapter 2). However, life span applied to a population or a species requires a modifier to avoid ambiguity (2). Maximum observed life span is the highest verified age at death, possibly limited to a particular population or time period. The overall highest verified age for a species is also called its record life span. The theoretical highest attainable age is known as maximum potential life span, maximum theoretical life span, or species-specific life span. Depending on the context, maximum life span can refer to either the observed or the potential maximum (Chapter 2).

Conceptual Aspects of Life Span

The life span concept is relevant only to species in which an individual exits—an entity circumscribed by distinct birth and death processes (3). Thus, the concept does not apply to bacteria that reproduce by binary fission, to plant species that reproduce by cloning, or to modular organisms with iterated (or repetitive) growth such as coral or honeybee colonies. When a single reproductive event occurs at the end of the life course that results in the death of the individual, then life span is linked deterministically to the species' natural history. This occurs with the seed set of annual plants (grasses), in drone (male) honeybees as a consequence of the mechanical damage caused by mating, in many mayfly species when a female's abdomen ruptures to release her eggs after she drops into a lake or stream, and in the anadromous (i.e., ascending the river from the sea for breeding) salmon that dies shortly after spawning. Life span can be considered indeterminate for species (including humans)

that are capable of repeated reproduction (iteroparous). That life span is indeterminate in many species is consistent with everything that is known about the lack of cut-off points in biology—all evidence suggests that some species do not have an internal clock for terminating life (Chapter 3).

Changes that occur in organisms that enter resting states such as dormancy, hibernation, and aestivation reduce mortality rates and thus increase longevity. This also occurs when individuals are subjected to caloric restriction (CR) or when their reproductive efforts are reduced (Chapter 23). A species' life course may consist of many phases such as infant, juvenile, and pre- and postreproductive period and, therefore, a change in overall life span will correspond to a commensurate change in the duration of one or more of the stages. When environmental conditions are drastically improved such as for animals kept in zoos or laboratories or for contemporary humans, mortality rates usually decrease and, thus, longevity increases. Whereas earlier stages such as prereproductive periods are evolved life history traits, the added segment(s) arising at the end of the life course are byproducts of selection for robustness or durability at earlier stages and are thus not evolved traits, per se (4). Rather, these additional segments are due to "ecological release" and referred to as "post-Darwinian" age classes.

■ Comparative Demography of Life Span

The literature contains descriptions of only a small number of life span correlates, including the well known relationship between life span and both body mass and relative brain size (1,5–8), and the observation that animals that possess armor (e.g., beetles, turtles) or capability of flight (e.g., birds, bats) are often long lived (9). But, major inconsistencies exist within even this small set of correlates. For example, there are several exceptions regarding the relationship of extended longevity and large body size (e.g., bats are generally small but most of their species are long lived) and this positive relationship may be either absent or reversed within orders. Likewise, the observation that flight ability and extended longevity are correlated does not provide any insight into why within-group (e.g., birds) differences in life span exist, nor does it account for the variation in longevity in insects where adults of the majority of species can fly.

An alternative approach for identifying broad correlates of longevity emerged from an examination of several large-scale databases containing the maximum recorded life spans of both vertebrate and invertebrate species (10–12). Many long-lived species across a wide taxonomic spectrum appear to cluster within one of two general ecology and/or life history criteria:

1. Species that live either in unpredictable environments (e.g., deserts) or where food resources are scarce (e.g., caves; deep water, etc.)
2. Species that exhibit extended parental care and/or live in groups with complex or advanced social behavior (Chapter 2)

These criteria led to a classification system regarding the life span determinants of species with extended longevity (Table 1) that we believe is general and applies to a wide range of invertebrate and vertebrate species:

1. *Environmentally selected*. This category includes animals whose life histories evolved under conditions in which food is scarce and where resource availability is uncertain or environmental conditions are predictably adverse part of the time. The extended longevity of animals in this category evolved through natural selection.
2. *Socially selected*. This category includes species that exhibit extensive parental investment, extensive parental care, and eusociality. The extended longevity of animals in this category results from natural, sexual, and kin selection.

TABLE 1 The Two General Categories of Factors That Favor the Evolution of Extended Life Span and Examples of Species Within Each

Category	Examples
Environmentally selected	Tortoises, sea turtles, deep-water tube worms, tuatara; birds, beetles, *Heliconius;* butterflies, tree-hole mosquitoes
Socially selected	Elephants, killer whales, dolphins, most primates (including humans), naked mole rats, microbats (brown bat, vampire), parrots, hornbills, albatross, termite, ant and bee queens, tsetse flies

This classification system relates the life span to two conventional correlates, relative brain size and flight capability, in the context of life history. That is, brain size is related to the size of the social group and the degree of sociality (13) and these, in turn, are linked to an extended life span (Chapter 3). An intensive parental care is linked to flight capability in birds and bats.

No system of classification is perfect and the one presented in Table 1 is no exception—the categories are not mutually exclusive and therefore some species could be placed in either or both categories. However, this classification serves as a practical and heuristic tool for considering the evolution of animal life spans. In particular, this system provides a general background for closer examination of specific human attributes including the evolution of life span and sets the stage for addressing questions of process.

■ Life Span Patterns: Humans and Primates

Estimates based on regressions of anthropoid primate subfamilies or limited to extant apes indicate a major increase in longevity between *Homo habilis* (52–56 years) to *Homo erectus* (60–63 years) occurring roughly 1.7 to 2 million years ago. The predicted life span for small-bodied *Homo sapiens* is 66 to 72 years (14). From a catarrhine (Old World monkeys and apes) comparison group, a life span of 91 years is predicted when contemporary human data are excluded from the predictive equation. For early hominids (family of bipedal primate animals comprising recent man, his ancestors, and related forms) to live as long or longer than predicted was probably extremely rare; the important point is that the basic Old World primate design resulted in an organism with the potential to survive long beyond a contemporary mother's ability to give birth. This suggests that postmenopausal survival (Chapter 10) is not an artifact of modern life style but may have originated between 1 and 2 million years ago, coincident with the radiation of hominids out of Africa.

The general regression equation expresses the relationship of longevity to body and brain mass when 20 Old World

anthropoid primate genera are the comparative group. The predicted longevity for a 50-kg primate with a brain mass of 1250 g (conservative values for humans) when case deletion regression methods are employed (each prediction is generated from the equation excluding the species in question) is 91 years and 72 years when humans are included within the predictive equation (Table 2). Using the predictive equation presented in Table 2, a typical old world primate with the body size and brain size of *H.sapiens* can be expected to live between 72 and 91 years with good nutrition and protection from predation.

■ Theoretical Model of Longevity Extension in Social Species

Improved health and increased longevity in societies sets in motion a self-perpetuating system of longevity extension. This positive feedback relationship is based on the demographic tenet that (all else being equal) increased survival from birth to sexual maturity reduces the number of children desired by parents (15). Because of the reduced drain of childbearing and child rearing, parents with fewer children remain healthier longer and thus raise healthier children. The higher survival rate of these children fosters yet further reductions in fertility. Greater longevity of parents also increases the likelihood that they can contribute as grandparents to the fitness of both their children and their grandchildren. And, the self-reinforcing cycle continues.

In an essay on the formation of human capital, Abramovitz (16) noted that the decline in mortality rates during the early stages of industrialization in the United States was probably one of the forces behind the expansion of educational effort and growing mobility of people across space and between occupations. Whereas previous conditions of high mortality and crippling morbidity effectively reduced the prospective rewards to investment in education during the preindustrial period (17), prolonged expectancy for working life span must have made people more ready to accept the risks and costs of seeking their fortunes in distant places and in new occupations. The positive feedback of gains in longevity on future gains involves a complex interaction among the various stages of the life cycle with long-term societal implications in terms of the investment in human capital (16), intergenerational relations (18), and the synergism between technological and physiological improvements—so-called "technophysio evolution" (Chapter 1) (19). In other words, long-term investment in science and education provides the tools for extending longevity. In turn, it makes more attractive the opportunity and cost of long-term investments in individual education and thus help humans gain progressively greater control over their environment, their health, and overall quality of life. This concept is reinforced by the finding of Bloom and Canning (20) who noted that, whereas the positive correlation between health and income per capita is very well known in international development, the health–income correlation is partly explained by a causal link running the other way—from health to income. In other words, productivity, education, investment in physical capital, and what they term "demographic dividend" (positive changes in birth and death rates) are all self-reinforcing—these factors contribute to health

TABLE 2 Estimates of Longevity for Fossil Hominids

Hominid species	Life span (yr)[a]	Incremental change
Australopithicus afarensus	46.6	
		8.4
Homo habilis	55.0	
		7.0
Homo erectus	62.0	
		10.9
Homo sapiens (*prehistorical*)	72.9	
		49.1
Homo sapiens (contemporary)	122.0	

Note: Based on hominoid body size; relationships range from 42 to 44 years for Australopithecus to 50 years for *Homo erectus*. Incorporation of brain mass increased estimates for *Homo habilis* from 43 years to 52–56 years and for *Homo erectus* from 50 years to 60–63 years.

[a] When six genera of apes are used as the comparison group, the regression equation is: log_{10} LS = 1.104 + 0.072(log_{10} Mass) + 0.193(log_{10} Brain), yielding a predicted human longevity of 82.3 yrs.

TABLE 3 Classification and Brief Description of Main Theories of Aging

Biological level/ theory	Description
Molecular	
Codon restriction	Fidelity and/or accuracy of mRNA message translation is impaired with aging due to cell inability to decode the triple codons (bases) in mRNA molecules
Somatic mutation	Type of stochastic theory of aging that assumes that an accumulation of environmental insults eventually reaches a level incompatible with life, primarily because of genetic damage
Error catastrophe	Errors in information transfer due to alterations in RNA polymerase and tRNA synthetase may increase with age, resulting in increased production of abnormal proteins
Gene regulation	Aging is caused by changes in the expression of genes regulating both development and aging
Dysdifferentiation	Gradual accumulation of random molecular damage impairs regulation of gene expression
Cellular	
Wear and tear	Intrinsic and extrinsic factors influence life span
Free radical accumulation	Oxidative metabolism produces free radicals that are highly reactive and thus damage DNA and/or proteins and thus degrade the system structure and function
Apoptosis	Process of systematically dismantling key cellular components as the outcome of a programmed intracellular cascade of genetically determined steps
System	
Rate-of-living	An old theory that assumes that there is a certain number of calories or heart beats allotted to an individual and the faster these are used, the shorter the life
Neuroendocrine	Alterations in either the number or the sensitivity of various neuroendocrine receptors give rise to homeostatic or homeodynamic changes that result in senescence
Immunologic	Immune system reduces its defenses against antigens, resulting in an increasing incidence of infections and autoimmune diseases
Evolutionary	
Antagonistic pleiotropy	Alleles that have beneficial effects on fitness at young ages can also have deleterious effects on fitness later in life
Mutation accumulation	The force of natural selection declines at older ages to a point where it has little impact on recurrent deleterious mutations, with effects confined to late life
Disposable soma	Preferential allocation of energy resources for reproduction to the detriment of maintenance and survival of somatic cells

and better health (and greater longevity) contributes to their improvements.

■ THEORIES OF AGING

In contrast to the life span of a species, which is an evolved, positively selected life-history trait analogous to a biological "warranty period," aging is a byproduct of evolution analogous to the progressive deterioration of any system that increases the likelihood of failure. Inasmuch as the concept for designing a machine is fundamentally different from the concept for understanding its deterioration, it follows that a theoretical framework for understanding life span must necessarily be different from the theoretical framework for understanding aging. Therefore, the purpose of this section is to provide a brief overview of the main theories of aging. We begin with a description of the theory concerning why all sexually reproducing organisms grow old, followed by the more conventional theories ordered hierarchically—molecular, cellular, systemic, and evolutionary.

■ Evolution and Origin of Aging

Bell (21) established the deep connection between the two invariants of life—birth and death—by demonstrating that protozoan lineages grow old as the result of an accumulated load of mutations. The senescence can be arrested by recombination of micronuclear DNA with that of another protozoan through conjugation. Conjugation (sex) results in new DNA and in the apoptotic-like destruction of old operational DNA in the macronucleus (Fig. 1). Thus, rejuvenation in the replicative DNA, and senescence of operational DNA are promoted by sexual reproduction. When this concept is extended to multicellular organisms, sex and somatic senescence are inextricably linked (22). In multicellular, sexually reproducing organisms, the function of somatic cells (i.e., all cells constituting the individual, except the germ cells) is survival of the replicative DNA—the germ cells (or gametes, either of two mature cells which, when they unite, form a new individual). Prior to bacteria, the somatic DNA was the germ line DNA; prior to multicellular animals, the somatic cell was the germ cell. Like the macronuclei in the paramecia, the somatic cells senesce and die as a function of their mitotic task of ensuring the survival and development of the germ cells. The advent of sex in reproduction allowed exogenous repair of replicative DNA (22); in multicellular organisms, the replication errors of somatic growth and maintenance are segregated from the DNA passed on to daughter cells and these errors are discarded at the end of each generation. Senescence is built into the life history concept of all sexually reproducing organisms. Thus, modifying senescence can alter death rate but death itself can never be eliminated. This evolutionary argument concerning senescence can be regarded not only as one of the most basic principles of biogerontology but also as one of the fundamental canons in the emergence of all sexually reproducing organisms.

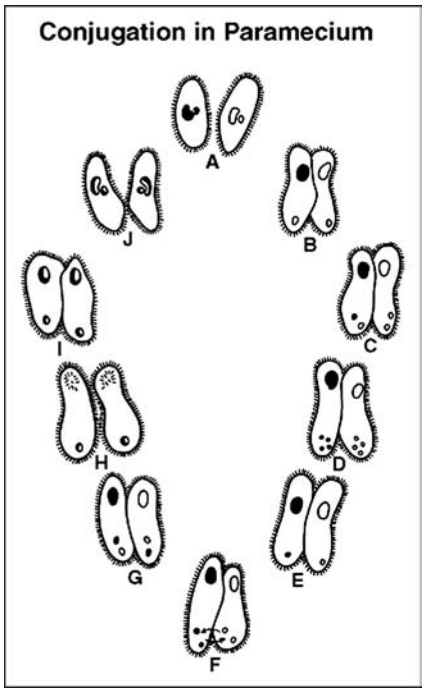

FIGURE 1 Schematic of sexual reproduction in the protozoan Paramecium aurelia (re-drawn from p70 in W. Clark 1996. Sex and the Origin of Death, Oxford). (**A**) Two genetically different protozoa, each with macronucleus (large oval in individual protozoa) and a micronucleus (small oval in protozoa). (**B**) The two protozoa fuse in the first step of conjugation, and the macronuclei and micronuclei move to opposite ends of the cell. (**C**) Each micronucleus divides once by meiosis, and (**D**) the daughter micronuclei each divide again, to produce four haploid micronuclei. (**E**) three of the four haploid microcuclei disappear. (**F**) The remaining micronucleus divides once more, to produce two identical micronuclei, and then (**G**) the two conjugants exchange one micronucleus. (**H**) the two haploid micronuclei fuse to produce a single diploid micronucleus. (**I**) The new micronuclei each direct the production of a new macronucleus; the old macronucleus begins to disintegrate. (**J**) the two protozoa disengage, and the nuclei assume their starting positions in the cell. The exconjugates are now genetically identical to one another, but genetically different from either of the two starting cells. Each will go on to produce genetically identical daughters by simple fission.

■ Molecular Theories of Aging

Codon Restriction

A codon consists of the three contiguous bases in an mRNA molecule that specify the addition of a specific amino acid to the growing amino acid chain as the ribosome moves along the mRNA (23). The process by which the enzyme RNA polymerase makes an RNA molecule complementary to the sequence of the template strand of the DNA is known as transcription. This transcribed message then undergoes a process know as translation in which a ribosome makes a protein by "reading" the sequence of an mRNA codon by codon. The codon restriction theory of aging is based on the hypothesis that the accuracy of translation is impaired with aging (24). Experimental evidence in support of this theory includes studies from Ilan and Patel (25) who reported alterations in mRNA and corresponding synthetases during the development period in a Tenebrionid beetle from the free-living nematode *Turbatrx aceti* (26) and in the fly *Drosophila melanogaster* where it was shown that the efficiency of some synthetases of old flies is only 50% as that of the young flies (27). The fetal rat liver contains six isoacceptor for tRNA (tyr) whereas the adult has only three (28).

Gene Regulation

Although every cell contains the same genetic information as every other cell in an organism, a given cell or type of cell expresses only a distinct subset of its genes at any one time. For example, only red blood cells need to produce hemoglobins and only cells in the retina need to produce light-sensitive proteins. Clearly, cells in the tongue do not need to produce hair. Genes need to be regulated to achieve this diversity of protein function during development. Gene regulation occurs primarily at the level of transcription within a cell that transcribes only a specific set of genes and not others.

The gene regulation theory, proposed by Kanungo (29), hypothesizes that senescence results from changes in the expression of genes after reproductive maturity is reached. Recent evidence that gene expression changes with age was reported by Helfand et al. (30), using a DNA sequence in *Drosophila* that interacts with "activator proteins" to stimulate gene expression (i.e., called enhancer traps) and which secretes into the cell a stainable marker substance whenever their gene is active (31,32). These studies enabled the scientists to visualize the gene activity patterns in the antennae of the fly during its adult life. The results revealed that around 10 to 49 genes examined are constant, while the remainder showed a changing expression with age. Of these, slightly over half increase their activities from an initially low level, and around a quarter decrease their activity from an initially high level. The activity patterns of the genes were linked to chronological and not to physiological age (33).

Dysdifferentiation

A theory of aging that is closely related to the gene regulation theory is that coined by Cutler (34) as the dysdifferentiation theory, that is, the gradual accumulation of random molecular damages that impair the normal regulation of gene activity while potentially triggering a cascade of injurious consequences (35,36). Whereas the gene regulation theory of aging (described above) hypothesizes that mistakes in protein synthesis (and hence aging) are an outcome of mistakes in the transcription process itself, the dysdifferentiation theory hypothesizes that the mistakes in protein synthesis are due to molecular damage, which causes aberrant expression of genes. Cutler suggested that the cell might synthesize proteins other than those characteristic of its differentiated state due to the lack of stringent gene control. The dysdifferentiation theory postulates that cells from old donors will synthesize more proteins that are uncharacteristic of their differentiated state than will similar cells from young donors. Ono and Cutler (37) reported a twofold age-dependent increase in the amount of α- and β-globin mRNA synthesized in mouse brain and liver. However, other studies (38) reported no evidence of age-dependent increases in uncharacteristic globin gene expression in young and old cultures of normal human fibroblast cells. Despite the absence of supporting evidence, the dysdifferentiation theory of aging is of interest partly because it provides testable predictions and partly because it allows for the stochastic modulation of a programmed process by means of a known genetic mechanism (36).

Error Catastrophe Theory

The basic idea of this theory, first introduced in 1963 (39), is that the ability of a cell to produce its complement of functional proteins depends not only on the correct genetic specification of the various polypeptide sequences but also on the competence of the protein-synthetic apparatus. This theory differs from DNA-based theories in that it postulates an error in information transfer at a site other than in the DNA. The summation of many small developing errors in the synthetic and enzymatic machinery of the cell mounts to a point beyond which conditions for the cell life become impossible.

One of the virtues of this theory is that it is testable—proteins obtained from cells of old donors should exhibit a higher frequency of errors than would proteins taken from cells of younger donors. Rothstein (40) concluded that it is unlikely that transcriptional or translational errors are one of the mechanisms responsible for aging and senescence. However, his conclusions do not rule out errors in the replication of the DNA itself (36). Murray and Holliday (41) reported that selected DNA polymerases obtained from older cells have a greater error rate than polymerases taken from younger cells; and Srivastava et al. (42) reported that the degree of loss in fidelity is less in calorically restricted mice than in ad libitum–fed mice (Chapter 23). These observations are thus consistent with the formal theory of error catastrophe.

Somatic Mutation and DNA Damage

The broad concept of this category of aging theories is that the integrity of the genome is the controlling factor in the aging process and therefore that either mutations (changes in the polynucleotide sequence that remain uncorrected) or DNA damage (chemical alterations in the double-helical structure that are not fully repaired) underlies the aging process and determine its rate. The theory that aging occurs due to either somatic mutation or DNA damage belongs to the class of stochastic theories of aging that assumes that an accumulation of environmental insults eventually reaches a level incompatible with life, primarily because of genetic damage. Somatic mutation occurs in day-to-day cell replication (43). It is generally concluded that there is currently little evidence to support the notion that either somatic mutation or DNA damage underlies the diseases and dysfunctions of aging.

■ Cellular Aging: Free Radical Hypothesis

The free radical hypothesis of aging states that free radicals in the cell damage cell macromolecules and lead to senescence and, eventually, to cell death (44–46). Free radicals are molecules that contain unpaired and reactive electrons. Various kinds of free radicals are present in the cell and come from either endogenous or exogenous sources (45). There are two major kinds of free radicals (Table 4). One is the extensively studied reactive oxygen species (ROS), which are metabolites of molecular oxygen (O_2) and are the predominant species of free radicals (46,47). Examples of ROS include weakly active superoxide radical ($O_2^{\cdot}$), weakly reactive nonradical hydrogen peroxide (H_2O_2), and highly reactive hydroxyl radical (OH). These ROS can be generated and reduced to stable water through sequential reactions of oxygen with electrons (e^-) and protons.

The other type of free radicals is reactive nitrogen species (RNS) (Table 4). Nitric oxide (NO) is a major RNS, which is not reactive with nonradical molecules but reactive with many other free radicals such as the superoxide radical (48). NO can react with an organic-free radical and form stable nonradicals that can essentially terminate free radical chain reactions. Additionally, NO can react with superoxide radicals to produce

TABLE 4 Biologic Oxidants and Oxidation Catalysts

Name	Structure	Oxygen activation
Superoxide radical	$O_2^{\cdot -}$	$O_2 + e^- \rightarrow O_2^{\cdot -}$ (weakly reactive)
Hydrogen peroxide	H_2O_2	$O_2^{\cdot -} + e^- + 2\,H^+ \rightarrow H_2O_2$ (weakly reactive)
Hydroxyl radical	$OH^{\cdot}$	$H_2O_2 + e^- \rightarrow OH^- + OH^{\cdot}$ (strongly reactive)
Singlet oxygen	$^1O_2$	–
Nitric oxide	$NO^{\cdot}$	Synthesized by NOS
Peroxynitrite	$ONOO^-$	$NO^{\cdot} + O_2^{\cdot -} \rightarrow ONOO^-$
Hypochlorite	HOCl	–
Transition metal ions	Fe^{+m}, Cu^{+n}	–

Abbreviation: NOS, nitric oxide synthase.

strongly reactive nonradical peroxynitrite, which can nitrate aromatic compounds; e.g., convert the amino acid tyrosine to nitrotyrosine. In the presence of oxidation catalysts such as iron or copper, peroxynitrite can be broken down to two new free radicals less reactive but toxic; they are nitrogen dioxide and highly reactive hydroxyl radicals (Table 4).

Sources of Free Radicals

Free radicals are continuously produced from various biological processes regulated by a variety of enzymes in multiple subcellular compartments within the cell (49). Under normal physiological conditions, most of the cellular ROS are generated in the mitochondria through "leakage" of oxidative phosphorylation, a biological process that produces ATP, the major form of energy in cells. Nicotinamide adenine dinucleotide (NADH) or flavin adenine dinucleotide (FADH), the reduced form of coenzymes for a variety of biochemical reactions in the cell, facilitates the formation of ATP, responsible for energy-transfer reactions. In oxidative phosphorylation, the electrons from NADH or FADH flow sequentially through a mobile electron acceptor such as coenzyme Q, and through acceptors such as cytochrome c1 and cytochrome c, which form protein complexes I to IV in mitochondria. The electrons are then transferred to molecular oxygen to form water and produce a proton gradient, which is used to generate portable ATP energy via the F1-F0 ATPase. During the transferring processes, some of the electrons are "leaked" out of the mitochondrial protein complexes and are absorbed directly by molecular oxygen to generate superoxide radical (O_2^-). The "leakage" occurs predominantly in two complexes, complexes I and III, where the potential energy of the electrons has large changes relative to the reduction of oxygen (49). The rate of ROS generation in the mitochondria is believed to be regulated by

1. the reduction-oxidation (redox) potential of these complexes, that is, the ability of a redox couple to accept or donate electrons,
2. the oxygen tension, and
3. the number of electron-transfer sites (48).

In addition to the mitochondria, ROS can also be produced in other subcellular compartments and by multiple enzymes. These include enzymes within the plasma membrane, such as NAD(P)H oxidase (50), and peroxisomal oxidases in peroxisomes (51). Endogenous biological processes such as oxidative phosphorylation as well as various exogenous stimuli such as radiation, exposure to toxins, pathogen infections, heat, and ultraviolet light regulate ROS production (52–54).

NO is an important free radical of RNS and is produced predominantly through oxidative deamination of *L*-arginine by NO synthase in the cell (48,55,56). Not much is known about regulation of RNS production in the cell. However, when NO is produced in excess, it can react with ROS to generate reactive peroxynitrite, which, in turn, can be broken down into nitric dioxide and highly reactive hydroxyl radicals.

Targets of Free Radicals

Due to their reactive nature, free radicals can indiscriminately attack macromolecules such as DNA, protein, and lipid to cause damage to these molecules. Much is known about oxidative damage of these molecules caused by ROS, but less is known about damage by RNS.

ROS can react with DNA to generate various oxidation products, which include the abundant forms of 8-oxoguanine (8-oxodG) and thymine glycol (55–57). Oxidation of DNA can lead to mutations and deletions of DNA and can block DNA replication and transcription by interfering interactions between DNA and proteins.

ROS, when unchecked, can also cause oxidation of proteins in various ways. For example, highly reactive fragment OH can react with the protein polypeptide backbone through sequential reactions to generate a carbon-center radical, which in turn can react with molecular oxygen and superoxide to form peroxyl radicals, alkyl peroxide, and alkoxyl radical and the hydroxyl derivative (55). The most abundant forms of protein oxidation are protein carbonyl derivatives, which are produced by a number of mechanisms, including amidation and glutamic acid oxidation pathways (35,47,55,56). Most susceptible amino acid residues to carbonyl formation include lysine, arginin, proline, and threonine.

RNS can also inflict damage to proteins through the formation of peroxynitrite from the reaction between NO and superoxide anion (48). Peroxynitrite can (*i*) nitrosate cysteine sulfhydryl groups and tyrosine and tryptophan residues, and (*ii*) oxidize methionine residue to methionine sulfoxide and generate various radicals, e.g., $ONOOCO2^-$ formed by reacting with CO_2. Oxidation of proteins can lead to alterations of function, stability, and degradation of proteins.

Lipids are the most sensitive molecules to free radicals due to the presence of bisallylic structures in polyunsaturated fatty acids. Lipid peroxidation can produce a variety of end products by chain reactions with free radicals. The end products include malonaldehyde, 4-hydroxy-2-nonenol, and F2-isoprostanes, which tend to be accumulated in the cell, especially in the membranes (48,55,56,58). F2-isoprostanes generated from the peroxidation of arachidonic acid are the biomarkers often used to determine the level of lipid peroxidation (59). β-Formation of isoprostanes is initiated by formation of peroxyl radicals, which are produced by abstraction of a bisallylic hydrogen atom and the addition of oxygen to arachidonic acid. Peroxyl radicals can react with another molecule of oxygen to produce prostaglandin-like compounds, which are then converted to isoprostanes (56). Lipid peroxidation can lead to damage to cellular membranes, lipid packing, and loss of cell integrity since lipids are essential components of cell membranes and various lipoproteins (47,58–60).

Antioxidant Systems

The presence of free radicals in the cells poses a huge dilemma to the biological systems within for the following reasons:

- Free radicals can randomly inflict damage to essential macromolecules; this damage leads to dysfunction of physiological systems in the organism (47,58–60).
- Compelling evidence indicates that free radicals play a positive physiological role in various biological processes such as immune response to pathogens (Chapter 14) and cellular signaling transduction (48,59).

Cells have developed a variety of ways to balance the oxidant level by regulating production of oxidant and removal of oxidant by the antioxidant systems in order to defend themselves from oxidative stress while maintaining normal physiological functions.

One antioxidant mechanism is to scavenge free radicals with various enzymes and proteins, of which one major player is superoxide dismutase (SOD), which is present in all the aerobic organisms (60). SOD is uniquely dedicated to scavenge superoxide radicals by converting them to H_2O_2 (61–63), which can be further detoxified into water and oxygen by catalase in the cell (47).

Oxidants can also be removed by various other kinds of proteins such as glutathione-*S*-transferase (64) for detoxification of xenobiotics, endogenous toxins, and hydroperoxides, and ferritin for sequestration of oxidation catalyst iron (62–64).

A second antioxidant system consists of small antioxidant molecules, which include ROS scavengers such as tocopherol, ascorbic acid, glutathione, bilirubin, lipoic acid, and urea (53,65,66). While some of these ROS scavengers are produced in the cells, some of them can only be obtained through food intake. These antioxidant molecules often interact with each other in scavenging oxidants. Such interactions can be exemplified by removal of free radicals in the plasma membrane, in which tocopherols reduce free radicals and then tocopherols can be regenerated by ascorbic acid to take electrons from oxidized tocopherols.

The balance between oxidants and antioxidants is tightly regulated in the cell (Table 5). Increased level of oxidants can lead to increased production of endogenous antioxidants, while increased intake of exogenous antioxidant can result in deceasing production of endogenous antioxidants (47).

Oxidants as Causes of Aging

The free radical theory of aging predicts that oxidants are the cause of aging. There are numerous experimental data supporting this hypothesis (67). However, there are also compelling data that are inconsistent with this hypothesis (68). There are several observations consistent with the free radical theory of aging, including the following:

- An age-related increase in oxidative damage to a variety of molecules (DNA, protein, and lipid) is present in organisms ranging from single cellular organisms and invertebrates, to humans. For example, a significant increase of oxo8dG levels with increasing age has been observed in nuclear nDNA and mitochondrial mtDNA isolated from various tissues in rodents (69).
- For protein oxidation, the widely studied oxidative modification is the formation of carbonyl derivatives on lysine, arginine, proline, histidine, cysteine, and threonine residues (56). The level of global protein carbonyl in the cell increases with age in different tissues of various organisms, such as rat liver, skeletal muscle of rhesus monkey, and human dermal fibroblasts, lens, and brains (67).
- The level of protein carbonyl in mitochondria also increases with age. In addition, carbonylation to specific proteins such as aconitase, gucose-6-phosphate dehydrogenase, and adenine nucleotide dehydrogenase (70–74) increases with age. Many of these selectively carbonylated proteins are involved in mitochondrial functions. Oxidative damage to mitochondrial proteins such as aconitase may lead to increased production of free radicals (75) and affect mitochondrial integrity (76).

TABLE 5 Agents Involved in Protection Against Oxidants

Name	Mechanism(s)
Ascorbic acid (vitamin C)	FR scavenger
Glutathione	FR scavenger, enzyme cofactor
Tocopherol (vitamin E)	FR scavenger
Carotenoids	Singlet oxygen scavengers
Bilirubin	FR scavenger
Lipoic acid	FR scavenger
Uric acid	FR scavenger
Enzyme mimetic agents	Superoxide, hydrogen peroxide scavengers
PBN	FR scavenger, cell-signaling effects
(−) deprenyl (selegiline)	Monoamine oxidase B inhibitor
Protein methionine groups	FR scavengers
Superoxide dismutases	Superoxide scavengers
Catalase	Hydrogen peroxide scavenger
Selenium peroxidases	Hydroperoxide scavengers
Heme oxygenases	Heme decomposition
Ferritin	Iron sequestration
Quinone reductase	Quinone scavenging
Glutathione-S-transferases	Detoxification of xenobiotics, endogenous toxins, hydroperoxides
Peroxiredoxins	Cell-signaling effects
Metallothionine	Transition metal sequestration

Abbreviations: FR, free radical; PBN, *N*-tert-butyl-α-phenylnitrone.

As highly sensitive molecules to free radicals, lipid peroxidation in aging has been extensively studied. The level of a biomarker for lipid oxidation, F2-isosprostane, dramatically increases with aging in blood, and tissues, e.g., liver and kidney, in rodents (59). Although the levels and extent of oxidation to various macromolecules vary among tissues and organisms, almost all the oxidative damage shows an age-related increase. This association is consistent with the free radical hypothesis of aging according to which free radicals cause damage to macromolecules and this damage is, in turn, manifested by aging-associated diseases (e.g., atherosclerosis, diabetes).

The free radical theory of aging has been tested in a number of experiments that are designed to alter the life span, especially to increase the life span. Life span and aging are modulated by various genetic and environmental factors (77). The free radical theory of aging predicts that the manipulations that extend life span should lead to reduction of the age-related increase in damage to macromolecule by free radicals. Studies of life span modulations in model organisms ranging from single-cellular organisms such as yeast (*Saccharomyces cerevisiae*), to multicellular organisms such as nematodes (*Caenorhabditis elegans*), flies (*D. melanogaster*), and rodents have identified a number of long-lived mutants with alterations in a number of genes (78–81). These life span–related genes fall into a number of pathways including

- Evolutionarily conserved genes, e.g., insulin/insulin-like signaling (IIS) pathway (78),
- Nutrient-sensing target of rapamycin (TOR) pathway (79,80),
- The sirtuin pathway (82), and
- And the Jun kinase (JNK) (83) pathway.

A common feature of these long-lived mutants is that most of them have increased resistance to exogenous oxidative challenging, such as H_2O_2 and paraquat, a free radical–-generating chemical (84). In addition, these mutants have lower rates in the age-related increase of oxidative damage compared to the wild-type control. For example, lipofuscin, a product of protein and lipid oxidation, increases with aging and is often used as a biomarker of aging (85,86). In long-lived *C. elegans* mutants, such as those with mutations in the IIS pathway, accumulation of lipofuscin with increasing age is significantly reduced compared to the control (87). Mutations in antioxidant defense system, such as mutations in the enzyme SOD in invertebrates and rodents, often result in either lethality or reduced life span, accompanied by increased sensitivity to oxidative stress and elevated levels of oxidatively damaged macromolecules (88).

Dietary restriction (DR) or caloric or calorie restriction (CR) is one of the extensively studied interventions that extend the life span in almost all the species tested so far (89,90). A number of studies have shown that CR reduces the level of oxidative damage represented by F2-isoprostane, global protein carbonyl content, and oxo-8-dG in a variety of tissues in rodents and nonhuman primates (67). The reduction of oxidative damage appears to be at least partially due to the effects of CR to reduce the ROS generation by mitochondria and to enhance the repair and turnover of macromolecule by the biological processes including the autophagy protein degradation process (91). These genetic and environmental manipulations of life span strongly support the role of free radicals in causing aging.

A number of studies described above have established a strong correlation between free radicals and aging. To directly prove the free radical hypothesis of aging, one needs to investigate the effect of interventions that alter metabolism of free radials on life span and aging. A direct approach to interfere with oxidative damage would test the administration of pharmacological antioxidants to reduce availabilities of oxidants (90). Based on the findings that DR or life span–related genes extend life span at least partially by reducing oxidative stress, a number of strategies to retard aging have been proposed, one of which is to supplement pharmaceutical or nutraceutical compounds into a regular diet to mimic the effects of DR or longevity genes. In the case of the former, dietary supplements are called "caloric restriction mimetics" (Chapter 23) (92). Indeed, a few such compounds and natural plant extracts when supplemented into a regular diet extend the life span and retard the aging-related decline of physiological functions in several model organisms (90). One of these compounds is resveratrol (found in red wine), which extends the life span in *S. cerevisiae*, *C. elegans*, *D. melanogaster*, and a short-lived fish *Nothobranchius furzeri* (82). It has been proposed that resveratrol extends the life span through activation of a conserved protein sirtuin, specifically Sir2 in *S. cerevisiae* and SIRT1 in mammals; these proteins may be involved in the beneficial effects of CR (Chapter 23) (81,93). However, the function of sirtuins and resveratrol on life span extension remains controversial, since Sir2 does not mediate DR response in *S. cerevisiae* in some experimental conditions (94).

Other antioxidant compounds exhibiting effects of life span extension include

- antioxidant vitamin E (95–98) in *C. elegans* and *D. melanogaster*,
- 4-phenylbutyrate (99) in *D. melanogaster*,
- anticonvulsant drugs (100),
- SOD/catalase mimetics (101), and
- blueberry extracts (102) in *C. elegans*.

In a number of these treatments, the levels of oxidatively damaged molecules decrease compared to age-matched controls. However, in most of the cases when life span extension is observed, only mean and not maximum life span is affected, and not all of these compounds show consistent beneficial life span effects. Moreover, few compounds consistently extend life span and retard aging in multiple evolutionarily distant organisms. Therefore, the results from these direct manipulations of life span and aging with antioxidants are not all consistent with the free radical theory of aging.

A number of enzymatic antioxidant systems have been identified in the cell. Attempts have been made to evaluate the effects of these genes when mutated or overexpressed on life span using genetic approaches in genetically amenable model systems such as yeast, nematodes, flies, and rodents. Using *D. melanogaster*, overexpression of various antioxidant genes encoding Cu/ZnSOD, manganese superoxide dismutase (MnSOD), catalase, and methione sulfoxide reductase A (MsrA), respectively, extend the life span (103–105). Overexpression of catalase alone did not extend life span. Overexpression of Cu/ZnSOD either ubiquitously or selectively in motor neurons was shown to extend life span. However, these effects of life span extension depended on the genetic background of fly strains, as in some strains, overexpression of Cu/ZnSOD alone or in combination with catalase failed to extend life span. Overexpression of *MnSOD* alone was shown to increase life span. Overexpression of *MsrA* ubiquitously or specifically in the nervous system increased resistance to paraquat and lengthened life span. MsrA functions in repairing oxidized methionine by reducing methione sulfoxide formed by reaction of methionine with oxidants. In mice, several research groups genetically altered various components of the antioxidant defense system to evaluate the free radical theory of aging. Transgenic mice with overexpression of Cu/ZnSOD were more resistant to cerebral ischemia but showed similar life span as the wild-type control (106). Overexpression of thioredoxin I (*TRX1*) increase mice's resistance to ischemia/reperfusion injury and reduce the levels of protein carbonyl after ischemia injury in brain (107). Life span of *TRX1* transgenic mice appeared to be longer but the results were obtained using a small number of animals. The knockout mice null for the *MsrA* gene have approximately 40% shorter life span than the wild-type control (108). Interestingly, mice with 30% to 80% reduction of MnSOD activity have similar life span and similar levels of several age-sensitive biomarkers as the wild-type control, although mutant mice have elevated oxidative damage in DNA. Overall, from these genetic studies that directly target the antioxidant system, some results are consistent with the free radical theory of aging and some are not. This ambivalence suggests that free radicals are not the only causing factors in aging and strengthens the view that aging is a multifactorial process. Additional evidence that supports or argues against the role of free radicals as causal factors in aging is shown in Table 6. However, it should be noted that normal functions of the cell require maintaining an intricate balance of the oxidant and the antioxidant levels. The direct

TABLE 6 Are Oxidants the Cause of Aging? Some Pro and Con Arguments

Pro	Con
Caloric restriction appears to reduce oxidative stress	Vitamin C, a superb free radical scavenger, is not synthesized by long-lived primates
Life span extension in mutants is often associated with stress resistance	Chronic ionizing radiation at low doses does not shorten life span but may increase it
Knockout mice lacking MnSOD or HO-1 have severely restricted survival	Dietary supplementation with natural antioxidants (vitamins C and E) does not extend life spans
Enzyme mimetics extend maximum life spans in some aging models	Tissue comparisons, e.g., brain vs. muscle, seem incompatible with simple oxidant and antioxidant models of aging
Certain drugs, PBN, (−) deprenyl, possibly acting as antioxidants, have been claimed to extend life spans	Exercise, often claimed to increase oxidant stress, exerts beneficial effects, at least on mean life spans

Abbreviations: MnSOD, manganese superoxide dismutase; HO-1, heme-oxygenase-1; PBN, *N*-tert-butyl-α-phenylnitrone

manipulations of the antioxidant system by pharmaceutical, nutraceutical compounds, or genetic approaches may disrupt this balance and mask the beneficial effects of the antioxidant systems.

Free Radical Accumulation

Initially, the mechanistic link between metabolism and aging was unknown. However, in the mid-1950s, Harman (45) articulated the "free radical theory" of aging, speculating that endogenous oxygen radicals were generated in cells and resulted in a pattern of cumulative damage (47). The standard explanation for this damage is that it is the result of cellular damage caused by free radicals—any number of chemical species that are highly reactive because they possess an odd number of electrons. Molecules that have unpaired electrons are thermodynamically unstable since they seek to combine with another molecule to pair off their free electron. The theory postulates that the physiological decrements characteristic of age-related changes can be ascribed to the intracellular damage done by the various free radicals (36,109). The net damage to various cell components (e.g., lipids, protein, carbohydrates, and nucleic acids) is the result of different types of free radicals present, their production rate, the structural integrity of the cells, and the activity of the antioxidant defense systems present in the organism. Oxidative damage is a candidate for what has been referred to as "public" mechanisms of aging—common mechanisms among diverse organisms that are conserved over the course of evolution (110). Structural and regulatory genes modulating genesis of free radicals have been identified in a wide range of species ranging from yeast, nematodes, and insects to mice and humans. The oxidative damage theory is supported in CR studies, which reveal that CR in individuals lowers the steady-state levels of oxidative stress and damage and extends life expectancy (Chapter 23).

■ Cellular Aging: Additional Theories

Wear-and-Tear Theory

This theory postulates that ordinary insults and injuries of daily living accumulate and thus decrease the organism's efficiency (e.g., loss of teeth leads to starvation). Although there is little question that some wear and tear plays a role in mortality risk and individual life span, this theory has been rejected by most gerontologists as a more general explanation for aging because (*i*) animals raised in protected environments still age; (*ii*) many of the minor insults that accumulate are essentially time dependent and thus cannot logically serve as an underlying causal mechanism of the aging process; and (*iii*) the theory is outdated inasmuch as cellular and molecular advances point toward more specific mechanisms.

Mitotic Clock

The majority of cell types grown in laboratory cultures have a finite ability to proliferate. After a number of population doublings, the cell cultures enter the terminally nondividing state referred to as replicative senescence (Hayflick Limit) (111). Many investigations have established a link between aging in vivo (live animals) and the proliferative potential of cells in culture. For example, cell cultures derived from one of the longest-lived species of animals, the Galapagos tortoise, doubled up to 130 times whereas cultures derived from mice with maximum life spans of three years were capable of doubling only 10 or fewer times.

One of the most compelling theories for explaining replicative senescence is that incomplete replication of specialized structures at the ends of chromosomes called telomeres account for the gradual loss of proliferation potential (112). Telomeres are essential for proper chromosome structure and function including complete replication of the genome—if organisms could not overcome the "end-replication" problem, they would fail to pass their complete genetic complement from generation to generation (Chapter 4). The simplest theory for accounting for the Hayflick Limit is one in which permanent cell-cycle arrest is due to a checkpoint mechanism that interprets a critically short telomere length as damaged DNA and causes cells to exit the cell cycle. This telomere hypothesis of aging provides a molecular mechanism for counting cell divisions in the normal somatic cell. According to the telomere-shortening model of cell senescence, to avoid telomere loss and eventual cell-cycle arrest, it is necessary for cells to synthesize telomeric DNA by expressing the enzyme. Therefore, a prediction of this model and one that is borne out in recent studies (113–115) is that telomerase activity will be absent in normal somatic cells but present in germ line cells and carcinoma cells. Although telomere length has historically been used as a means to predict the future life of cells, a new model frames the connection between telomere shortening and cellular senescence by introducing the concept of a stochastic and increasing probability of switching to the uncapped/noncycling state (116).

It is widely believed that senescence evolved to limit the number of available cell divisions and therefore that it serves as a brake against the accumulation of the multiple mutations needed for a cell to become malignant (117). Results of new studies (118,119) support the notion that DNA repair pathways and antioxidant enzymes are adequate for protecting against the accumulation of mutations and development of cancer in small organisms (mice) whose cells do not seem to have a cell division–counting mechanism but that replicative senescence evolved in long-lived species to ensure that they would have the greatly increased protection that their longevity necessitated.

Apoptosis Theory

Apoptosis or programmed cell death is a process of systematically dismantling key cellular components as the outcome of a programmed intracellular cascade of genetically determined steps (Chapter 4). It describes the orchestrated collapse of a cell and involves

- membrane dissolution,
- cell shrinkage,
- protein fragmentation,
- chromatin condensation, and
- DNA degradation followed by rapid engulfment of corpses by neighboring cells.

It is an essential part of life at all levels of multicellular organisms; it is conserved as the way cells die from worms to mammals (120). Alzheimer's and stroke-damaged cells die due to apoptosis (Chapters 7 and 8). Evidence suggests that all cells of multicellular organisms carry within themselves the information necessary to bring about their own destruction and that this process has been evolutionarily conserved. However, the key to understanding this process in the context of aging concerns when and under which conditions the process can be invoked. In particular, the possibility exists that one major function of apoptosis is to serve as a precisely targeted defense mechanism against dysfunctional and/or potentially immortal (cancer) cells. More generally, apoptosis provides us with a controllable process that is clearly important in regulating cell number. Apoptosis and mitosis are controlled by gene-based signaling systems, which can interact at the population and cell

levels to bring about the net gain or net loss of cells in a particular tissue (36).

■ System Theories of Aging

Rate-of-Living

The rate-of-living hypothesis of aging states that the metabolic rate of a species is inversely proportional to its life expectancy (Chapter 3). The original theory makes two predictions (36) including the following:

1. There is a predetermined amount of metabolic energy available to the organism that can be expressed equally well in terms of oxygen consumption or kilocalories expended per life span, and when this energy is gone, the organism dies.
2. There is an inverse relationship between metabolic rate and aging. Recent data show that the metabolic potential does not stay at a constant value for different populations of a species (36). Long-lived strains spend about the same number of calories per day; yet, they may live significantly longer. Thus, during their life time, the long-lived strains expend around 40% more calories than the normal-lived strains (36).

Neuroendocrine Control Theory of Aging

The endocrine system evolved to coordinate the activities of cells in different parts of the body by releasing hormones from major endocrine glands and/or organs into the bloodstream to be transported to other parts of the body where it affects particular target cells (Chapters 9–13). The major endocrine glands in mammals are the hypothalamus-pituitary-adrenal complex, the thyroid and parathyroid glands, the pancreas, the sex organs, and the adrenal glands (121). The neurological system is inextricably linked to the endocrine system because of the central role of the hypothalamus in synthesizing and realizing hormones (hypophysiotropic hormones) that control the pituitary gland to release hormones that act on target cells throughout the body (Chapters 9–13). Normal functioning requires that nervous and endocrine signals be synchronized and responsive to the needs of the many functions they regulate. However, some of the efficiency of the neurological and endocrine systems decreases with age, leading to decreased function and increased frequency of disease. Thus, the neuroendocrine control theory of aging hypothesizes that the effectiveness of homeostatic adjustments declines with aging and leads to the consequent failure of adaptive mechanisms, aging, and death (122). Functional loss includes deterioration of reproductive organs and loss of fertility, diminished muscular strength, lesser ability to recover from stress, and impairment of cardiovascular and respiratory activity (Chapters 9–13).

Immunological Theory of Aging

The cells and molecules responsible for immunity constitute the immune system and their collective and coordinated response to the introduction of foreign substances is called the immune response (123). This includes both the innate response (mechanisms that exist before infection) and the adaptive response (mechanisms that develop after infection), also called specific immunity (Chapter 14). Innate and adaptive immune responses are components of an integrated system of host defense with important links: (*i*) the innate immune response influences the nature of the adaptive responses; and (*ii*) the adaptive immune responses use many of the effector mechanisms of innate immunity to eliminate microbes. The immunological theory of aging rests on three key findings (36):

- A quantitative and qualitative decline in the ability of the immune system to produce antibodies
- Age-related changes in the ability to induce particular subsets of T-cells and to produce different types of cell-mediated responses
- Correlative evidence linking these alterations to the involution of the thymus

■ Evolutionary Theories of Aging

The evolutionary biology of aging theory postulates that the force of natural selection will always decrease with age with either replacement-level or positive-population growth (124). This is the theoretical basis for the disposable soma theory of aging, named for its analogy with disposable goods with a limiting warranty period. This theory postulates that fitness is maximized at a level of investment in somatic maintenance, which is less than would be required for indefinite survival. The disposable soma theory serves as the conceptual foundation for two population genetic hypotheses of aging. The first is termed negative pleiotropy and is based on the concept that alleles that have beneficial effects on one set of components of fitness also have deleterious effects on other components of fitness (125,126). It is based on the selection for forms of genes that confer beneficial effects early in life, in contrast to the ineffective selection to remove such forms of genes that are associated with deleterious effects late in life (postreproduction). The underlying concept is one of trade-offs—a beneficial effect at young ages may have a deleterious effect at older ages. The declining force of natural selection leads to a tendency for selection to fix alleles that have early beneficial effects but later deleterious effects. This biases evolution toward the production of vigorous young organisms and decrepit old organisms. Support for antagonistic pleiotropy is based on the consistent observation of a negative genetic correlation between early reproduction and longevity both in selection experiments (127) and in manipulative studies such as those of Sgro et al. (128,129). Support for antagonistic pleiotropy is also derived from the observation that longevity quantitative trait loci often appear to have antagonistic effects on life span in different environments and sexes (130–132). Although antagonistic pleiotropy is an important possible mechanism for the evolution of aging, it probably plays a limited role in explaining the persistence of genetic variation in fitness components (133,134). The second population-genetic hypothesis concerning the evolution of senescence is the mutation accumulation that arises when the force of natural selection has declined to a point where it has little impact on recurrent deleterious mutations with effects confined to late life (135–137). This theory hypothesizes that mutations producing a deleterious effect at postreproductive ages are not removed from populations by natural selection. Thus, mutations conferring late age-specific deleterious effects will accumulate in populations, causing aging and ultimately mortality.

■ Reliability Theory of Aging

A mathematical theory of aging is outlined by Gavrilov and Gavrilova (138) who build on the reliability theory—a body of ideas, mathematical models, and methods aimed at predicting, estimating, understanding, and optimizing the life span and failure distribution of systems and their components. They note that reliability theory allows researchers to predict age-related failure kinetics for a system of given architecture and reliability

of components. Considerations of the reliability theory of aging lead to several conclusions (138):

- Redundancy is a key notion for understanding aging and the systematic nature of aging in particular. Systems can be redundant in numbers of irreplaceable elements but still deteriorate over time even if they are built of nonaging elements.
- Apparent aging rate is higher for systems with higher redundancy levels. The author's note that this is important because it helps puts a correct perspective over observations of negligible senescence since death rates is also demonstrated to be negligible.
- Organisms running out of cells as they live lose reserve capacity and experience redundancy depletion. This explains the observed "compensation law of mortality" in which mortality converges at older ages, regardless of the initial levels of redundancy.
- Living organisms may be formed with a high load of initial damage, and, therefore, their life span and aging patterns may be sensitive to early-life conditions that determine this initial damage load during early development.
- Early-life programming may have important implications for developing early-life interventions promoting health and longevity.

■ CONCLUSIONS

Theories in aging research, as with theories in all of science, must be considered as means and never as ends. They provide a plan for searching and, even if wrong, a specific theory regarding the mechanism(s) of aging can be useful provided it is based on new observations and suggests an original path for scientific thought (139). But theory can be used not only in a mechanistic (causal) context but also to broaden the disciplinary scope, as was done by Sacher (140) when he rejected the standard view of biological gerontology as synonymous with "the biology of aging." Sacher expanded the domain of the field by defining biological gerontology as "the biology of the finitude of life, in its three aspects of longevity, aging, and death." He noted that these three aspects constituted an "irreducible triad" and that the ultimate goals of gerontology cannot be attained if attention is confined to one aspect to the exclusion of the others. The theoretical framework outlined by Sacher is important to aging science for at least two reasons. First, it provides conceptual coherence by linking the evolutionary by-product (aging) with the evolutionary "product" (longevity). Second, it helps to vertically integrate the various discoveries at different biological levels by providing a hierarchical context from the molecular and cellular to the organ and the whole organism.

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Part II

Systemic and Organismic Aging

6

The Nervous System: Structural, Biochemical, Metabolic, and Circulatory Changes

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INTRODUCTION

The human nervous system, its changes during development, adulthood, and old age, and its alterations with disease represent one of the most intriguing challenges of our time. Despite rapidly increasing advances in our understanding, we still have few direct answers to the many questions concerning the activities of its three major divisions [the peripheral, the autonomic, and the central nervous system (CNS)], their interrelationship with each other and with the entire organism. Comparison of the adult and elderly brain, with or without neurologic and psychiatric diseases of old age, reveals specific, morphologic, biochemical, metabolic, and functional differences under normal and diseased states.

Aging of the CNS will be presented in this and the following two chapters (Chapters 7 and 8), under normal aging conditions and in a few diseases prevalent in old age. The location, mechanisms, and consequences of changes with aging are summarized in Table 1 and Figure 1. These changes are multiple and involve all levels of organization. After a brief introduction, this chapter addresses morphologic changes (see section entitled Structural Changes) including brain size and weight, number of cells, synapses, and some neural pathology. This will be followed by biochemical changes in general (see section entitled Biochemical Changes), with examples from Parkinson's disease (PD) pathogenesis and therapy. Metabolic and circulatory changes with aging conclude the chapter (see section entitled Metabolic and Circulatory Changes).

We are currently witnessing a remarkable shift in the way physiologists think about aging of the CNS. The view formulated at the beginning of the twentieth century was of severe and inexorably progressive deterioration of structure, biochemistry, and function (e.g., the dire threat of dementia with longer life expectancy) (1). It is now considered that normal brain function can persist into old age with adaptive, compensatory, and learning capabilities occurring at all ages (2–7). Extension of mental and neurologic competence in old age is a characteristic of successful aging (Chapter 3) (Box 1).

TABLE 1 Aging in the Central Nervous System Induces Structural and Biochemical Changes Resulting in Functional Consequences

Structural changes
Regional selectivity
Neuronal loss/gliosis
Reduced dendrites and dendritic spines
Synaptic susceptibility
Vascular lesions
Biochemical changes
Neurotransmitter imbalance
Membrane alterations
Metabolic disturbances
Intra-intercellular degeneration
Cell adhesion alterations
Neurotropic changes
Functional consequences
Sensory and motor decrements
Circadian (sleep) alterations and EEG changes
Cognitive impairment
Increased neurologic and psychiatric pathology
Impaired homeostasis

Abbreviation: EEG, electroencephalogram.

STRUCTURAL CHANGES

Brain Weight

Brain weight, size, volume, and metabolism measured by imaging techniques do not differ significantly in adult and old

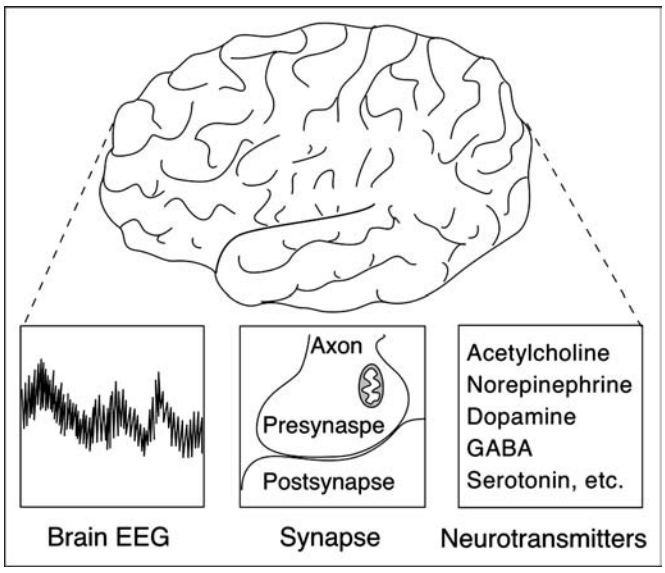


FIGURE 1 Diagram of electrophysiological (brain EEG), functional (synapse), and chemical (neurotransmitters) sites for changes in the brain with aging. *Abbreviations*: EEG, electroencephalogram; GABA, γ-aminobutyric acid.

BOX 1 *Persistence of Brain Plasticity in Old Age*

One of the outstanding properties of the central nervous system (CNS) is its "plasticity," that is, its capacity to be "shaped or formed or influenced" by external and internal stimuli as well as to learn and to recover from damage. In response to stimuli, neurons can change their signals, transforming operations to adapt to new requirements. If the neuron has undergone injury or loss of snapses, adaptation is reflected in the sprouting of new dendrites, axons, and synapses as in "reactive synaptogenesis" (8). Glial cells, specifically astrocytes, in addition to increasing neuronal stability, may also be involved in regulating the number of neurons and synapses (9–13).

Plasticity, defined as long-term compensatory and adaptive change, is most effective during CNS development. During embryonic (in humans) and early postnatal (in rodents) development, a number of factors, including thyroid and sex hormones, when present at "critical periods" of ontogenesis, determine CNS differentiation and maturation (14 –18). In the aging brain, the degree of plasticity declines, but is not entirely lost.

Programs for prevention or treatment of CNS disorders exploit the current advances in molecular biology and genetics to locate and to target for intervention specific molecules responsible for CNS pathology as well as to identify susceptibility genes and risk factors for complex CNS diseases (■ Chapter 3). The possible use of embryonic neural transplants, cultured neurons, and stem cell implants to replace lost or impaired neurns offer promising avenues of treatment (19–21). For example, embryonic cells implanted in the area of basal ganglia survive, grow, and differentiate into neurons (22). While restoration of normal motor function has not yet been achieved (23,24), these implantations and similar studies underline the brain's capability to sustain new cell growth and to promote cell differentiation even in old individuals, and raise new, exciting research prospects (25). However, this approach may not be as effective as previously thought, and current results suggest that caution should be exercised (22–25).

individuals without neurologic or mental disorders (Fig. 2) (26–29). The 6% to 11% decrease in brain weight registered in some healthy elderly individuals contrasts with the severe cortical atrophy (Fig. 3) reported in many (but not all) patients with Alzheimer disease (27–30). The shrinkage of a healthy brain in later decades of life does not appear to result in any significant loss of mental ability.

The close association of a larger brain with enhanced functional and behavioral capabilities is well justified in evolutionary and phylogenetic terms (31). Heavier brain weight (relative to body weight) and larger cerebral and cerebellar cortex have been implicated in the longer life span of humans compared to other species (Chapter 3). Correlation of size and function began to be appreciated in the late nineteenth century, when, for example, it was observed that primarily "visual" animal species had enlarged superior colliculi (involved in vision), and primarily "auditory" species had enlarged inferior colliculi (involved in hearing) (32). However, association of brain size with intelligence does not apply to the relatively minor differences in structure and function among individuals of different body size, within the same species; in humans, given the redundancy of neural cells and their interconnections, the differences in brain size among individuals of adult and old age have little functional significance. Experimental animals vary with respect to aging and brain size: rats do not show any changes with aging (33), but dogs and monkeys demonstrate cerebral atrophy (34).

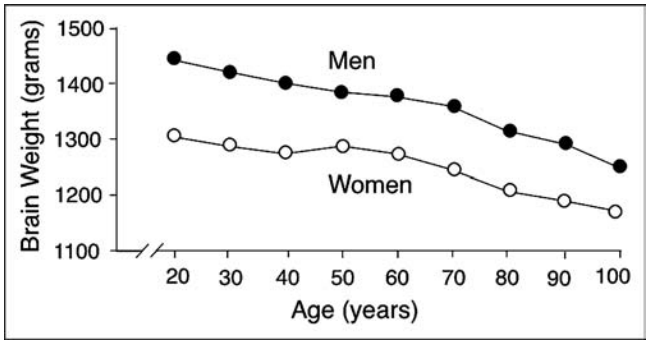


FIGURE 2 Changes in brain weight with aging in human males and females.

■ Neural Cell Number

The adult human brain contains approximately 10^{12} neurons and 10 to 15 times that number of glial cells. Each neuron has, on average, 10,000 connections (from a few thousand to over 100,000), resulting in an extraordinarily large total number (about 10^{15}). It is through these connections that the nervous system exerts its essential role in directly communicating with the internal and external environment, and, as an intermediary, between the two environments. CNS cells include neurons, and glial and endothelial cells (Box 2).

Aging affects nervous structures differentially. One striking example of this diversity involves neuronal loss. In the brain of old persons without apparent functional or pathologic deficits, loss of neurons is limited to discrete areas and shows considerable individual variability (35). In some areas of the cerebral cortex and in the cerebellum, the number of neurons remains essentially unchanged throughout life, except perhaps at very old ages (36). Other areas where losses may occur are the locus ceruleus (catecholaminergic neurons), the substantia nigra (dopaminergic neurons), the nucleus basalis of Meynert, and the hippocampus (cholinergic neurons). In all brain areas, neuronal loss appears modest compared to the redundancy of existing neurons.

Although new neurons actually continue to form in some areas of the brain in adult primates (37), neuronal loss may become quite severe in some aging-associated diseases, as for example, the marked loss of cholinergic neurons in the frontal cortex, nucleus basalis of Meynert, and hippocampus in about half of patients with Alzheimer's disease (AD) (38) and of dopaminergic neurons in the substantia nigra in patients with PD (39).

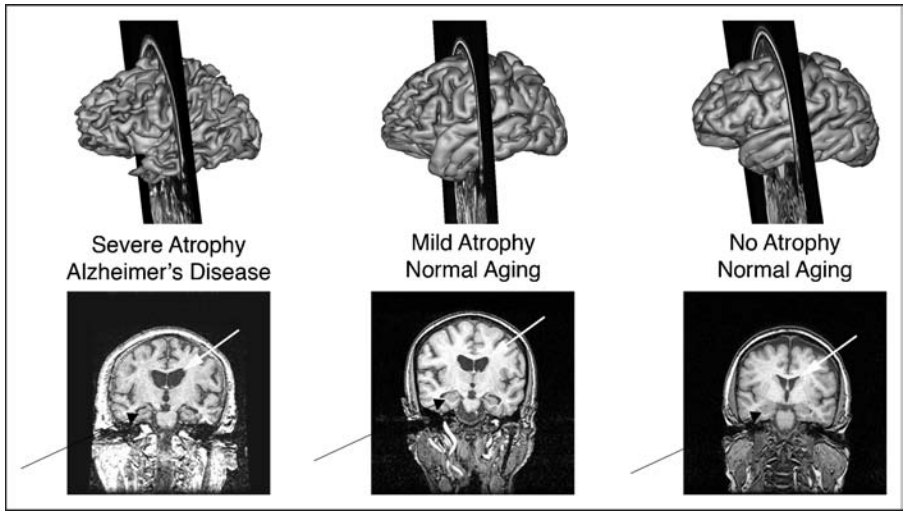


FIGURE 3 Magnetic resonance images from two normal older individuals, one not demonstrating significant cortical atrophy and one demonstrating mild cortical atrophy. A third image is from a patient with Alzheimer's disease, demonstrating severe cortical atrophy. The cortical atrophy is seen in the topmost images that display the brain surface. In addition, the hippocampus (*black arrow*) in all three individuals, as seen on the image slices in a coronal plane, shows varying degrees of atrophy that generally parallel the atrophy of the cortex. Likewise, note that the ventricles are significantly enlarged (*white arrows*) in both mild and severe atrophy in comparison to the individual with no atrophy. *Source*: Courtesy of Dr. W. J. Jagust.

Neuronal number in experimental animals decreases slightly with aging, depending on the area considered, the type of cell, and the animal species. For example, in the cerebral cortex of rats, the number of neurons remains unchanged with age (33). Reduced numbers of neurons have been reported in discrete brain areas of aged monkeys, guinea pigs, and mice (18,34). When the number of neurons is examined in these animals at progressive ages throughout their life spans, a major reduction in cell number often occurs at young rather than old ages.

In contrast to the possible loss of neurons, the number of glial cells increases with aging in most areas, and this increase, or gliosis, involves primarily the neuroglial cells (of the same embryonic ectodermic origin as the neurons, Box 2). Gliosis is a

BOX 2 *Cells of the Central Nervous System: Changes with Aging*

Neurons are regarded as the primary functional cells even though the glial cells are more numerous. Dendrites and axons are cell processes branching from neurons and conducting impulses to or from the cell body. Communication between neurons is through synapses and synaptic spines (the latter, small knobs projecting from dendrites). Synapses are composed of the presynaptic membrane (often enlarged to form terminal buttons or knobs), a cleft, and a postsynaptic membrane. Present in the spines are vesicles or granules containing the synaptic transmitters synthesized in the cell. The axons may be surrounded by a specialized membrane, myelin, or may be unmyelinated. The myelinated fibers transmit the nerve impulse more rapidly than do the unmyelinated ones.

While most neurons do not divide in the adult brain, some do: the neurons that are capable of regeneration are located in the olfactory bulb and hippocampus. Additionally, the ependymal cells located in proximity to the central ventricles have the capacity to transform into multipotent cells, capable of generating neurons. Also, neuroglial cells, particularly astrocytes, classically considered part of a committed astroglial lineage, can de-differentiate into neuroblasts.

The glial cells comprise the neuroglia, of ectodermal origin like neurons, and the microglia, of mesodermal origin, and part of the immune system. Both neuroglial and microglial cells continue to divide throughout life. *Neuroglial cells,* long shunned as the mere "glue" of the central nervous system (CNS), as their Greek etymology implies, in fact play critical roles in brain plasticity by modulating neuronal transmission and regeneration. These cells may assume a neuronal precursor role in addition to their support of neurotransmission and metabolism (astrocytes) and myelination (oligodendrocytes). *Microglial cells* play a defensive role in CNS phagocytosis and inflammatory responses.

Endothelial cells of cerebral capillaries, in contrast to those in other tissues, form tight junctions that do not permit the passage of substances through the junctions. Cerebral capillaries are enveloped by the end-feet of astrocytes, a special feature that hinders the exchanges across capillary walls between plasma and interstitial fluid. The resulting, so-called blood-brain barrier prevents the entry of endogenous metabolites and exogenous toxins and drugs of the blood into the brain, and reciprocally, the entry of the neurotransmitters into the general circulation.

normal response to neuronal damage at all ages and may represent a compensatory response not only to the modest neuronal loss, but also to the neuronal, metabolic, and functional impairment (33). *Gliosis persists in old age and is considered to be part of a repertory of compensatory responses that protect neuronal function and plasticity.* The number of microglia cells (part of the immune system) remains essentially unchanged, except in the presence of inflammation, when the cell number increases (33).

■ Dendritic and Synaptic Losses

The normal organization of neuronal networks is maintained in many healthy elderly, but for some older individuals, the number of dendrites and dendritic spines may be reduced (the so-called "denudation" of neurons). For example, the cortex of a typical young individual shows large pyramidal cells with abundant dendrites rich in dendritic spines. A corresponding zone, in a typical old individual, shows a striking loss of dendrites and spines (40). Dendrites function as receptor membranes of the neurons and represent the sites of excitatory or inhibitory activity. Dendritic spines, tiny and numerous on each dendrite, amplify such activity, and by doing so, control increases in synaptic calcium transport that may serve for induction of information storage. Loss of dendrites and spines results in neuronal isolation and failure of interneuronal communication. With reduced dendrites, synapses are lost, neurotransmission is altered, and communication within and without the nervous system is impaired. However, further reflecting the persistence of plasticity of the brain, it is also known that increased density of dendritic growth and length of individual dendrites may occur in different brain areas—including the cerebral cortex—in humans in their seventies (41,42).

Inasmuch as dendrites undergo a certain degree of continuous renewal, the denudation of the neurons may not be a true loss, but rather a slowing of the renewal process. When the loss of dendrites is viewed in a network of neurons, the consequences of diminished connectivity become apparent (43,44). In normal aging, with continued environmental stimulation, dendritic loss may be minimal, absent, or even supplemented with a degree of dendritic outgrowth. In dementia (Chapter 7), the dendritic loss is severe and progressive (Fig. 4).

The decreased number of synapses in discrete areas of the aging brain follows the corresponding loss of dendrites and dendritic spines. Alterations in synaptic components—membrane, vesicles, and granules—have been variously reported. The "reactive synaptogenesis" or axonal sprouting that follows the loss of a neuron is not entirely lost but decreases with aging (8). *Reactive synaptogenesis represents a compensatory reaction to neuronal loss or damage and is characterized by an increase in the number of synaptic contacts provided by the nearby neurons.* Such a compensation, although less efficient, can persist in the old brain.

As "hyperconnectivity" resulting from neurologic causes (e.g., temporal epilepsy) may lead to heightened attention, perceptions, memories, and images, progressive loss of interneuronal communication may cause a downward shifting of neurologic and mental processes. Relatively impaired CNS function in normal old age may be due to a multifactorial "hypoconnectivity" and increasing "rigidity" (consequent to vascular and metabolic alterations) rather than to regional and moderate neuronal losses. Several functions [e.g., electrocardiogram (ECG), electroencephalography (EEG), pulsatile hormonal secretions] that were formerly thought to be relatively periodic show a complex type of variability reminiscent of "chaos" (i.e., unpredictable behavior arising from internal feedback from interconnected loops of nonlinear systems) (45). Aging, by reducing the interconnectedness and complexity of these systems, might diminish the plasticity and dynamics of brain processes (46–48).

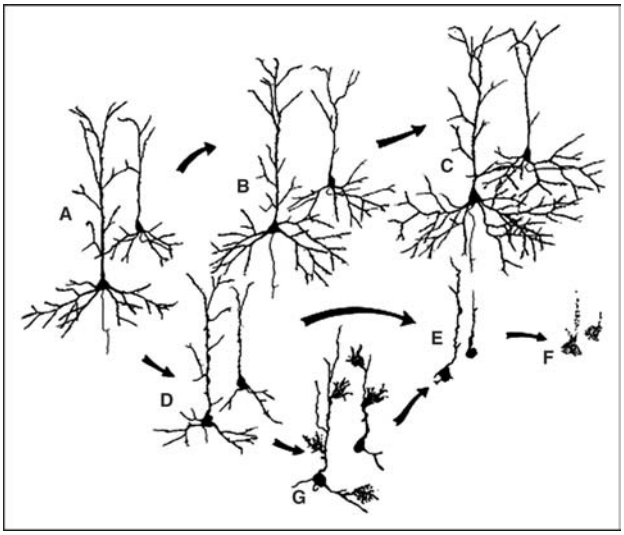


FIGURE 4 Semischematized drawing summarizing the changes that may occur in pyramidal neurons of the aging human cerebral cortex. Sequence **A**, **B**, **C** follows the changes that may occur in the normal aging cortex under the effects of continued physical and cognitive "challenge" in the probable presence of continuing small amounts of neuronal loss. Increasing dendritic growth, especially in the peripheral portions of the basilar dendrites, reflects dendritic response to optimal cortical "loading," plus presumed supplementary growth to fill neurophil space left by dendrite systems of those neurons that die. Sequence **A**, **D**, **E**, **F** epitomizes the progressive degenerative changes that characterize senile dementia of the Alzheimer type and includes progressive loss of dendritic spines and dendritic branches culminating in death of the cell. Basilar dendrite loss precedes loss of the apical shaft. Sequence **A**, **D**, **G**, **E**, **F** represents a unique pattern of deterioration of the dendrite tree found only in the familial type of presenile dementia of AD. During the period of dendritic degeneration, bursts of spine-rich dendrites in clusters appear along the surface of the dying dendrite shafts. Such changes have been seen in neocortex, archicortex (hippocampus), and cerebellar cortex. The cause and mechanisms of these eruptive attempts at regrowth, even as the neurons are in a degenerative phase, are unknown. *Abbreviation*: AD, Alzheimer's disease. *Source*: Courtesy of Dr. A. B. Scheibel.

A number of other harmful changes in brain structure, usually quite moderate and with considerable individual variability, may occur in normal aging. These include alterations in the axons that may contribute to disruption of neural circuitry, demyelination, axonal swelling, and changes in the number of neurofilaments and neurotubules. Changes occurring with aging in the neural cytoskeleton [as in neurofibrillary tangles (NFTs)], accumulation of amyloid proteins [as in neuritic plaques (NPs)], impaired cell survival (reduced antiapoptotic synaptic proteins), alterations in the cerebrospinal fluid composition and volume, and other changes are discussed below and in ■ Chapter 7 in relation to their repercussions (e.g., dementia).

■ Neural Cell Pathology

As in the case of cell and synaptic loss, cell pathology also occurs in the normal aging brain, but the number of affected

structures is quite small and confined to discrete areas (e.g., the hippocampus). This contrasts with the extensive pathology associated with most neurodegenerative diseases (e.g., AD, PD, multi-infarct dementia) (49).

Major cell pathologic changes are of a degenerative nature (i.e., progressively leading to impaired function and death) and are manifested by the following:

- *Intracellular accumulation* of abnormal inclusions such as lipofuscin, melanin, Lewy bodies, and neurofibrillary and amyloid proteins
- *Extracellular accumulation* of abnormal amyloid proteins in NPs and surrounding cerebral blood vessels [perhaps deriving from the systemic circulation through defects of the blood-brain barrier (BBB) permeability], and of ubiquitin, hyperphosphorylated tau proteins, and other abnormal proteins
- *Vascular (atherosclerotic)* alterations that may induce hemorrhages and infarcts (strokes) consequent to rupture or obstruction of blood vessels (as in multi-infarct dementia)

Lipofuscin and Melanin

Lipofuscin or "age pigment," the by-product of cellular autophagia (self-digestion) and lipid peroxidation due to free radical accumulation, has a protein and a lipid component. It accumulates with aging in most CNS cells, both neurons and glial cells, where it follows a regional distribution (e.g., in the hippocampus, cerebellum, anterior horn of spinal cord). It increases linearly with age also in other cells of the body (e.g., cardiac and muscle cells, macrophages, interstitial cells). Lipofuscin can be visualized as autofluorescent material (Fig. 5A and B) and, with the electron microscope, as dark granules, either scattered in the cytoplasm or clustered around the nucleus (Fig. 6A and B) (50). Lipofuscin's functional significance (if any) is unclear. The claim that lipofuscin accumulation interferes with intracellular function or that reduction of brain lipofuscin through the administration of antioxidants may lead to improved neuropsychologic behavior remains controversial.

Melanin pigment is localized primarily in the locus ceruleus and substantia nigra, where it imparts a dark coloring to the catecholaminergic, especially dopaminergic, cells of these regions. Melanin increases until about 60 years of age and then decreases, probably in parallel with the progressive loss of the heavily pigmented cells.

Accumulation of lipofuscin or melanin is not greater in the brain of old people affected by Alzheimer's disease, Down's syndrome, progeria, and most degenerative diseases compared to old people without these diseases.

Lewy and Hirano Bodies

The Lewy and Hirano bodies are eosinophilic, cytoplasmic inclusions, derived from the differential expression of particular proteins responsible for selective neuronal vulnerability (51). Lewy bodies are usually spheroid in shape with a dense central core (Fig. 7), and the Hirano bodies may often appear as spindle shaped and fusiform.

Lewy bodies may be present in aged individuals (60 years of age and older) without clinical evidence of PD, but are more numerous in those individuals affected by this disease. Indeed, the accumulation of such bodies, especially in the dopaminergic cells of the locus ceruleus and the substantia nigra, is considered a hallmark of PD (52). The accumulation of larger numbers of Lewy bodies in the cerebral cortical neurons of many demented individuals has led to the identification of a "diffuse Lewy body dementia," in which the severity of the dementia correlates with the increasing number of Lewy bodies (53).

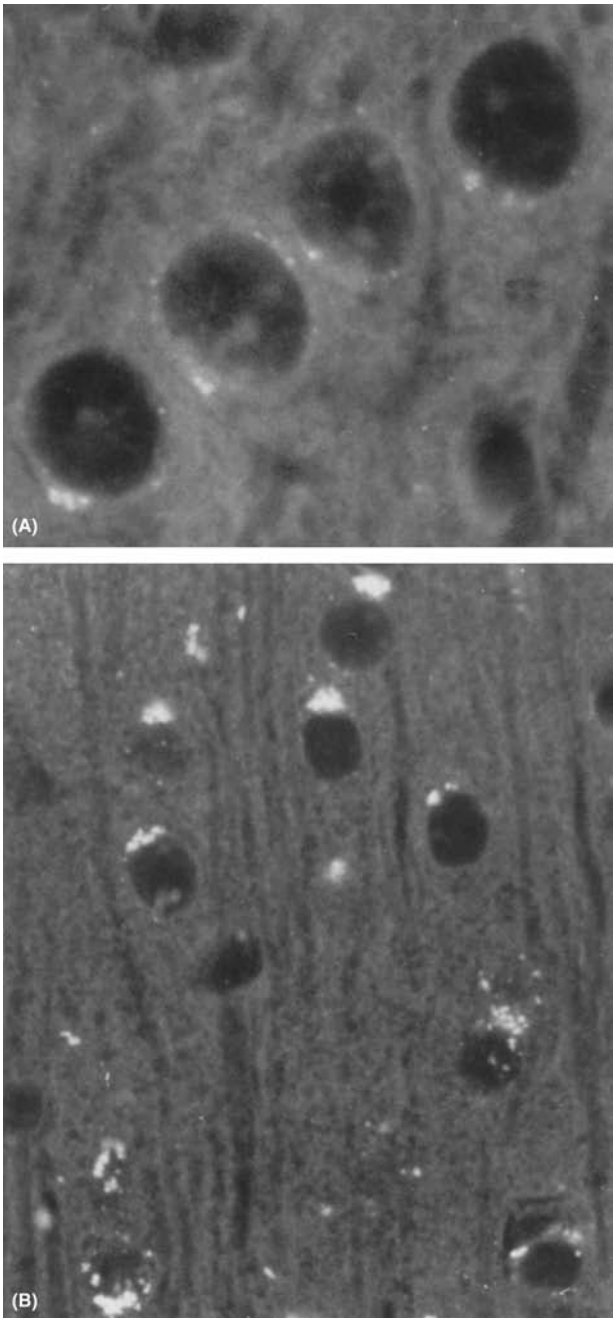
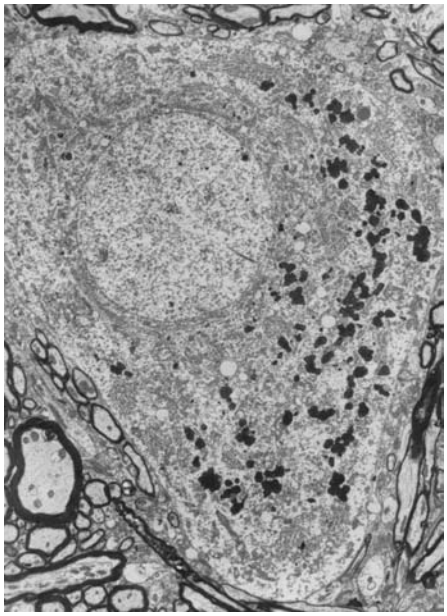


FIGURE 5 Photomicrograph of unstained sections under blue light fluorescence illustrating lipofuscin pigment in neuron somata in cerebral cortex of male Long–Evans rats (50). (**A**) Young adults (109–113 days) (original magnification ×32); (**B**) aged rats (763–972 days) (original magnification ×14). *Source*: Gerontological Society of America.

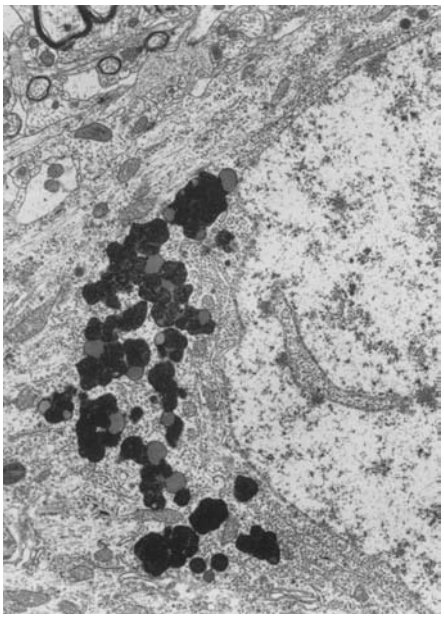
Hirano bodies in humans develop as changes related to old age; they are predominant in the hippocampus and more numerous in subjects with dementing illnesses, including Alzheimer's disease, Pick's disease (frontotemporal dementia), and amyotrophic lateral sclerosis (Chapter 7).

Neurofibrillary Tangles (NFTs) and Neuritic Plaques (NPs)

NFTs consist of intracellular tangled masses of fibrous elements, often in flame-shaped bundles, coursing the entire cell body



(A)



(B)

FIGURE 6 (**A**) A neuron from the cerebral cortex of a 605-day-old male Long-Evans rat (50). There are numerous dense bodies (lipofuscin) irregularly distributed in the perikaryon (original magnification ×1440); (**B**) a portion of a neuron from the cerebral cortex of the same 605-day-aged animal (50). The lipofuscin granules are clustered at one pole of the nucleus (original magnification ×6000). *Source*: Gerontological Society of America.

(Fig. 8A and 8B). The tangles are present in the normal aging brain, frequently in the hippocampus, and their accumulation in the cortex and other brain areas is one of the diagnostic signs of senile dementia in AD (Chapter 7). In AD, tangles would be in greatest number in the associative areas of the cortex, and their distribution may reflect a primary toxin/infection spread through the olfactory system (54,55). The density of the tangles has also been correlated with the dementia severity, cell loss, and cholinergic deficits (56,57).

Under the electron microscope, each fiber is seen to consist of a pair of filaments wound around each other to form a paired helical filament (PHF). *Chemically, PHFs are highly insoluble and are composed predominantly of the microtubule-associated protein (MAP) tau*. They react with monoclonal antibodies specific for neurofilament proteins or for MAPs (58). Because neurofilaments and microtubules are normal constituents of the cytoskeleton, the immunologic reactivity of PHF supports the hypothesis that several cytoskeletal proteins are linked in the formation of these abnormal, helical filaments (59).

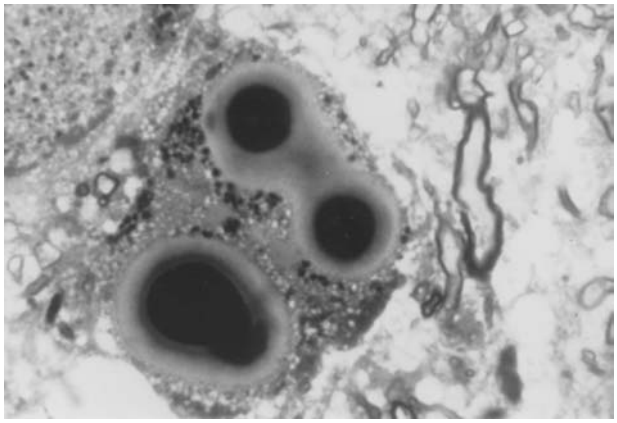


FIGURE 7 Lewy bodies, intraneuronal cytoplasmic inclusions. The dense core is composed of apparently random, tightly packed aggregations of filaments, vesicular profiles, and poorly resolved granular material containing neurofilament antigens. These bodies are found in substantia nigra as well as in other CNS regions. *Abbreviation*: CNS, central nervous system. *Source*: Courtesy of Dr. L. S. Forno.

Alterations of tau protein (i.e., a microtubular protein that enhances the polymerization of tubulin subunits) and of the enzyme protein kinase that catalyzes its phosphorylation are involved in the PHF generation. Tau proteins have attracted special attention because of their crucial role in regulating balance between stability and plasticity of the neuronal cytoskeleton (60). Tau proteins have several isoforms that result from alternative splicing during transcription from a gene located on chromosome 17. Tau undergoes phosphorylation at several sites along its length. Excessively phosphorylated tau leads to aggregation of tau molecules into PHFs and subsequently into insoluble NFTs. Although the cause of hyperphosphorylation is not known, a likely mechanism in AD may be the induction by elevated levels of amyloid-β-42 peptide (60–65). More discussion on the possible central role of tau protein in the pathogenesis of AD is presented in the next chapter (Chapter 7).

The NPs are situated extracellularly. Present in normal aging, they are abundant in AD, where they are found throughout the depth of the cerebral cortex, especially in the posterior neocortex—particularly the parietal and temporal regions—as well as in the frontal cortex and the hippocampus. Their distribution is similar to that of the NFTs, and the two structures are often found in close proximity (Fig. 9A and B). Typically, the plaque consists of a central core of amyloid. Amyloid is a generic term for proteinaceous fibrillar deposits characterized by:

- The properties of yellow to green birefringence in crossed polaroid illumination after Congo red dye staining
- A large amyloid precursor protein and its cleavage product, β-amyloid peptide (considered by many researchers to be the pathogenic molecule in AD)
- Insolubility and resistance to enzymatic digestion

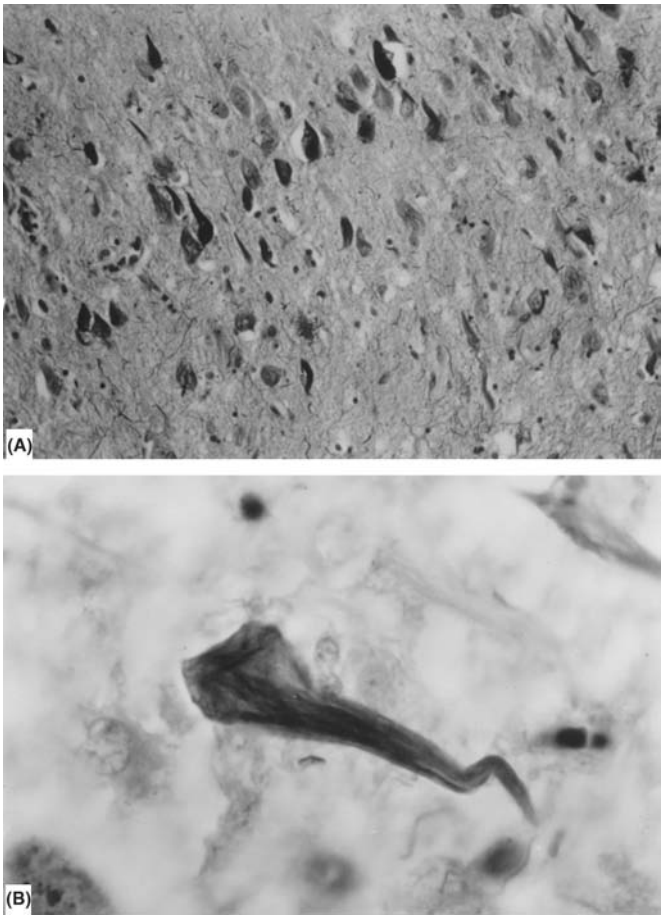


FIGURE 8 Neurofibrillary tangles in hippocampus pyramidal layer. (**A**) Neurofibrillary tangles (low magnification) accumulate in neuronal bodies and stain very darkly; (**B**) a neurofibrillary tangle (high power) shows the characteristic flame shape. *Source*: Courtesy of Dr. L. S. Forno.

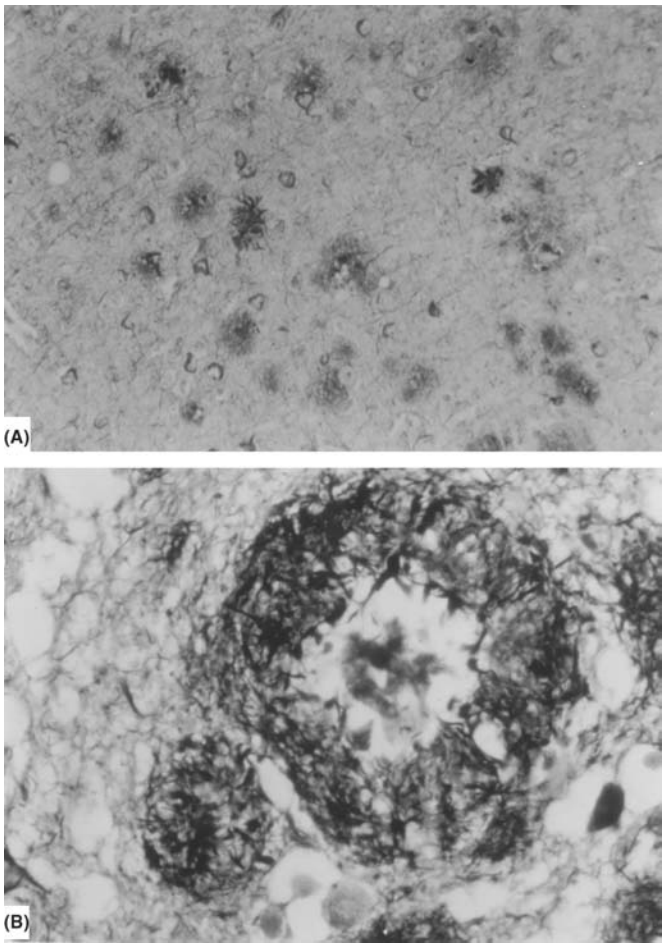


FIGURE 9 Neuritic plaques in frontal cortex, silent area: (**A**) presence of neuritic plaques (*large*) and neurofibrillary tangles (*small*) (low power); (**B**) neuritic plaques (high power). *Source*: Courtesy of Dr. L. S. Forno.

- Coarse, silver-stained fibers surrounding the plaque and represented, under the electron microscope, as many dystrophic axon segments with degenerated mitochondria, synaptic complexes, and dense bodies

Glial, especially microglial, cells react to the NP and accumulate about the abnormal nerve cell processes (Fig. 10). One theory is that development of the plaque proceeds first from abnormalities of the neural processes, is then followed by deposition of amyloid, and, later, by stimulation of reactive microglial proliferation. Another view proposes that the primary formation of the plaque depends on the leakage of amyloid from the capillaries or of a precursor substance of blood origin that diffuses into the cerebral tissue. This amyloid deposit would accumulate extracellularly, leading to the destruction of neurons and simultaneous proliferation of microglial cells (Chapter 7).

The presence of abnormal protein aggregates, such as those found in the NFTs and NPs (and abundant in AD), stimulates the ubiquitin-dependent protein degradation system. Thus, ubiquitin accumulates not only in the brain of AD patients (66,67), but also in PD patients (67). In normal aging, where fibers, plaques, and Lewy bodies are few, ubiquitin is only minimally activated. Oxidation of proteins is prominent in many cells, and free-radical–mediated oxidation affects the activity of a number of brain enzymes as well. With aging,

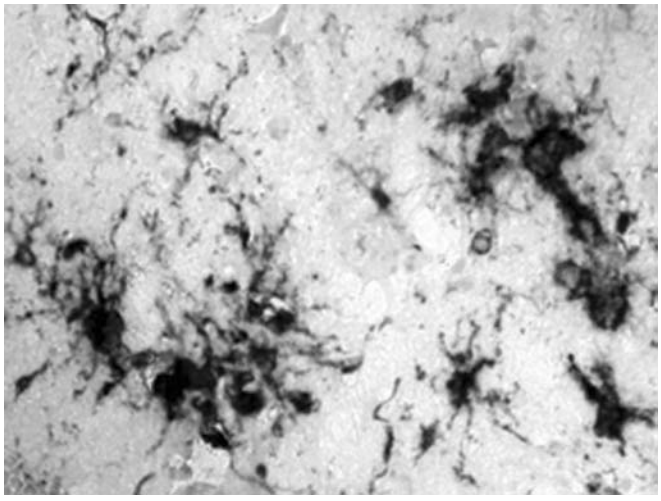


FIGURE 10 Microglia cells surrounding a neuritic plaque. The neuritic plaque is usually surrounded by microglial cells trying to surround the neuritic plaque and limit the possible damage to the interstitial tissue. The increased number of microglia generate an inflammatory reaction around the neuritic plaque. The use of anti-inflammatory drugs in Alzheimer's disease treatment is based on reducing the inflammatory involvement in the neuritic plaque. *Source*: Courtesy of P.C. McGeer.

oxidative damage and glycosylation of proteins result from the decreased rate of protein turnover.

Amyloid and Amyloidoses

Amyloid deposits are not confined to the brain but may occur in several tissues and organs, where their presence is noted in a number of conditions classified under the general term "amyloidoses," which represent a number of diseases characterized by extracellular accumulation of insoluble fibrillar proteins. These diseases show pronounced age dependence, and their prevalence increases rapidly toward the end of the life span. Inasmuch as amyloid is present in some of the neurodegenerative diseases prevalent in old age, considerable attention is being given to understanding the structure of the deposits in the brain and to finding therapies to prevent their formation or to induce their dissolution.

Amyloidoses are classified on the basis of the biochemical composition of the amyloid subunit protein in the following:

- *Primary systemic amyloidosis*: As in myeloma, amyloid is produced by proteolytic cleavage of the N-terminal variable region of the immunoglobulin (Ig) light chain in phagocytic cells.
- *Secondary amyloidoses*: These occur in chronic inflammation with accumulation of amyloid A protein.
- *Familial amyloidoses*: Prealbumin is the major accumulating protein.

Senile cardiac amyloidosis is present in 65% of hearts from persons 90 years of age and older. Cardiac focal deposits of amyloid have few functional consequences, but extensive distribution is associated with congestive heart failure and fibrillation (Chapter 20). Other forms are found in senile lungs, liver, kidneys, and, in general, in senescent or injured tissues. Controversy still exists regarding the origin, deposition, and toxicity of amyloid (Chapter 7).

■ BIOCHEMICAL CHANGES

■ Neurotransmission and Cell Communication

Information processing in the nervous system involves neurons "talking" to each other or with target cells. Research in neurotransmission, including neurotransmitter turnover, release, and binding to receptor is central to our understanding of CNS aging. Chemical transmission requires a series of events (Box 3). One of the most studied aspects of aging of the nervous system involves neurotransmitter changes at the synapse. In the healthy elderly, neurotransmitter levels and number, and the affinity of their receptors, and the activity of their metabolic enzymes undergo modest changes circumscribed to specific brain structures and individual neurotransmitter systems. In some diseases, however, a definite relationship exists between loss of one (or several) neurotransmitter(s) and abnormal brain function. For example, dopamine (DA) deficit in the nigrostriatal pathway is associated with PD, and acetylcholine (ACh) deficit in the Meynert nucleus is associated with AD.

Many synapses are classified according to the major neurotransmitter released at their site. Examples of synaptic transmitters include ACh, norepinephrine (NE), DA, serotonin (5-hydroxy-tryptamine), and γ-aminobutyric acid (GABA). Synthesis, storage, release, postsynaptic interactions, and inactivation of some neurotransmitters are summarized briefly (Fig. 11) (Box 3) (Table 2).

Several transmitters, often a classic neurotransmitter and a peptide, may coexist within the same neuron. Such

BOX 3 *Chemical Classification of Synapses and Neurotransmitters*

Known transmitters include amines, amino acids, peptides, and gases (Table 2). Of these, some may be viewed as classic, our knowledge of them extending back almost one century; they are *acetylcholine*, the *catechol-amines*—norepinephrine, epinephrine and dopamine—and *serotonin*.

Others include *γ-aminobutyric acid (GABA)*, certain amino acids such as *glycine, glutamate, and aspartate*. GABA transmits inhibitory impulses and aspartate and glutamate usually mediate excitatory synaptic transmission. Their role is crucial to several normal functions of the nervous system, including memory and long-term modification of synaptic transmission, perception of injurious stimuli, activation of membrane receptor, and transmembrane calcium and sodium fluxes (68).

Among the peptides, a few of the more extensively studied are enkephalin, substance P, and hypothalamic neurohormones. Some peptides such as cholecystokinin and somatostatin are found both in the brain and the gastrointestinal tract.

The soluble gas *nitric oxide (NO)*, viewed originally as a toxic molecule (in cigarette smoke and smog), is now considered to be a biologic messenger in mammals that acts by nonsynaptic intercellular signaling (69,70). NO is generated by the enzyme NO synthase (acting on arginine), sensitive to calcium and calmodulin modulation. Unlike other neurotransmitters, NO is not stored in or released from vesicles in the neuron, but rather diffuses out from the cell of origin. NO is also found in genitalia (where it is essential for penile erection) and in the gut (where it is involved in peristalsis). Carbon monoxide (CO), originally viewed as an exclusively toxic gas (70), and metals such as zinc (71,72) are now considered to be putative neurotransmitters in some specific neurons (in the olfactory bulb and hippocampus) often affected by aging.

A membrane-associated, synaptic vesicle protein, *synapsin*, may also influence neurotransmission by increasing the number of synapses, synaptic vesicles, and contacts (73). There are four synapsins, each with similar amino acid sequences, generated by two alternative gene splicings. All four are associated with the cell cytoskeleton and, like some of the other cytoskeletal proteins, they may undergo phosphorylation during synaptic transmission. Synaptic vesicles may be destabilized in the absence of synaptins and, during synaptic plasticity, upregulation of release may be defective.

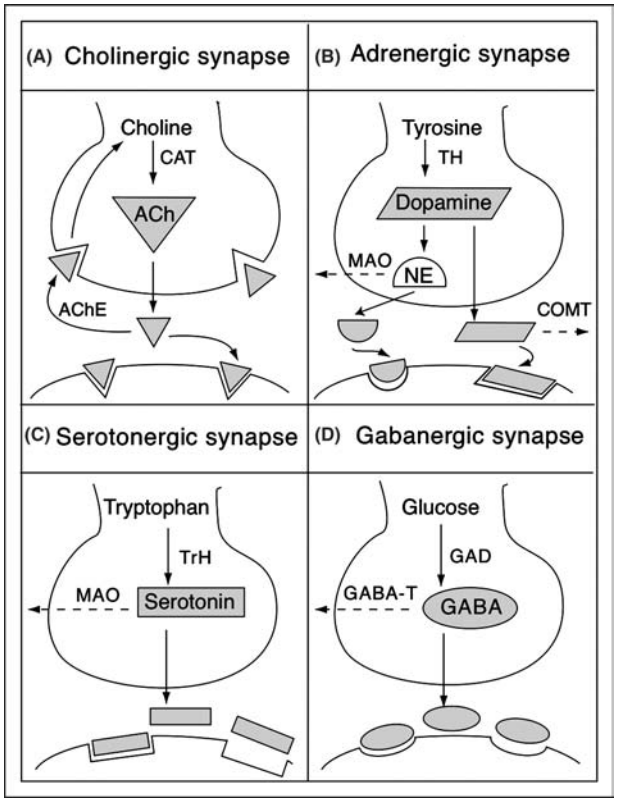


FIGURE 11 Diagrams of established CNS synapses illustrating presynaptic synthesis, storage, metabolism, synaptic release, and postsynaptic binding. (**A**) Cholinergic synapse. Choline is the precursor of the transmitter ACh through the actions of the synthesizing enzyme CAT. ACh is free in the cytoplasm of the prejunctional axonal ending and is also contained in vesicles. Release of cytoplasmic ACh initiates transmembrane events. Several distinct pre- and postsynaptic ACh receptors have been described. Receptor-bound ACh is inactivated by the enzyme AChE. (**B**) Adrenergic synapse. NE precursor is tyrosine, and the enzymes involved in NE synthesis are TH, DOD, and DBH. The primary degrading enzyme is MAO. NE is stored in vesicles. Once released, transynaptic degradation is initiated by COMT. Several pre- and postsynaptic adrenergic receptors have so far been identified. Released NE not degraded by COMT may be taken up by the presynaptic neuron and internalized. Note the simultaneous production of dopamine at its release at the synapse. (**C**) Serotonergic synapse. The precursor of serotonin is tryptophan, and the synthesizing enzyme is TrH; the degrading enzyme is MAO. Several different types of cell surface receptors are known. (**D**) GABAergic synapse. GABA is formed in the GABA-shunt pathway of the Krebs cycle. It is formed by the action of GAD from glutamate and is metabolized by deamination. At least two receptors for GABA have been identified in the CNS. *Abbreviations*: ACh, acetylcholine; CAT, choline acetyltransferase; AChE, acetylcholinesterase; NE, norepinephrine; TH, tyrosine hydroxylase; DOD, dopa-decarboxylase; DBH, dopamine-β-hydroxylase; MAO, mitochondrial monamine oxidase; COMT, catechol-*o*-methyltransferase; TrH, tryptophan hydroxylase; GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; CNS, central nervous system; GABA-T, GABA-transaminase.

"coexistence," resulting in "coutilization" of several transmitters, vastly expands the potential diversity of synaptic communications. Indeed, the peptide often "modulates" or "modifies" the action of the classic neurotransmitter. For example, serotonin coexists with substance P and thyrotropin-releasing hormone in neurons of the rat medulla and spinal cord, where the three neurotransmitters collaborate to regulate some motor and behavioral responses. Receptors may increase in number or affinity for the respective neurotransmitter and its agonists in response to a decrease in concentration of the neurotransmitter, and vice versa (up- and down-regulation of receptors).

TABLE 2 Neurotransmitters and Modulators in the Nervous System

Amines	Amino acids	Peptides	Others
Acetylcholine	Glutamate	Enkephalin	Nitric oxide
Catecholamines	Aspartate	Cholecystokinin	Carbon monoxide
Norepinephrine	Glycine	Substance P	Zinc
Epinephrine	GABA[a]	VIP[a]	Synapsins
Dopamine	Taurine	Somatostatin	Cell adhesion molecules
Serotonin[a]	Histamine	TRH[a]	Neurotropins
			Others

[a] Serotonin, 5-hydroxytryptamine, or 5-HT.
Abbreviations: GABA, γ-amino butyric acid; VIP, vasoactive intestinal polypeptide; TRH, thyrotropin-releasing hormone.

Synaptic diversification and multiplicity of neurotransmitters result in increasing complexity of neuronal communication and the need for a fine tuning of all chemical messengers, transmitters, and their modulators (Box 4) (Table 3). Neurotransmitters have excitatory or inhibitory actions, and it is most important to the function of the nervous system that these actions must remain in balance. With aging, alterations in function are more likely to occur as a consequence of imbalance among neurotransmitters, rather than as a consequence of a global alteration of a single neurotransmitter. Such imbalance could involve the classic neurotransmitters for which evidence is already available, or the neuropeptides, or both. In the human brain, knowledge and understanding of age differences in neurotransmitter levels, activities of their synthesizing and degrading enzymes, and receptor number and affinity are still fragmentary and await further clarification.

■ Neurotransmitter Imbalance

Imbalance among neurotransmitters, rather than severe depletion or excess of a particular one, may contribute to functional disorders with aging. In early studies in rats, neurotransmitters were compared at progressive ages in the cerebral hemispheres, the hypothalamus, and the corpus striatum. Overall, two major changes were noted: serotonin concentrations remained unchanged until very old age when they then increased, and NE and DA concentrations progressively decreased, starting at relatively less advanced ages. Thus, in the aging rat brain, the ratio of serotonin to the catecholamines progressively increases. For the resulting imbalance in the rat brain to become functionally manifest, it is sufficient for a single neurotransmitter, rather than several simultaneously, to be altered to induce a cascade of changes.

A final point to emphasize here is that each neurotransmitter has its own regional timetable of aging. For example, in rats, serotonin concentration remains unaltered in the cerebral hemispheres until three years of age, while dopamine levels start declining at one year of age (74). It is this differential change that may be responsible for creating an early neurotransmitter imbalance, well before significant decrements in each transmitter may be detected.

■ Aging of Dopaminergic Systems and Parkinson's Disease

The dopaminergic system undergoes changes with normal aging and with aging-associated diseases. Dopamine is present

BOX 4 *Analysis of Synaptic Chemical Events: Difficulties of Measurement in Humans*

Chemical transmission requires the following series of events:

- Synthesis of the neurotransmitter at the presynaptic nerve terminal
- Storage of some neurotransmitters in vesicles
- Release of the neurotransmitter into the synaptic space (between pre- and postsynaptic neurons)
- Presence of specific receptors for the neurotransmitter on the postsynaptic membrane and binding of the neurotransmitter to the receptors, thereby effecting nerve stimulation
- Termination of the action of the released neurotransmitter by its diffusion from the synaptic space, its metabolism, or its reuptake by the presynaptic neuron

In humans, information is often gained by indirect measurement of neurotransmitters and their metabolites in urine, blood, and cerebrospinal fluid and, more recently, directly in the brain by labeled probes and imaging devices. The use of pharmacological agonists and antagonists of the neurotransmitter under study can provide an alternate approach. Under the best conditions, the difficulty in controlling the clinical setting with humans and/or accurately measuring subtle physiological changes in status suggest that much is to be gained by the use of animal models in studying cellular and molecular mechanisms of synaptic function. Even in animals, the information on aging is still scarce and needs to be pursued vigorously.

throughout the brain, but its levels vary significantly from region to region. Currently, four major dopaminergic pathways are recognized, the most prominent of which originates in the substantia nigra and extends to the corpus striatum and other structures of the basal ganglia; the other three dopaminergic pathways connect with the limbic system, the hypothalamus, and the cortex (Box 5) (Table 4). DA is also secreted by interneurons in sympathetic ganglia; hence, its administration stimulates several tissues and organs, thereby causing the undesirable side-effects often seen (e.g., nausea, constipation, cardiac irregularities, involuntary movements) with the therapeutic use of *L*-dopa.

With normal aging, DA content steadily decreases, particularly in the corpus striatum, probably as a consequence of the loss of dopaminergic neurons, perhaps due to free radical damage (39,75). This decrease in dopaminergic neurons may or may not be associated with motor disturbances, depending on the degree of neuronal loss or the severity of the action of excitotoxins (such as glutamate) (76–79). When neuronal loss is severe, the neurologic alterations, which become more pronounced, are associated with progressively more severe motor decrements and other neurologic and clinical manifestations and are categorized as signs and symptoms of PD. This disorder involves rigidity (of the whole body), tremor (at rest), and akinesia (loss of motor function) or bradykinesia (slowed movement) (39,80,81). PD is a progressive disease of the elderly (about 85% of those affected are aged 70 years or older) in which the dopaminergic neurons projecting to the caudate nucleus and putamen degenerate due to reduced stimulation from the damaged substantia nigra (Fig.12). This damage is characterized by loss of neurons, gliosis, and the presence of Lewy bodies (52). In addition to PD, several forms of motor disabilities of similar etiopathology, but with only some of the motor deficits of PD (for example, lacking the resting tremor), are grouped under the term "parkinsonism."

TABLE 3 Major Chemical Events at Various Locations in the Synapse

Presynaptic events
Availability of neurotransmitter precursors
Enzymatic synthesis and degradation
Storage or release of neurotransmitters
Regulation of neurotransmitter release
Neurotransmitter re-uptake
Ionic regulation
Transynaptic events
Neurotransmitter released in synaptic cleft
Neurotransmitter degraded enzymatically
Postsynaptic events
Receptor binding
Enzymatic degradation
Ionic regulation
Second-messenger stimulated

As already mentioned, Lewy bodies may be found in normal aging, but their incidence is much greater not only in PD but also in AD patients (52). There is an overlap in pathology between PD and AD (Chapter 7). The prevalence of histological changes of the Alzheimer type is higher in Parkinson's patients than in age-matched unaffected populations. In Lewy body dementia (Chapter 7), the major symptom is cognitive failure, along with the motor disturbances, which may present differently than in AD patients (53).

Drug-Induced PD

A contaminant, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), of illegally synthesized heroin acts specifically to induce a motor-deficit syndrome in experimental animals (mice, rabbits, monkeys) similar to PD in humans (81). In young animals, the syndrome is only incompletely produced, whereas in old animals, the neuropathology and behavior of PD are completely reproduced. Only in primates is the presence of Lewy bodies observable following MPTP administration. Older neurons appear more vulnerable to the mitochondrial toxicity of MPTP, either (*i*) because of their lowered ability to recover from toxic damage or (*ii*) because the damage is cumulative with other damage that has occurred over time. While old age is the primary factor of AD, a variety of environmental agents and stressors may contribute to inducing other CNS degenerative diseases (Chapter 7). This MPTP drug model is particularly useful in transplantation studies to assess the efficiency of the transplanted tissue in overcoming the MPTP-induced damage.

While motor abnormalities dominate the clinical picture of PD, current DA replacement therapy permits more patients to live long enough so that some develop more advanced features of the disease, such as cognitive dysfunction, dementia, and postural instability, which are difficult to treat. Indeed, PD and AD may be viewed as part of clinical cases of which neuronal damage is the common feature.

BOX 5 *Major Dopaminergic Pathways in Human Brain*

The nigral-striatal pathway participates in the central control of motor functions by regulating the planning and programming of movement; in broad terms, it is involved in the process by which an abstract thought is converted into voluntary action. It consists of three nuclei—caudate nucleus and putamen (often referred together as corpus striatum) and globus pallidus—and the functionally related substantia nigra. Dopamine released at the caudate nucleus, inhibits the stimulatory (possibly cholinergic or glutaminergic) input from the motor cortex.

In the caudate, a balance is established between the inhibitory and excitatory control of motor activity. A deficit of dopamine and an increase in the cholinergic output result in rigor (state of extreme rigidity) and tremor (regular alternating contracting of antagonistic muscles) whereas an increase of dopamine or a decrease in the excitatory input results in writhing movements, as in chorea (rapid involuntary dancing-like movements).

Also involved in motor control is the nucleus accumbens in the septal region which controls locomotion (particularly forward movement).

Within the limbic system, other dopaminergic fibers synapse and mediate emotionality, sexual and aggressive behavior, and some neuroendocrine regulation.

In the hypothalamus, a rich dopaminergic network regulates several endocrine-releasing and inhibitory hormones. This dopaminergic activity will be discussed along with the endocrine system (■ Chapter 9).

A neocortical dopaminergic system is apparently activated by stress. Lesions of the system induce locomotor hyperactivity and inability to suppress behavioral "stereotypic responses." These responses can be blocked by drugs that act as dopamine inhibitors. Uncontrolled stimulation of dopamine receptors would induce a euphoric action or a psychotic action, and might be involved in manic and schizophrenic syndromes.

■ Treatment of PD

A number of symptomatic treatments are available. The cause of this disease as well as of other neurodegenerative ones remains unknown, although a number of environmental risks have been identified, including trauma, toxins, and pesticides (suggesting that PD symptoms may involve defective phosphorylation). In younger patients, genetic risks may be involved. A cure or a method of prevention for PD has not yet been found. The brain is a most complex structure—even incremental, less than perfectly successful therapies should be welcomed. Thus, while most of the neurologic and psychiatric diseases prevalent in old age are not yet "curable," they certainly are treatable to some degree (e.g., the ancillary symptoms such as aggressiveness). Current therapeutic approaches are listed in Table 5 and are briefly discussed below.

Pharmacological Strategies

Neurosurgery was the first therapeutic approach to parkinsonism. Replacement by L-DOPA of the lost striatal DA (adopted in the early 1960s) was welcomed as a safer alternative to surgery. In the neuronal pathway of catecholamine biosynthesis, the enzyme tyrosine hydroxylase catalyzes the conversion of the amino acid tyrosine to dihydroxyphenylalanine (*L*-dopa, levodopa), which is then converted to DA by the action of dopa-decarboxylase. *L*-Dopa, unlike DA, can pass the BBB (see below), and when administered as a drug, can then serve as a precursor of neuronal DA. Although treatment of patients with *L*-dopa may not provide a "cure," the treatment ameliorates some of the more disturbing symptoms and has been viewed as a model therapy for this aging-associated neurologic disorder. In clinical use, *L*-dopa must be administered concomitantly with a peripheral decarboxylase inhibitor (carbidopa); this is necessary (*i*) to minimize the peripheral (gastrointestinal, cardiovascular, and motor) side

TABLE 4 Major Dopaminergic Pathways

Substantia nigra to corpus striatum	Regulates control of motor function from cortex
Substantia nigra to nucleus accumbens	Controls locomotion (forward movement)
Limbic system	Regulates emotion, behavior (sexual and aggressive)
Hypothalamus	Regulates secretion of releasing and inhibiting hormones
Neocortex	Regulates locomotor activity

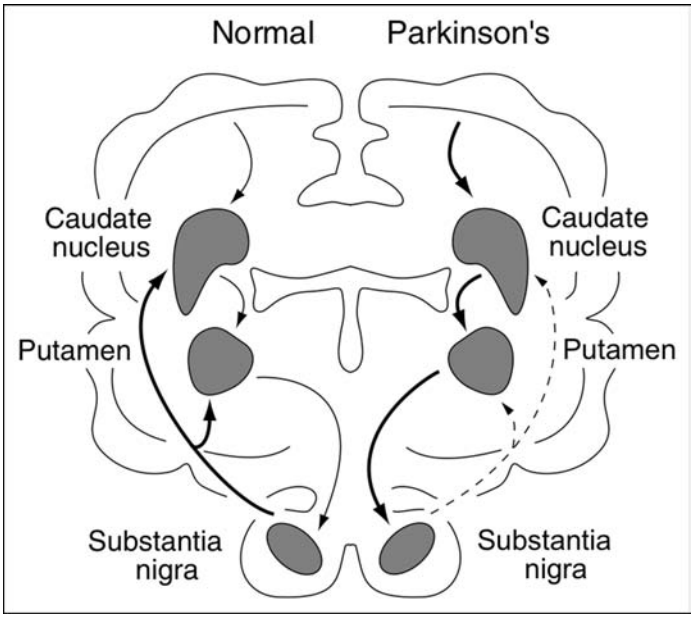


FIGURE 12 Dopaminergic nigral-striatal pathway in the normal (*left dark arrow*) and in parkinsonism (*right dashed line*). In parkinsonism, dopaminergic neurons of the substantia nigra are lost, reducing dopamine (*right dashed line*) to putamen-caudate (striatum) and subsequent control of cortical stimulatory effects. Increased excitatory transmission to extrapyramidal system (*broad open arrow*) is associated with tremor and rigor.

TABLE 5 Strategies for Treatment of Parkinson's Disease

Pharmacological
Neuroprotective
Surgical and electrostimulatory
Cell therapies

effects of DA, and (*ii*) to prevent increasing tolerance to the drug and, therefore, (*iii*) its decreased availability to the brain.

The original pharmacological treatment had been designed to reduce neural activity with cholinergic inhibitors. The loss of dopaminergic input to the striatum, in the presence of persisting cholinergic excitatory activity, disrupts basal ganglia circuitry. Cholinergic inhibitors are still used as an adjunct to replacement *L*-dopa therapy.

Despite its beneficial effects, *L*-dopa treatment has some contraindications such as motor complications, negative general and systemic effects such as gastrointestinal disturbances. In addition, there is a gradual loss of effectiveness after three to five years of administration, probably due to continuing loss of dopaminergic neurons and reduction of dopa-decarboxylase, with consequent deficit in DA synthesis. Because neurotoxicity from *L*-dopa may be due to the generation of oxidative species (79,82), it is often recommended that antioxidants (e.g., tocopherol) be coadministered to prevent or reduce accumulation of free radicals (eventually capable of destroying the dopaminergic cells).

Treatment with *L*-dopa is often used in combination with other drugs such as DA agonists (e.g., bromocriptine, pramipexole, ropinirole) (83,84), or inhibitors of the two enzymes involved in DA breakdown, monoamine oxidase (e.g., selegiline, rasagiline) (85), and catechol-*o*-methyltransferase (entacapone) (86), thereby extending the duration of action of *L*-dopa, or with a variety of anticholinergic drugs such as benzatropine and trihexyphenadyl.

Current pharmacological therapy will remain essentially ineffectual on a long-term basis until we understand more about the cause of dopaminergic cell loss. Recent evidence from postmortem brain and animal models of the disease (87) has suggested that early depletion of the antioxidant glutathione (88) and iron-mediated oxidative stress (89) may contribute to the loss of dopaminergic neurons of the substantia nigra (87–89). Basic research into the cause of dopaminergic cell death is needed to prevent the occurrence of cell loss.

Neuroprotective Strategies

Another approach in Parkinson's therapy is the administration of substances that may prevent death of striatal neurons. Such preservation has been accomplished by injection of growth factors into the brain (humans) or by their delivery with a lentiviral vector (in aged rhesus monkeys). One such promising growth factor candidate is of glial origin, the "glial cell line–derived neurotrophic factor" (GDNF), related to other members of the transforming growth factor superfamily (90,91). GDNF can facilitate the survival of dopaminergic cells in vitro and possibly also in vivo.

Nurr1 is a molecule that plays a critical role during embryonic development in the formation of DA-producing cells that are lost in PD. Nurr1 also appears to help keep these cells active throughout life and to assist them in producing appropriate amounts of DA. It may be possible to find drugs that may increase or decrease Nurr1 activity and prevent or cure PD (92). However, to date, a specific therapy has not been found to engender specific neuroprotection.

Surgical and Electrostimulatory Strategies

Surgical techniques in Parkinson's patients leading to ablation of the pallidum nucleus or the thalamus were employed for many years but were superseded by *L*-dopa treatment. Currently, focus on surgical interventions has awakened again. Ventromedial pallidotomy reduces overactivity of the globus pallidus, which had been implicated in the motor disability associated with PD (93). Deep brain stimulation (DBS) has become the surgical treatment of choice. Despite the effectiveness of the surgery, it is important to bear in mind that such surgeries are drastic measures; a part of the brain, once ablated, cannot be replaced. So although these therapies may yield symptomatic alleviation of the disease, they may cause other complications later or eventually become ineffectual as dopaminergic neurons continue to be lost.

Another approach to addressing Parkinsonism uses electrical stimulation to specific brain areas with the intent of reestablishing normal electrical circuitry. These techniques include electroshock, high frequency, DBS, and transcranial magnetic stimulation (94–97).

Cell Therapies

The promise of cell therapies has captured the imagination of scientists (98–103). Attempts to replace lost dopaminergic cells in Parkinsonians by various methods (e.g., transplantation/injection/grafting) with fetal nerve tissue, adrenomedullary and carotid-body cells, and stem cells are being actively pursued despite uncertain results and ethical questions. We have already mentioned recent studies in which precursors of dopaminergic cells from fragments of embryonal mesencephalon were transplanted into the brains of PD patients, with mixed results (22–24). Other studies explore the use of transplanted cells: these include pluripotent stem cells, more restricted neuronal and glial precursors, or differentiated neurons with or without the help of growth factors to stimulate cell proliferation and survival. With the ongoing technological advances, more progress is expected (25). It is important to understand that the implanted cells must retain their functional state and that their mere survival does not indicate success of the treatment.

■ Cell Adhesion Molecules

Cell adhesion molecules (CAMs) act on cell surfaces and play an important role in all aspects of CNS function, from development to adult maintenance. During development, they are involved in the formation of the neural tube and neural crest, cell migration, axonal outgrowth, and guidance. In adulthood, they regulate synaptic stabilization and plasticity, myelination, and nerve regeneration after injury and in learning (104,105). They are members of the (immunoglobulin) Ig gene superfamily (Chapter 14) and are subdivided into three groups:

- Integrins, found in the nervous tissue
- Cadherins, found in the nervous tissue
- Selectins, found in the immune system

CAMs interact with each other and with nonadhesive cell surface and cytoplasmic molecules. When acting together, they may often produce effects opposite to those of the individual factors, e.g., encourage movement or growth along a preferred pathway or immobilize a cell and prevent its movement and growth (106).

The few studies on CAMs in old age indicate that in several brain areas (hippocampus, and frontal and occipital cortices) of neurologically normal elderly humans, CAMs levels closely resemble those of adult individuals; however, there were significantly fewer CAM-positive neurons in the frontal cortex

of elderly individuals with AD (107). In rats, the CAM upregulation that usually occurs with learning and regeneration is reduced in adult animals as compared to younger animals. This reduction would suggest that neural CAMs promote structural and functional remodeling and that this action may be less efficient in old individuals, especially in those affected with AD (Chapter 7) (108,109).

■ Neurotropic Agents

The best known among the neurotropic agents is nerve growth factor (NGF), a basic protein that resembles in structure the hormone insulin (Chapter 13). NGF promotes growth and maintenance of sympathetic and sensory neurons as well as neurons in the brain during developing and mature ages (110). In the brain, NGF is particularly effective in promoting growth and reducing or preventing damage to cholinergic neurons of the basal forebrain (nucleus basalis of Meynert) drastically affected by neuronal loss in AD. In AD, NGF levels, receptors, and its mRNA are reduced. Intraventricular administration of NGF, in young and adult rats, prevents these cholinergic neurons from dying after axonal transection or chemical damage and, in fact, may promote regeneration. However, clinical trials with NGF in AD patients were ineffective.

NGF is abundant in salivary glands, where it is taken up by terminals of neurons and transported in retrograde fashion to their cell bodies (111). NGF has been purified and sequenced, and a number of similar growth-promoting proteins have been identified. A mouse fibroblast cell line capable of secreting recombinant NGF has been established (110).

Other neurotrophic agents involved in the development and maintenance or repair of neuronal tissue in adult and aged animals include, in addition to GDNF [discussed above, for its action on dopaminergic neurons (90,91)], gangliosides (112,113) and several compounds of the fibroblast and epidermal growth factors' family effective on neurons, glial cells, specialized sensory cells [e.g., photoreceptors (113)] in health and disease, and on some components of the cytoskeleton. While clinical use of growth factors is still in the experimental stage, the possibility that neurotropic factors may benefit neuronal survival in CNS degenerative diseases represents a physiologic and hopeful therapeutic intervention.

As suggested at the beginning of this chapter, brain plasticity persists in old age but is less effective than at younger ages. An attempt to generalize the current views suggests that neurons in old age are capable of displaying compensatory responses (reminiscent of those occurring in ontogenesis) when they are provided with an appropriate environment or when the conditions that curtail their growth are removed. With aging, surviving neurons are capable of remodeling their configuration [e.g., reactive dendritic sprouting (8), axonal growth (109)] in response to functional challenges and with the help of neurotropic agents. However, neuronal loss, isolation of one structure from another, and neurotransmitter deficits are more difficult to repair in the aged brain unless growth-promoting factors again become available and inhibitory factors are removed. Just as neuronal modeling is gene regulated during ontogenesis, similarly, future techniques to provide differential gene expression in aged neurons may direct the morphological and biochemical compensatory events that follow aging or damage and lead to functional recovery.

■ METABOLIC AND CIRCULATORY CHANGES

In addition to neurotransmitters, other chemical constituents of the CNS are known to change with aging. A number of these are listed in Table 6. Some generalizations may be drawn from clinical and experimental data: changes are specific to discrete regions and structures, follow differential timetables, and differ according to the constituent considered. Because of the lack of consistent studies in humans during normal aging, only a few of these constituents will be discussed here. A good model of regional, age, and constituent specificity is presented by some putative amino acid neurotransmitters. For example, in the rat, of the excitatory ones, glutamic acid declines with aging in the cerebral cortex and brain stem but not in the cerebellum and spinal cord; glycine and GABA, inhibitory amino acids, increase with age in the cerebral cortex, cerebellum, and brain stem, but remain unchanged in the spinal cord (114). These observations support the concept of a neurotransmitter imbalance with aging.

Other changes with normal aging include the following:

- Decreased extra- and intracellular water content of the brain (as of most other organs), while electrolyte distribution remains essentially unchanged
- Regional decrease in protein content and synthesis, perhaps associated with slow turnover (to maintain steady state), increased oxidation of proteins and their consequent glycosylation, an increase in complexity of RNA molecules, an increase (perhaps related to gliosis) or no change in DNA content, and an accumulation of intraneuronal proteins (such as NFTs)
- Decreased lipid synthesis, primarily decreased synthesis of membrane phospholipids due to increased variation in the structure of lipid substrates, rather than reduction of synthesizing enzyme activity or concentration of substrates; changes in membrane lipids would alter membrane fluidity and, in turn, nerve conduction and receptor binding
- Circulatory changes related primarily to atherosclerosis (Chapters 15 and 16) and a decrease in cardiac output (Chapter 20), and characterized by a progressive reduction in cerebral blood flow and a corresponding decrease in oxygen uptake, glucose utilization, and, consequently, energy metabolism

■ Metabolic Changes

Parameters of cerebral energy metabolism and circulation, such as cerebral blood flow, oxygen consumption, and glucose metabolism (as a measure of cerebral metabolic rate), have been studied in humans and experimental animals utilizing a number of techniques (e.g., radioactive tracers, autoradiography, CT scans, and other imaging techniques). Energy metabolism is dependent on normal glucose utilization, normal membrane function, and normal supplies of high-energy phosphate compounds. The brain, unlike most other tissues, normally derives almost all of its energy from the anaerobic oxidation of glucose. Metabolic changes vary considerably with age, from prenatal development, to birth, to old age.

TABLE 6 Possible Metabolic and Circulatory Changes Targeted by Aging in the Central Nervous System

Total water	Carbohydrates
Extra- and intracellular spaces	Circulation
Lipids	Energy metabolism
DNA, RNA, and protein	Oxygen and glucose uptake
Amino acids	Blood-brain barrier

Abundant data from experimental animals and some data from humans show that cerebral oxygen consumption and the activity of the associated enzymes are low in fetal life and at birth, rise rapidly during the period of cerebral growth and development, and reach a maximal level at about the time brain maturation is completed. Whole brain blood flow and oxygen consumption remain essentially unchanged between young adulthood and old age in the absence of disease; however, in the presence of incipient atherosclerosis, blood flow is reduced, and, in severe atherosclerosis, blood flow and oxygen consumption are lower than in healthy controls of the same age (Chapter 15).

Metabolic and circulatory changes vary not only with the health of the subjects, the animal species, and the brain area considered, but also with the sensory, motor, or mental task performed (115). The clinical significance of these changes is unknown, particularly in terms of oxygen consumption, because, in decreased blood flow due to ischemia, more oxygen is extracted per unit of blood (an increased arteriovenous oxygen difference) in an attempt to compensate for the reduced flow.

The relative stability of the energy metabolism of the aging brain in the absence of neuropathology must be contrasted with the severe deprivation that occurs in the aged brain affected by degenerative diseases such as AD. For example, in AD, the reduction in rate of cerebral formation of adenosine triphosphate from oxidized glucose and oxygen ranges (compared to controls of the same age but without AD) from 7% to 20% in incipient AD, and from 35% to 50% in stable, advanced dementia (116). Thus, the AD brain may be characterized by:

- Regional hypometabolism
- Oxidative stress
- Alterations of the glucose-fatty acid cycle

The view that, in AD, accumulation of free radicals, amyloid, and other modified proteins (118) and alterations in neurotransmission may be secondary to the main cause of progressively worsening energy deprivation/ischemia is not new (116,117,119). Neurodegenerative diseases may best be considered to be the consequences of sequential alterations of biochemical processes (119). For example, glucose deprivation would elicit the counter-regulatory mechanisms to preserve glucose for anabolic needs by switching from glucose to ketone body utilization (Chapter 13). Soluble amyloid-β peptide, which inhibits glucose utilization and stimulates ketone body utilization, would serve as the agent mediating this adaptive switching. However, while this metabolic switch is successful during early brain development, it is not sufficient to maintain normal energy metabolism in the aged brain. *With old age, exhaustion of the metabolic adjustments to the stress of glucose deprivation and ischemia would occur, and this failure would precipitate the manifestations of AD (Chapter 7).*

Circulatory Changes

Brain circulatory changes may occur due to atherosclerotic lesions common throughout the arterial vascular system. One of the potential consequences of atherosclerosis is the production of infarcts—areas of dying tissue and scar formation following interruption of circulation due to obstruction or rupture of blood vessels. The occurrence of multiple small infarcts over time leads to progressive destruction of brain tissue, which may be responsible for one form of vascular dementia, called "multi-infarct senile dementia," and contrasted with Alzheimer's dementia (Chapter 7). Ischemic injury to the brain can result from several processes, ranging from *focal ischemia* due to occlusion of an artery supplying a region of the brain, to *global ischemia*, which occurs during cardiac arrest and resuscitation and reflects a transient loss of blood to the entire brain. *Hypoxia* and *hypoglycemia* usually accompany ischemic injury. Neurons are more susceptible than glial cells to *ischemia, hypoxia*, and *hypoglycemia*. These three conditions activate glutamate receptors and induce excitotoxicity, that is, an abnormal increase in the levels of the excitatory amino acids such as glutamate, with consequent neuronal cell death. The same conditions may also damage the blood brain barrier (BBB). This barrier is a functional concept based on specific mechanisms of exchange (e.g., tight junctions, numerous mitochondria, presence of astrocytes) across the endothelium of the cerebral capillaries. *The concept is that of a barrier that protects the brain (and the spinal cord) from fluctuations in many blood constituents and from transport of potentially toxic or larger molecules from the blood into the brain and spinal cord*. It preserves homeostasis in the CNS by blocking the entry of unnecessary metabolites and toxic substances while facilitating the exchange of necessary metabolites.

The cerebral capillaries are much more permeable at birth than in adulthood, and the BBB develops during the early years of life. The BBB efficiency increases during early development and peaks in adulthood. With age and disease, the BBB may again become permeable at least to selected substances, and the passage of blood-borne substances may represent one of the causes of such dementias as AD (120–121). Studies of the structure of cerebral capillaries at progressive ages, in experimental animals, reveal an increase in the capillary wall thickness and a decrease in mitochondrial content of endothelial cells. Although direct evidence for increased permeability of the BBB as the primary pathogenic factor in AD is still lacking, defects of the BBB may represent a common pathogenic mechanism linking many different risk factors. *It is to be noted also that, due to its protective action in preventing substances from entering the CNS, the BBB also limits the effectiveness of several medications in crossing the barrier and pass from general circulation into the brain parenchyma.* Such restriction hinders the treatment of CNS diseases at all ages, including old age: one illustrative example of this handicap, discussed in this chapter, is the BBB permeability to *L*-dopa but not to DA, and, consequently, the use of *L*-dopa, but not DA for PD treatment.

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7

The Nervous System: Functional Changes with Aging

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INTRODUCTION

The functional integrity of the nervous system is well maintained in most elderly persons despite the morphologic and biochemical changes described in the previous chapter. This ability to conserve neurologic and intellectual competence into old age attests to *the nervous system's plasticity and its capability, under certain conditions, to repair itself and compensate in response to damage and injury*. As noted in Chapter 6, the demonstration of new cell growth and new sprouting of dendrites in the brains of adult and old animals, including humans, suggests that compensatory mechanisms and some regenerative capacity persist in the aging brain. Indeed, ongoing research supports the prospect that neurodegenerative diseases may eventually be treatable or prevented altogether. *The difficulty for nerve cells—perhaps more than for any other cells of the body—is that their function depends not only on their number and integrity but also on their inherent capacity to connect with other cells, to reach their synaptic target, and to be able to secure appropriate neurotransmitter signaling*.

Despite the persistence of central nervous system (CNS) plasticity in old age, we must recognize that in many elderly individuals, especially those in the 75- to 85-year-old group and older, the incidence of neurologic and mental impairment increases. It remains to be evaluated whether normal aging is simply an early stage of pathology without obvious clinical expression or whether aging-associated diseases represent cases of "accelerated aging" or of "distinctive disease processes" (Chapter 3). The prevalent view is that the senescent cell may represent a favorable environment for pathological changes to occur (Chapters 3 and 4). CNS disorders responsible for impairment of memory, intellect, strength, sensation, balance, and coordination account, in the Unites States, for more than 90% of the cases of total dependency in this age group.

One characteristic of the aging nervous system that came to light in the previous chapter is the resistance of discrete structures or biochemical pathways to the aging process and, similarly, the differential effects of aging on various CNS functions. In this chapter we discuss the effects of aging for three major functional areas of the nervous system:

- Motor function: gait and balance
- Rhythmicity of daily activity: wake/sleep
- Cognitive function: memory

Disruption of these functions can lead to neurologic (e.g., locomotor, as in Parkinson's disease) and mental (e.g., cognitive, as in dementia) deficits. *Dementias are characterized by an insidious, global, and persistent decline in cognitive function from a previous higher level of activity often associated with emotional changes, neurologic deficits, and vascular disturbances*. These impairments have catastrophic consequences for those who are demented, their families, and the society in which they live. Despite some optimistic trends that suggest a current decrease in the prevalence of dementia in those over 85, at the end of the last century, this disease was, and still is, the fourth leading cause of death in the 75- to 84-year-old age group in the United States (Chapter 3). Deaths are secondarily due to the concurrently occurring common causes of mortality in the elderly, including cardiovascular disease, stroke, sepsis, and pneumonia (1).

MOTOR CHANGES: POSTURE, GAIT, AND BALANCE

Control of Posture and Movement

Posture or "station" (the bearing of one's body that provides a stable background for movement) and movement (the ability to change posture and position) are regulated by a number of structures and functions within and without the nervous system (Table 1). In this chapter, nervous regulation is considered primarily with respect to CNS control of movement, and in Chapter 8, with respect to reception and control of sensory (e.g., visual, auditory, and vestibular) inputs.

In addition to the CNS input, major structures involved in the execution of movement and balance are the skeleton, joints, and skeletal muscles (Chapter 20) and, indirectly, for metabolic support, the cardiovascular (Chapter 15) and endocrine (Chapters 9–13) systems. The beneficial role of good nutrition

TABLE 1 Structures/Systems Controlling Posture, Balance, and Mobility

CNS
Cerebral cortex
Basal ganglia
Cerebellum
Vestibular-ocular and proprioceptive pathways
Limbic system
Spinal cord
SKELETAL MUSCLES
BONES AND JOINTS
HORMONES
BLOOD CIRCULATION

Abbreviations: CNS, central nervous system.

and physical exercise in improving mobility and balance and in promoting a better quality of life in old age and longer survival is discussed in Chapters 23 and 24.

With aging, skilled motor movements are slowed, and gross movements, particularly those related to maintenance of posture and gait (i.e., manner or style of walking), are altered (2–6). These changes affect the speed of movement, which may be accelerated or slowed. Alternatively, these changes may affect the contraction of specific muscles, resulting in abnormal movements (as in dyskinesias) or in abnormal posture (as in dystonias).

Alterations of movement and posture lead to imbalance and, thus, to a high incidence of falls, one of the most frequent and life-threatening accidents of old age (7–10)(Chapters 20 and 25). Falling is a serious public health problem among the elderly because of its frequency and its association with morbidity and mortality. Serious injuries that may result from falls represent the sixth leading cause of death in the United States among individuals 65 years of age and older (11–16). Furthermore, unhealthy behavioral changes succeeding the fall (e.g., not going out of the house, limitation of social participation because worry of falling again) increase the cost of follow-up care, deprive the affected individual of independence, and drastically impair well-being and survival.

With advancing age, the typical adult gait changes to a hesitant, broad-based, small-stepped gait with many of the characteristics of early parkinsonism (Chapter 6), often including stooped posture, diminished arm swinging, and turns performed en bloc (i.e., in one single, rigid movement). Some frequently used measures of balance and gait are listed in Table 2. Rarely do individuals over the age of 75 walk without the marks of age. Indeed, it is the ability to walk without serious limitations or falling that distinguishes a normal, aged gait from a dysfunctional one. Alterations of locomotion are due primarily to CNS impairment. The peripheral changes seen in normal aging, including minor decreases in nerve conduction velocity, decrease in muscle mass, and increased muscle rigidity, are insufficient to account for the disability.

Despite the frequency of these age-related changes in the regulation of gait and balance, relatively little is known about the mechanisms responsible for them. Nevertheless, much can be learned about the overall physiologic competence of the aging individual by observing gait and balance: disturbances of gait and repeated falls, in the very old particularly, are signs that herald or reflect serious ill health (11–16).

TABLE 2 Key Measures of Functional Assessment of Balance and Gait

Balance	Gait
Sitting balance	Initiation of gait
Rising from a chair	Step height/foot clearance
Immediate standing balance	Step length
Standing balance with eyes closed (feet close together)	Step symmetry
Turning balance (360°)	Step continuity
Nudge on sternum	Path deviation
Neck turning	Trunk stability
One-leg standing balance	Walking stance
Back extension	Turning while walking
Reaching up	Width of base
Bending down	Arm swing
Sitting down	
Recovery from spontaneous loss of balance	

■ Changes in Gait

Typical walking is characterized by the following:

- head that is erect (without spinal curvature)
- Arms that swing reciprocally (without grabbing at furniture)
- Stepping without staggering or stumbling movements and
- Feet that clear the ground at each step

Several of the above characteristics are altered with aging:

- The gait slows
- The step length shortens and
- Both irregularity and hesitancy in the walking pattern increase

Such changes are indicative of impaired stability and may depend on multiple neurologic and extraneurologic causes. Sex differences in gait are found in all ethnic groups In adult and older ages, females almost always perform less well than males on all gait parameters, especially when coincident with impaired vision or cognition or under conditions of poor illumination (15). Demented patients show significant decrements in all gait parameters.

Measurements of a subject's gait, such as speed and length of stride (stride is the distance between two successive contacts of the heel of the same foot with the ground), are not obtrusive and can provide useful information on the degree of competence or damage of the central and peripheral nervous system. Several pyramidal and extrapyramidal structures, including visual, vestibular, and proprioceptive sensors, impart finely graded instructions to the muscles of the neck, trunk, and limbs for maintenance of normal posture, balance, and gait (Box 1). Alteration in any of these functions will reflect impairment and failure of integration of one or more of the CNS structures involved (17–19). Further, impairment of gait and balance may also indicate disturbances of the vascular (Chapter 15) and mental status (see below) as well as the conditions of the skeleton and joints and orthopedic disorders (20,21) (Chapter 20).

Gait changes may be useful in providing additional clinical insights to those mentioned above, as follows:

- Gait asymmetry gives a clue to hemiplegia or arthritis (both of which negatively affect movement)
- Impairment of shoulder movements in walking suggests parkinsonism
- Increase in stride width relates to cerebellar disease and arthritis and
- Trunk flexion (due to unstable balance) suggests impaired visual, vestibular, and proprioceptive controls

■ Changes in Balance and Falls

Balance or stable physical equilibrium can be studied clinically, as with gait, by simply observing individuals as they rise from a chair, stand, walk, or turn. Do the subjects examined sway, sweep, and stagger when performing these movements? In the elderly, the fear of falling and pain, or limitation of joint movement, are all reflected in their carriage (21–24). The main adaptation to a balance disorder is the shortening of step length accompanied by slowing of gait and increasing of time between steps. This pattern is particularly noticeable in people who have fallen repeatedly, and indeed, it is called "post-fall syndrome" or "3 Fs syndrome" (fear of further falling) (23).

The immediate consequence of a worsening in balance is an increased frequency of falls. There is a good deal of reserve in the postural system, and young adults can fairly well tolerate

BOX 1 *Pyramidal and Extrapyramidal Motor Pathways*

Skilled movements (e.g., fine finger movements) are regulated in the brain by nerve fibers that originate in the motor cortex and form the "pyramids" in the medulla, hence the term pyramidal tracts. Grosser movements and posture are regulated by central nervous system (CNS) areas (e.g., basal ganglia) other than those connected with the pyramidal tracts, hence, by exclusion, the term extrapyramidal pathways. Coordination, adjustment, and smoothing of movements are regulated by the cerebellum, which receives impulses from several sensory receptors. Impulses from all of these brain structures ultimately determine the pattern and rate of discharge of the spinal motor neurons and neurons in motor nuclei of cranial nerves, thereby controlling somatic motor activity. Axons from these neurons form motor nerves that travel from the spinal cord to the various skeletal muscles throughout the body. Once the muscle has been reached, motor nerves synapse at the myoneural junction and transmit the nerve impulse to the muscle fibers. Contraction or relaxation of muscles will, in turn, direct bone and joint movements to maintain posture and promote mobility.

experiments in which they are tested in moving platforms with sensory input (primarily vision) absent. The elderly are much less tolerant of any loss or decline of sensory input (such as vision) (15,24). Falls of the elderly occur when engaging in ordinary activities, most often indoors. Trips and accidents account for the largest number of falls. It is to be noted that the incidence of falls declines with further aging, probably due to the reduced mobility of the very old. Some falls occur without any external cause and may be due to impaired peripheral (ocular, vestibular, and proprioceptive) and central (cerebellar and cortical) coordination (24) or, especially in postmenopausal women, to bone fractures due to osteoporosis (21) (Chapters 10 and 20).

Falls are often fatal in the elderly, but even the nonfatal falls have serious consequences, including

- physical injury,
- fear (the 3 Fs syndrome, "fear of further falling"),
- functional deterioration, and
- institutionalization (23).

Performance-oriented evaluation of falls shows that their occurrence may be related to impaired central information processing (e.g., decrements in selective attention and choice reaction time as in central processing) as well as in sensory input (e.g., vision) and motor activity (e.g., muscles).

Women are at greater risk for falls and the consequences thereof than men because of a number of factors, including (21)

- more severe osteoporosis and bone fragility, especially after menopause (Chapter 10),
- less muscle strength,
- more sedentary, physically less strenuous way of life (Chapters 20 and 24), and
- greater degree of comorbidity and disability (Chapter 3).

Comparison among ethnic groups in the United States indicates few differences in the increasing frequency and severity of motor disabilities, fractures, and falls with old age. Mexican-American women present a profile similar to that of non-Hispanic Caucasian women (25). However, Japanese women (in Hawaii) (26) and African-American women (27) have lower rates of falls and fractures than Caucasian women of the same age. These differences seem, in the main, to depend on a better neuromuscular performance in Japanese women and on lower incidence of osteoporosis in African-American women.

■ Get Up and Move: A Call to Action for Older Men and Women

There is considerable overall functional reserve in the locomotor system: hence, the loss of one of the sources of control of postural maintenance may be of little consequence (28). However, in elderly individuals, in whom impairment tends to occur simultaneously at several functional levels, this reserve is easily depleted. It is not difficult to understand why a fall is the consequence of the simultaneous involvement of two major factors: neurologic (e.g., extrapyramidal damage) and extraneurologic or environmental (e.g., drugs, cardiovascular, skeletal, and social). As our methods for measuring gait and balance become more sophisticated and quantitative, the close examination of these two factors—neurologic and environmental—becomes increasingly more important as a means of providing a comprehensive picture of the physiologic or pathologic condition of the older individual. At the same time, improving the mobility of the old individual, if not vital for survival, will considerably increase the well-being and overall health of the elderly (29).

Benefits of physical exercise versus a sedentary life are discussed in Chapter 24. A regimen of regular physical activity is recommended for all ages; when it is started at a young age and continued throughout life, it may confer significant benefits on health and longevity. Among these benefits, not least are those promoting better neurologic and mental activity in old age, as suggested by the impact of running in "boosting" neural cell number. Studies in mutant mice affected by a rare neurodegenerative disease (ataxia telangiectasia, characterized by a progressive loss of brain cells, first in the cerebellum and then throughout the brain), with consequent loss of motor control show that when these mice are placed on running wheels, the miles they log correlate directly with the increase in the number of brain cells; in contrast, in nonrunning control mice, most brain cells continue to die (30,31). In humans, the growth of new cells also occurs in adults and elderly under specific conditions, including physical exercise and learning (32,33) (Chapters 6 and 24).

■ CHANGES IN SLEEP AND WAKEFULNESS

■ Biologic Clocks and Sleep Cycles

Changes in several cyclic functions with aging may be ascribed to changes in so-called biologic clocks—the biological timepieces that govern the rhythm of several functions, such as the 24-hour (circadian) hormonal rhythms (Chapters 9–13) and several behaviors (34–36). The cessation of menstrual cycles and of ovarian function at menopause (Chapter 10) exemplifies alterations occurring in one such rhythmically recurring event. Cyclic functions are thought to depend on specific signals located primarily in the brain and coordinated by the

suprachiasmatic nucleus in the hypothalamus, the so-called circadian pacemaker. These signals are required for the clock to progress through the synthesis of gene-regulated molecules (e.g., RNA, proteins, and neurotransmitters).

The most usual change with aging in the biological rhythmic systems is a reduction in the amplitude of circadian rhythms (36). Usually, the level of motor activity and the amplitude of the activity rhythm are greatly reduced with advancing age as are learning and memory (37) as well as blood pressure, heart rate, and temperature regulation (38,39). The deteriorating activity rhythm of old hamsters may be rejuvenated by a surgical implantation of fetal tissue from the same hypothalamic area that contains the circadian pacemaker; the brain grafts not only would restore the amplitude of the activity rhythm but also increase the longevity of the animals (40).

A prime example of a neural cyclic function is the circadian sleep/wake cycle, which undergoes characteristic changes with aging, including phase advancement (a shifting of the circadian rhythm to an earlier period). With respect to the changes in sleep, this "phase advancement" means that older people tend to go to bed earlier and to wake up earlier in the morning (41). A precise delineation of sleeping changes intrinsic to normal aging is still lacking. However, the incidence of chronic sleep-related abnormalities (e.g., poor sleep efficiency, frequent and prolonged nocturnal arousals, and day sleepiness) becomes increasingly prevalent in old age (42–44) and does not show significant differences among ethnic groups (44). Changes in sleep patterns have been viewed as part of the normal aging process; they are often associated with altered responses to external (light) (45) or internal (body temperature) (46) cues. Many of these common disturbances may also be related to the development of pathological processes that disturb sleep (47,48).

As we all know, sleep is a regular and necessary phenomenon of our daily lives. Lack of sleep leads to a desire for sleep. The pressure to sleep is strongest at night, particularly in the early hours of the morning. Yet, the mechanisms and purpose of sleep still elude us. Researchers have identified genes upregulated specifically during sleep; expression of such genes would vary depending on the state of sleep and of wakefulness (49). A popular assumption is that sleep provides a quiescent period during which the body recovers from the strains of the waking hours and that it represents a descent to a lower level of consciousness, but such a hypothesis remains to be proved.

There are, however, certain facts that are recognized:

- Sleep is closely related to the electrical activity of the brain, as measured by the electroencephalogram (EEG), which records the variations in brain electrical activity.
- Sleep is also closely associated to whole-body visceral manifestations such as changes in heart rate, respiration, basal metabolic rate, endocrine function, etc.
- There are two kinds of sleep: the rapid eye movement (REM) sleep, with an EEG resembling the alert, awake state and a four-phase, non-REM or slow-wave (SW) sleep with unique EEG patterns (Fig. 1). Each sleep phase has its EEG pattern.

Sleep patterns change during growth and development as well as in old age (50). In old age, major changes include

- decrease in total sleep time (TST),
- increase in length of slow wave (SW) stages 1 and 2 sleep,
- decrease in length of SW stages 3 and 4 sleep,
- essentially unchanged rapid eye movement (REM) sleep time, and
- possibly modified sleep patterns in neuropsychiatric disorders and by the administration of psychotropic drugs (see below) (Chapter 22).

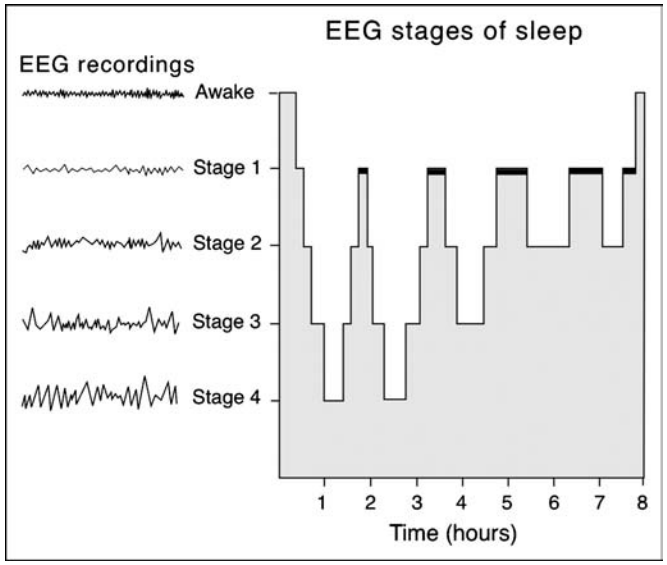

FIGURE 1 Diagrammatic representation of an eight-hour sleep period. The dark areas represent REM sleep. The EEG recordings (on the left side of the diagram) show the different rhythms that accompany each sleep stage. *Abbreviations*: REM, rapid eye movement; EEG, electroencephalogram.

■ Sleep EEG Changes with Aging

The EEG represents the background electrical activity of the brain as characterized by wave patterns of different frequencies: α, 8 to 12 waves/sec; β, 18 to 30 waves/sec; θ, 4 to 7 waves/sec; and δ, less than 4 waves/sec. The α waves have the highest amplitude. With advancing age, the α rhythm (prominent in a person awake with eyes closed and mind at rest during the EEG test) slows throughout the brain as well as focally (temporal region). The β activity increases in aged persons.

With respect to the evoked potential (i.e., the electrical activity generated by stimulation of the cortex through internal or external stimuli), the data suggest that latency is prolonged after stimulation involving a variety of sensory modalities, perhaps due to decreased conduction velocity or a change in the sensory organs (Chapter 8). Alterations in spontaneous and evoked potential are aggravated in individuals with Alzheimer's disease (AD). As illustrated in Figure 1, when people fall asleep, they go from a state of quiet wakefulness through four consecutive stages of SW sleep and several episodes of REM sleep.

In the normal adult, the total amount of sleep per day is approximately seven hours, of which 23% is spent in REM sleep and 57% in stage 2 SW sleep. Of the other stages, stage 1 accounts for 7% and stages 3 and 4 for about 13% (Fig. 2). Generally, the episodes of REM sleep occur at about 90-minute intervals. They lengthen as the night progresses; this is the stage in which dreaming occurs.

With aging, the TST decreases, although not markedly. Nocturnal sleep is interrupted, and sleep time is often distributed more widely over the 24-hour period by adding short naps. Period of quiet wakefulness is lengthened: it takes longer to fall asleep, and the number of awakenings per night increases (sleep fragmentation). Stage 3 almost completely disappears and stage 4 disappears so that there is little deep sleep time.

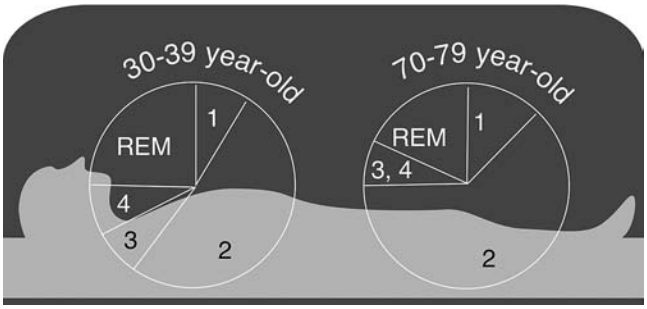

FIGURE 2 Relative distribution of sleep stages in adult (30-39 years) and aged (70-79 years) male individuals. Quiet wakefulness (preceding the onset of sleep) is followed by four (1 through 4) stages of slow-wave sleep with periods of REM sleep. In the aged, the period of quiet wakefulness and stages 1 and 2 are lengthened, while stages 3 and 4 of deep sleep have almost completely disappeared. *Abbreviation*: REM, rapid eye movement.

The marked shortening of SW stages 3 and 4 may explain why older individuals complain of little sleep despite an overall normal amount and why they are much more easily aroused than the young. Additionally, although the statistics vary widely, there seems to be a progressive reduction of sleep time during the night, and about 40% of the elderly suffer from insomnia. Insomnia, or "failure to maintain sleep," is a complaint with multiple manifestations: for example, "early sleep insomnia"—inability to go to sleep before very late at night, or "early sleep offset"—awakening very early in the morning.

Insomnia has many diverse causes that require different treatments. Hypnotic medications to induce sleep, particularly when used chronically, should not be the mainstay of insomnia treatment; often the medications are ineffective, particularly over the long term, and many can be habit forming. They may be contraindicated when used with other medications (Chapter 22). The value of sleep hygiene (e.g., regularization of bedtime, that is, a length of sleep capable of restoring well-being), good nutrition, and regular physical exercise should not be underestimated in prevention and treatment of insomnia (51) (Chapters 23 and 24).

The relationship of the pineal hormone, melatonin, to sleep patterns and its potential value in the treatment of insomnia are discussed in Chapter 12.

■ Respiratory, Cardiovascular, and Motor Changes During Sleep in the Elderly

Periods of apnea (cessation of respiration) or hypoapnea (slowing of respiration) during sleep increase with aging from an average of five respiratory disturbances per night at 24 years of age to about 50 per night at 74 years of age. These periods of apnea are brought about by a collapse of the upper airway with temporary blockage of the breathing passages during the sleeping state; they are terminated only by an arousal from sleep that restores the activity of the upper airway muscles. Apneic incidents are more common in men than women and in overweight individuals. They account for a great deal of the fragmented sleep experienced by the elderly. They may be lead to daytime drowsiness, irritability, faulty memory, headaches, and possibly depression.

Although the nocturnal hypoxia (low oxygen in blood and tissues) attendant to apnea is transient, it is not known whether changes in oxygen saturation may have adverse effects on brain function. In old age, we observe:

- Disordered breathing during sleep, leading to hypoxia during the night and fatigue during the day, thereby increasing the risk of cognitive decline (even reversible dementia) (52,53)
- Sleepiness during the day, which also increases the risk of falls (54) and automobile traffic accidents (55)
- Another consequence of impaired function of the upper airway, that is, the increased prevalence of snoring. Current statistics indicate that 60% of males and 45% of females in their 60s are habitual snorers

The significance of respiratory dysfunction associated with sleep in the otherwise normal elderly is not well understood (56). Several factors appear to be involved, including aging-related alterations in neural and chemical regulation of respiration (Chapter 17). Abnormal rhythm of the heartbeat [cardiac arrhythmias (Chapter 20)] and pulmonary hypertension are common during apneic periods (57). Apnea, arrhythmia, and hypertension are usually most frequent during REM sleep; they have been related to an increased release of norepinephrine from sympathetic stimulation, with mental disorders such as depression, or with the use of some neurotropic medications (Chapter 22).

Sleep-related leg movements are also common in the elderly. One-third of elderly experience such "twitches" or leg discomfort every 20 to 40 seconds during a large part of the night; they often cause a brief arousal from sleep (58). The origin of these movements is little known, but they seem to be related to loss of coordination between motor excitation and inhibition. Sleep disturbances are also frequent in individuals suffering from arthritic pains (e.g., knee osteoarthritis) (59), although these interruptions stop once the arthritic episode is terminated.

■ The Role of the Reticular Activating and the Limbic Systems

The reticular activating system, formed of an afferent and efferent network of interconnecting neurons distributed in the core midbrain, controls conscious alertness and, thus, makes sensory, motor, and visceral perception possible. Changes in sleep patterns with aging may be related to alterations in the level of alertness as manifested by the EEG changes discussed above and shifts in neurotransmitters, primarily serotonin (60,61) (Chapter 6). Serotonin appears to function as an inhibitory neurotransmitter that modulates the effects of light on circadian rhythmicity and regulates several cyclic hormonal secretions (62,63). It is also the precursor of the pineal hormone melatonin, which, in some, has potent sedative and hypnotic (sleep-inducing) activity (Chapter 12). Insomnia, frequent in the elderly, may depend on several factors superimposed on the aging process. Anxiety, depression, and stress that often affect sleep, are prevalent among many elderly and may account for some of the sleep disturbances.

Changes in the reticular activating system with old age may not only modify sleep patterns but also alter alertness and behavior. A decrease in sensory input to the higher brain centers may result either from an electrochemical failure of the reticular formation to receive, integrate, and relay signals to the sensory cortex, or from decrements in peripheral sensory perception (Chapter 8), or from both. Any impairment of sensory input would impair motor responses and behavior, decrements that can be detected in EEG recordings and physiologic responses. *Such sensory-motor alterations among the aged may explain their decline in response time, that is, the speed with which one initiates a*

motor or behavioral reaction to a sensory stimulus. The greatest slowing of performance is seen in demented individuals.

Another brain system affected by old age is the limbic system, which regulates many types of autonomic responses (e.g., blood pressure and respiration) and behavior (e.g., sexual behavior, emotions of rage and fear, and motivation). The limbic system consists of a rim of cortical tissue around the hilus of the cerebral hemispheres and of deep structures such as the amygdala and the hippocampus. These structures are involved in memory, mood, and motivation and are frequently affected in old age.

■ Warning

Because depression, anxiety, agitation, and insomnia often complicate clinical disorders in the elderly, a variety of drugs are administered to stimulate or tranquilize, depending on the condition. Use of drugs, such as "psychotropics" to treat insomnia is part of the medicinal armamentarium for old people. None of these drugs are without side effects, and the distribution, metabolism, and excretion of these drugs—as of most drugs—may be impaired in the elderly (64) (Chapter 22).

■ MEMORY AND LEARNING

This and the following section address some of the functions regarded as "the high functions of the nervous system." Indeed, according to Rene' Descartes, a seventeenth century French philosopher, mathematician and physiologist, the very existence of humans depends on their ability to think; hence his well-known phrase "Cogito, ergo sum" (I think, therefore, I am). These functions of the mind are numerous and range from motivation to judgment, to cognition, and to language and others. This section will focus on some aspects of how we remember and how we learn, and, in the following section, on disorders of cognitive function (e.g., dementias).

An almost universal complaint among older adults is the experience of not remembering as well as they once did. Impaired memory, from "benign forgetfulness" (see below) to major memory loss, seems to affect a large proportion, but not all, of the elderly. Memory is indispensable to normal cognitive function; sadly, in some degenerative diseases of aging such as the dementias, all cognitive functions, starting with memory, are eventually lost.

■ Memory Acquisition, Retention, and Recall: Changes in Old Age

While disorders of memory may occur at all ages, they occur with increasing frequency in old age. As mentioned earlier, there are various types of memory. Their characteristics and underlying mechanisms are still being elucidated (65,66). The long-held idea that information storage was widely and equally distributed throughout large brain regions has been displaced by the view indicating that memory is localized in specific areas of the brain. *Actually, current theories hold that memory is localized in discrete brain areas involved in specific aspects of short-term memory as well as being widespread, with many areas communicating to form long-term memory.* Among the primary areas involved are the limbic system (especially the hippocampus), the thalamus, the cerebral cortex (temporal, prefrontal, and frontal lobes), and the cerebellum.

The view that memory resides in localized discrete brain areas evokes the concept of cell groups producing specific neurotransmitters, released into the synapse, and integrally involved in the memory process. While neurotransmission is undoubtedly involved in memory and learning processes [e.g., the role of acetylcholine (ACh)], the specific identification of which neurotransmitter or combination of neurotransmitters is responsible for particular kinds of learning and memory continues to elude us (65–67).

Memory is a complex process that involves the ability to sense (visually, audibly, by touch, by smell, etc.) an object or event, to formulate a thought, to retain this information, and to recall it at will. It is not surprising, therefore, that different types of memory have been identified: two currently accepted classifications are presented in Table 3. Additional kinds of frequently used memory classifications are "explicit or declarative memory" (when we consciously recollect previous experiences), "implicit or nondeclarative memory" (when past experiences influence current behavior and performance even though we do not consciously recollect them), "prospective memory" correlated with "executive function" (e.g., the ability to plan, organize, self-monitor, and use strategies to remember to perform future actions), and others.

As for many other functions (Chapter 3), memory and learning show considerable variability among individuals, and this variability increases with advancing age (Fig. 3). Some healthy elderly retain intact memory until very old age or are capable of positive compensatory adjustments (Fig. 3) (65). However, many aged subjects do not perform as well as younger ones on many tasks having a significant memory component, particularly when the task is timed; in these individuals, the impairment occurs regardless of the length of time the information (to be recalled) was retained or the type of cognitive skills required for its retention (65–67).

Memory loss in the elderly appears to be restricted primarily to memory for recent events, leaving immediate and remote memory essentially intact (Table 4). Age differences in memory recall, with less proficiency for the elderly, are found in some types of long-term memory (e.g., episodic memory) but not in others (e.g., semantic memory) (Table 4).

In the elderly, deficits in memory are associated with other cognitive deficits, an overall decline in body functions, and an increase in medical comorbidities (Chapter 3). For example, the demented patient shows progressive, severe memory

TABLE 3 Classifications of Kinds of Memory

Three stages	Five stages
Sensory memory An image is recorded rapidly, faster than 1 sec *Short term memory or primary memory* Information endures several minutes *Long-term memory or secondary memory* May need hours or days to develop but lasts a lifetime	*Nondeclarative memory* Corresponds to classic conditioning, usually unconscious *The perceptual representational model* Responsible for early processing of sensory and perceptual information *Primary memory* Corresponds to short-term memory *Episodic memory* Refers to the ability to recollect specific autobiographical events *Semantic memory* Refers to store of factual knowledge independently from episodic recollection. These two stages together correspond to long-term memory

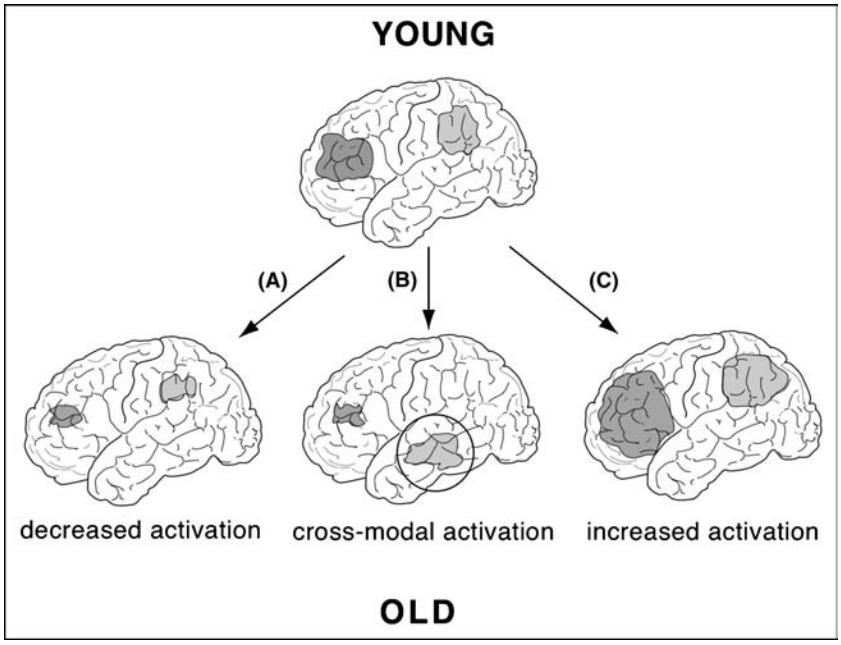

FIGURE 3 Different ways in which brain activation may vary between healthy young (ages 20–30) and healthy old (ages 60–79) individuals during the performance of a cognitive task (remembering a digit number) as detected by functional neuroimaging. Even though both groups performed the task well, the systems supporting performance differed between the two age groups. (**A**) A similar pattern of activation is seen in the two groups, but there is a greater activation in the young individuals in some of the brain regions. This suggests that these brain regions may not have been operating effectively as a network in older individuals. (**B**) A different pattern of activation is seen in the two groups, with new brain regions activated in old individuals that are not active in young individuals. (**C**) A similar pattern of activation is seen again in the two groups, but there is a greater activation in older individuals in some of the brain regions. The recruitment of new brain regions in old individuals (**B** and **C**) may have been the consequence of reduced neural interactions and can be considered compensatory, as the recruitment of these new brain regions apparently support performance. *Source*: Courtesy of Dr. M.T. D'Esposito.

impairment. Memory loss for recent events is the first severe problem encountered. This stage is associated with, or followed by, language dysfunction and, at this point, memory function is difficult to assess (68). There is an increasing clinical focus at the interface between memory changes associated with normal aging—the now called "mild cognitive impairment"—and the severe loss of all cognitive functions typical of dementia (69,70). It has been hypothesized that the cognitive deficits that occur with dementia progress in the same order as they are acquired in childhood, but in reverse. As functional stages merge and overlap at certain points of human development, the borderlines between the various clinical stages of dementia are subtle rather than abrupt.

■ Functional and Biochemical Correlates of Memory

Past and current studies have related memory changes in old age to impairment of the mechanisms that promote memory formation, such as reduced speed of elementary cognitive processes and reduced amount of attentional resources (e.g., decrease in alertness and awareness, sensory inputs, and information programming), all of which are required to accomplish complex cognitive tasks (67,68,71). *Some reports attempt to explain memory losses in the aged as a failure to remove irrelevant information rather than as a failure of acquiring and retaining new information (72–74).* In other words, information no longer needed remains active instead of being discarded. The failure to clear irrelevant information could easily disrupt memory. It is important to keep in mind that the elderly, by virtue of having lived longer and having accumulated more memories, have more memories through which to sort. Irrespective of whether memory impairment in old age is due to failure of information acquisition or removal or of more accumulated memories, the complexity of memory processes in older persons is further complicated by (*i*) their susceptibility to numerous factors; this is the case of changes in circadian rhythms as they affect responses that depend on testing time, (*ii*) of type of measures chosen to test memory, and (*iii*) of aging-associated changes in inhibitory control (74).

Role of Neurotransmitters in Memory Processes

The cellular and molecular mechanisms underlying memory processes from early development to old age are being actively studied, and our understanding of them continues to evolve (Table 5). Several neurotransmitters have been implicated in modulating and facilitating the acquisition and retention of information (Table 5). One of the first to be considered was ACh, the major transmitter in the cholinergic neurons of the septohippocampal and entorhinal areas and the nucleus basalis of Meynert (all areas associated with memory). In AD, where memory loss is an early sign of disease, several neurons appear to be missing in these brain areas, especially in the hippocampus (Chapter 6) (Fig. 4). Indeed, in about 50% of AD patients, ACh is virtually absent in the ascending pathways from the nucleus basalis. None of the subsequent attempts to restore to normal the lowered levels of ACh has been able to normalize impaired memory, whether due to old age, disease, drugs, or experimental lesions. Rather, the administration of the following drugs has had little or no effect on memory:

TABLE 4 Summary of Changes in Human Memory with Old Age

Type of memory	Changes with old age
Procedural memory Covers learning and retention of motor, counting, spelling, reading, other skills	*Unaffected by aging* However, confusion (false memories) between "real" and "intended" action is more frequent in the elderly
The perceptual representational system Responsible for early processing of sensory and perceptual information	*No proven change* May depend on overall body function, decreasing in parallel with impaired physiologic competence, e.g., declining sensory (visual, acoustic) input
Primary short-term memory Refers to information held in mind	*Decline with aging is minimal* when tasks performed are easy; more severe decline when tasks are complex
Episodic memory Refers to ability to recollect specific autobiographical events that have occurred recently	Declines starting at 30 yr of age and progressively deteriorates to 80 yr and older
Semantic memory Refers to storage of factual knowledge independently from episodic recollection	*Declines little with age* However, word-finding failures increase with age, especially retrieving names; spatial memory is reduced
Remote Memory Refers to memories of the remote past	*Remembering declines with the remoteness of the event to the same extent in younger and older adults*. However, childhood memories often are retained better in older adults

- An increase in the precursor, choline
- An increase in the activity of the synthesizing enzyme (choline acetyltransferase)
- A decrease in the metabolizing enzyme [acetylcholinesterase (ACHE)]
- The administration of ACh agonists

These failures have dampened but have not destroyed interest in the role of ACh in memory (75,76). Thus, the interest in galantamine, an ACHE inhibitor and potential ACh agonist. Galantamine acts not only by inhibiting the activity of the enzyme ACHE, thereby reducing the breakdown of ACh and prolonging its action at the synapse, but also increases ACh release from the presynaptic neuron through its agonistic role on CNS nicotinic cholinergic receptors (77,78). Other attempts to bolster cholinergic inputs have involved transplanting embryonal stem cells in critical cholinergic areas, akin to the transplantation of dopaminergic cells in Parkinson's disease (Chapter 6). As with Parkinson's, results of cholinergic cell transplantation are still uncertain. The neurotransmitter serotonin has also been implicated in activation of sensory neurons (79).

Among other neurotransmitters, glutamate and, to a lesser extent, aspartate are excitatory transmitters in the brain and spinal cord (Chapter 6) that may play a role in memory processes (80). Glutamate acts by binding to two types of receptors: (*i*) those that regulate intracellular cyclic adenosine monophosphate (cAMP) levels, and (*ii*) those that are ligand-gated ion channels such as the *N*-methyl-D-aspartate (NMDA) receptors. Activation of NMDA receptors may well be necessary for converting new memories into long-term memories (Table 5). In addition to their role in memory, excitotoxic amino acids, such as glutamate, have been ascribed a role in promoting free radical accumulation in neurons and consequent neuronal degeneration (Chapter 5). Introduction of memantine, an antagonist of glutamate-gated NMDA receptor channels, as a treatment for cognitive and functional decline in patients with moderate-to-severe AD has shown some clinical benefit in slowing deterioration (81).

Role of Neuropeptides, Hormones, and Metabolites

A number of neuropeptides and hormones have been implicated in normal memory processes and in AD. Two often-proposed candidates are the hypothalamic vasopressin (Chapter 9) and the intestinal cholecystokinin (Chapter 19); however, there seems to be more substantive evidence that the primary function of these neuropeptides may be in anxiety processes and satiety rather than in memory (82,83). Nerve growth factor (NGF) is another peptide important not only during neural development but also in later life, when it is involved in maintaining CNS plasticity (84,85). However, NGF production declines with aging. Replacement therapy with NGF is effective when it is administered directly in the brain (84), a difficulty that can be circumvented in the laboratory rat by noninvasive intranasal administration (86) or transplantation of NGF-producing cells or by genetic or environmental interventions (86,87). Secretion of NGF and other neuropeptides has been stimulated in laboratory rats by providing "enriched" conditions and, in the case of the brain-derived neurotropic factor, by making the rats run on a treadmill (86).

While some memory improvement has been reported following administration of these neuropeptides as well as that of hormones (e.g., estrogens) (Chapter 10) in animals, including humans, their efficacy in preventing or reducing memory loss in the elderly remains controversial. Vertebrates (e.g., mollusks, flies, and worms) offer useful models for the study of memory and learning because of the relative simplicity of their neural networks and genome. Although informative data have been

TABLE 5 Neurochemical Correlates of Memory Impairment in Old Age

Decreased activity of the brain cholinergic system, especially in the hippocampus and midbrain
Decreased activity of neurotrophic factors, NGF, neuropeptides, hormones, others
Decreased NMDA NR2 receptors possibly accounting, in part, for impaired memory/learning in adulthood and old age
Decreased protein synthesis due to: blocking of protein kinase activity responsible for amino acid phosphorylation; in the absence of protein phosphorylation failure of cAMP response element to bind to the CREB protein that promotes transcription; decreased synaptic synthesis impairs of long-term memory by preventing synaptic potentiation of repetitive presynaptic stimuli
Changes in posttranslational modification at the synapse may be responsible for impairment of short-term memory in old age
Decreased cortical activation, as measured by brain-imaging techniques, has been recorded in old individuals in some brain area (frontal, temporal), whereas, in others (frontal), activation is increased. It is unclear whether increased activation represents recruitment of neurons to compensate for difficulty of task or diffuse, nondifferentiated activity

Abbreviations: CRE, cAMP response element; CREB, CRE-binding; NMDA, *N*-Methyl-D-Aspartate; NGF, nerve growth factor.

generated by use of these models, it remains to be ascertained how these data in animals are applicable to humans.

A number of studies have tested the effects of stimulating or inhibiting neuronal protein synthesis, known to be implicated in long-term memory (88). Inhibition of protein synthesis occurs in two stages: (*i*) decrease of the activity of protein kinase enzymes reduces protein phosphorylation; and, (*ii*) when proteins are not phosphorylated, transcription is blocked because of the failure of the cAMP response element (CRE) to bind to the CRE-binding protein that promotes transcription (Table 5). While deterioration in long-term memory has been explained in terms of changes in genomic (transcriptional) expression, alterations in short-term memory have been ascribed to posttranslation modification (that is, translation of the mRNA signal into a polypeptide) at the synapse (88).

Another approach to uncovering the mechanisms at work in human memory uses imaging techniques, such as magnetic resonance imaging (MRI) and positron emission tomography (PET) with radioactive tracers (e.g., 14C-labeled 2-deoxyglucose) to measure increases that occur in metabolic activity in response to the presentation of familiar visual clues. The distribution of the metabolic changes is compatible with the view that memory is localized in specific brain areas (e.g., the hippocampus) (Fig. 4), but the large number of neurons involved suggests that most "plastic" (i.e., metabolically responsive) cells participate in multiple forms of memory (89). It is still unclear whether increased activation means that neurons are being recruited to compensate for the difficulty of the task at hand (Fig. 3), or whether it simply indicates diffuse, nondifferentiated activity (90). Currently, under special conditions (e.g., administration of growth factors, antioxidants, and anti-inflammatory substances, and retinoic acid), neuroglial cells dedifferentiate into progenitor cells and these, in turn, may transdifferentiate into neurons (91,92). Currently, there is a renewed interest in this area of research because neuroglial cells play a potentially critical role in brain plasticity and respond to a number of intrinsic (endocrine) and extrinsic (environmental) stimuli (92–94).

The crucial role of memory in cognition and learning—and, therefore in most aspects of life—has lead to the development of an abundance of memory-training techniques, aggressively marketed in radio and television programs and in books (e.g., *Mega Memory*, *Total Memory Workout*, *Page-a-Minute Memory Book*, and many more). Nearly all of these techniques can be traced to the Greek Simonides who, more than 2000 years ago, named them "mnemonics" and suggested them as remedies for specific neurologic problems (for which they may be still useful today) but not for overall memory improvement. In addition to various drugs such as neurotransmitters (see above) also being marketed as "cognitive enhancers," various food and food supplements are being marketed as indirectly enhancing memory by improvement in blood circulation or reduction of free radicals and inflammation (Chapters 5, 14, and 23). Most of these substances do not seem to provide any benefit in healthy or demented individuals, and in fact, they may have some dangerous side effects (Chapter 22).

■ Learning and Longevity

Learning has emerged as a factor in prolonging life and reducing disability and disease in old age (Box 2). Epidemiologic studies have reported that there is a positive correspondence between life expectancy and amount of schooling one receives (95,96). The benefit of education persists when "active" life expectancy or life free of disabilities is compared to "total" life expectancy or life with disabilities and this finding is irrespective of sex and race. As Katzman (97) quotes, "Scholars grow wiser with age, but the noneducated become foolish." A higher level of education seems also to be associated with a lower prevalence of AD (98). In a long-term investigation, started in l986 and continuing today (the "Nun Study"), 678 Catholic nuns are being studied for the relationship between their writing proficiency at a young age and continuing in middle and old age, and their longevity and incidence of dementia (99). Reports to date indicate that those with the higher writing proficiency at a young age lived longer and had a lower incidence of AD (99,100).

According to one Japanese proverb: "Aging starts when we stop learning." Learning may be defined as the ability of the individual to alter behavior on the basis of experience. Learning depends, in part, on memory, which may be defined, in a large context, as a reception–processing–storage–retrieval function of the brain and the mind. It is the ability to recall past events. While "learning" and "memory" are integral to each other, both come in different forms that are thought to depend on different neural mechanisms and sites in the nervous system. It is not surprising then that changes in different kinds of learning and memory do not necessarily parallel each other. Environmental

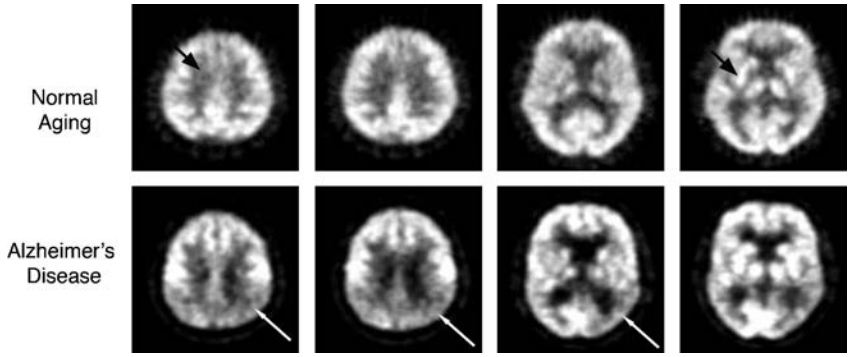

FIGURE 4 PET scans taken with the glucose metabolic tracer FDG. In these scans, whiter colors represent higher glucose metabolism. Two individuals, one with AD and one normal older person were studied, and each of four images represents a brain "slice" from the top of the head to the bottom (rostral to caudal). The AD patient shows reduced glucose metabolism in the parietal-temporal cortex, illustrated with white arrows. Also visible on these scans are the ventricles (*black arrow, upper left image*) and basal ganglia (*black arrow*, *upper right image*), which can be clearly seen in both subjects. Ventricular enlargement may be seen more clearly on MRI scans. Basal ganglia glucose metabolism is relatively preserved in AD. *Abbreviations*: PET, positron emission tomography; FDG, [18F]fluorodeoxyglucose; AD, Alzheimer's disease; MRI, magnetic resonance imaging. *Source*: Courtesy of Dr. W.J. Jagust.

BOX 2 *Continuing Education Throughout the Life Span*

The beneficial effects of education in prolonging life span and postponing the onset of disability and disease, although intellectually appealing, are in need of continuing experimental and clinical support. As private research and government health programs attempt to improve our understanding of biologic phenomena, so must all organizations devoted to improving quality-of-life at all ages recognize the value of continuing education to human health. By establishing a robust brain reserve at young ages, it is possible to draw from this reserve as we grow old and/or become disabled or ill. Given our present knowledge that the plasticity of the nervous system persists even in old age, the outlook for at least some degree of regeneration and compensation is brighter than it once was. Combined with other biologic and psychologic evidence, there is every reason to support education as an important tool in the eternal quest for a better and longer life.

changes trigger sensory responses that, in turn, modify the nervous system so that animals can learn and remember. This learning and remembering ability, which can be viewed as an expression of neural plasticity (Chapter 6) is altered with aging, despite the persistence of compensatory adjustments even in old individuals (Fig. 3) (101).

The beneficial effects of education on longevity and, indirectly, on normal and abnormal aging, has been ascribed to a number of factors, the most obvious being a better socioeconomic status (e.g., higher family income and greater employment opportunities) (Table 6) (Box 2). One of the most challenging interpretations of these observations is that extended learning may prevent (or at least, delay) the aging-related losses that occur in the nervous system with old age (102,103). The active process of learning is thought to build up a "brain reserve" in the form of an increased number and enhanced function of neurons and glial cells, better cerebral blood flow, higher oxygen levels, and glucose metabolism. Such an increased brain reserve may manifest itself as

- reduced or delayed neuronal losses or, conversely,
- reestablishment of neuronal and glial proliferation,
- increased synaptic density,
- changes in neurotransmitter levels (e.g., reduced sensitivity to excitotoxins),
- changes in receptor number and sensitivity (e.g., NGF low- or high-affinity receptors stimulate or inhibit apoptosis, respectively), and
- ion (e.g., Ca^{2+}) transport and distribution.

During normal brain aging as well as in the presence of disease, degenerative processes are often associated with adaptive growth and regeneration (101–103). Continued learning may act by inducing and strengthening these adaptive responses at all ages, including old age, thereby giving validity to the well-known adage "use it or lose it" (Box 2) (103).

TABLE 6 Mechanisms of the Effects of Increased Education on Successful Aging

Adequate income
Better access to medical care
Better access to recreational activity
Good nutrition
Responsible health behaviors
Moderate alcohol intake
Abstinence from smoking
Possibility of increased brain reserve capacity
More dendritic branching, more synapses
Better cerebral blood flow
Better neural cell efficiency, adaptability, survival, and growth

■ SENILE DEMENTIAS

■ Definitions and Prevalence

Dementia (from the Latin 'de-mens', without mind) is a clinical syndrome that refers to a global deterioration of intellectual and cognitive functions characterized by a decline of all five major mental functions—orientation, memory, intellect, judgment, and affect—but with persistence of a clear consciousness. Dementia caused by a variety of factors may occur at all ages and may be reversible or irreversible. The concept of a reversible dementia is sometimes a confusing one and needs to be accurately described, especially when dealing with individual elderly patients and their caregivers.

Reversible dementia is a subacute condition of impaired cognition to be contrasted to reversible acute changes in cognition, defined as delirium (the two concepts may be semantically confused). It is generally due to known causes, and once these are removed (e.g., drugs) or treated (e.g., infections), the dementia may be ameliorated. A handy mnemonic to remember the main causes of reversible dementia is presented in Table 7. A list of types of cognitive impairment in the elderly, to be differentiated from dementia, is presented in Table 8. Among these types, delirium is particularly alarming in the demented elderly, since the condition is associated with high morbidity and mortality (104).

In the elderly, dementia may occur due to multiple, but still little known factors. In most cases, dementia is irreversible and progressive (i.e., worsens with time). According to causes, pathology, and clinical manifestations, dementia has been categorized in several types, the distribution of which is illustrated in Figure 5:

- Dementia of AD accounts for 50% to 60% of all senile dementia cases.
- Vasomotor dementias and Lewy-body dementia account for 20% to 30% of all cases.
- Reversible dementias, as listed in Table 7, account for 10% to 20% of all cases.

TABLE 7 Underlying and Reversible Causes of Dementia

D	*D*rugs
E	*E*motional disorders
M	*M*etabolic or endocrine disorders
E	*E*ye and ear dysfunctions
N	*N*utritional deficiencies
T	*T*umor and trauma
I	*I*nfections
A	*A*therosclerotic complications, i.e., myocardial infarction, stroke, or heart failure

TABLE 8 Types of Cognitive Impairment in the Elderly To Be Differentiated from AD

Delirium: An acute alteration of mental status characterized by inattention and disordered thinking. Other symptoms include clouding of consciousness and fluctuation of symptoms. Severity ranges from mild to severe. Delirium is reversible, with improvement of mental function occurring after the underlying medical condition has been treated. Delirium is particularly problematic in the elderly, especially those who are already demented, inasmuch as it is responsible for high morbidity and mortality (104)
Depression: A specific psychiatric entity that can precede or be associated with dementia and that can be differentially diagnosed and treated
AAMI: Normal aging changes, not progressive and not of sufficient severity to interfere with daily function. Mild cognitive impairment is an entity describing those in the nonspecific interface area between AAMI and early AD (69)
Paranoid states and psychoses: Patients presenting with specific diagnostic psychiatric correlates
Amnesic syndrome: Characterized by inability to register new information but without delirium or dementia

Abbreviations: AAMI, age-associated memory impairment; AD, Alzheimer's disease.

- Depression or pseudodementias (i.e., condition in which symptoms of depression may mimic or mask those of dementia) (105) account for 1% to 5% of all cases.
- The remainder comprise miscellaneous disorders such as Parkinson's disease, and Pick's disease (see below).

■ Alzheimer Disease

AD is the most frequent type of dementia. Structural differences between the brains of demented and nondemented older individuals are essentially quantitative: in the aging brain of nondemented individuals, neurofibrillary tangles and neuritic plaques are few (Chapter 6), whereas they are numerous and widely distributed in the brain of the AD patient; in fact, their inordinate accumulation is accepted as a definitive diagnostic marker of the disease.

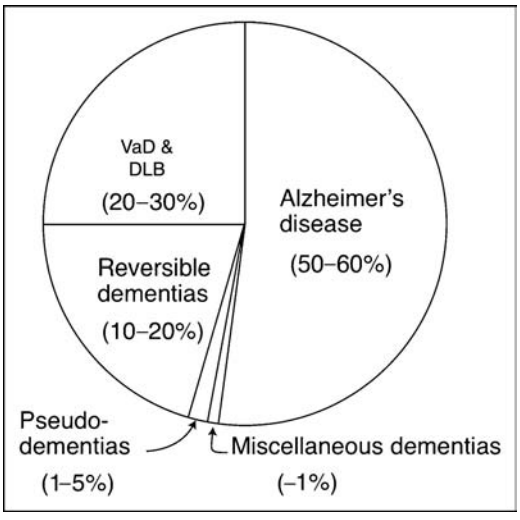

FIGURE 5 Percentage of major forms of dementias in the elderly. *Abbreviations*: DLB, Lewy-body dementias; VaD, vascular dementias.

It is important to recognize that dementia is not an inevitable consequence of aging; the greater proportion of the elderly remains lucid and mentally competent until death. One study showed that, in 1982, severe dementia affected only 4% to 5% of those aged 65 years and older and 10% of these patients were affected by mild-to-moderate forms. The incidence (that is, the number of newly diagnosed cases in a certain time interval) of severe dementia rises dramatically with age. Likewise, the prevalence (that is, the number of cases of a disease existing for a given population and at a given time) also increases with old age, doubling with each subsequent five-year interval from about 1% at ages 65 to 70 to 20% to 50% at ages 85 or older. However, when the prevalence of dementia is compared at selected intervals (1982, 1988, 1994, and 1999) (Box 3), these percentages, of the same age groups, show a significant decline (104–107). *Age and family history are the most important risk factors for dementia in older populations*. Although some studies seem to indicate a slight decline in AD after age 90, it is still unknown why, exactly, after age 90, other diseases become more common than AD. Recent studies show, in the United States, an increase in AD as cause of death in the 85 + age group from 1996 (1.9%) to 2003 (5.5%) (Chapter 3, Table 13).

The relatively young age of the patient (51 years of age), a woman, first described by Alzheimer, indicates that AD may also occur at younger ages than those so far discussed. Usually, those individuals diagnosed with the "early-onset" form of AD (40–50 years) present the same symptoms and pathology as those diagnosed with the "late-onset" form (65 years and older), but their symptoms are generally more severe, and the disease progresses more rapidly. Considerable genetic research with AD presently involves these familial early-onset cases.

About two-thirds of the patients in nursing homes have dementia; therefore, two-thirds of the enormous amount of money spent in nursing home care in the United States each year (many billions of dollars) is expended on patients with dementia. Each year, several hundred thousand Americans develop the disease, and at least a hundred thousand die with it. Presently, according to the AD Association, there are, in the United States, more than 4 million patients with AD, with an estimate as high as 14 million over the next 30 years, an estimate considered quite conservative by many. In 2003, in the United States, AD complications (e.g., pneumonia, falls, and accidents) represented the fourth leading cause of death within the 85 + age group (Chapter 3). Probably more so than other chronic diseases, the demented state leads to other morbid conditions that represent the immediate cause of death. The problem of care for demented patients is a serious one; it represents a significant burden for individual caretakers, health-care resources, and society at large (Box 4).

Pathogenesis of AD

In 1907, Alois Alzheimer, a German psychiatrist, described a case of a 51-year-old woman with a five-year history of progressive dementia (i.e., the adult or presenile, early-onset form), which led to her death. At autopsy, he found many neurofibrillary tangles and neuritic plaques in her cerebral cortex and, particularly, in the hippocampus. Today, with the significant advances in brain imaging techniques and the availability of better diagnostic neuropsychological and biochemical tests, the diagnosis of AD still requires a history of a progressive dementia in life and the presence of significant amounts of the two pathologic lesions, neurofibrillary tangles and neuritic plaques, which can be seen only at autopsy. A combination of these measures provides a high sensitivity and specificity of diagnosis (81% and 71%, respectively) (112). Major

BOX 3 *Possible Decline in Prevalence of Severe Cognitive Impairment Among Older Americans*

Despite some previous pessimistic predictions, a current study allows us to be more optimistic about our ability to reduce the prevalence of severe cognitive impairment, such as Alzheimer's disease (AD), by various health and societal interventions. Comparison of severe cognitive-impairment prevalence among participants of National Long-Term Care Surveys (U.S.) shows a significant decline in cognitive impairment from l982 to 1999 (105). Contrary to the sobering forecast of a few years ago of a steady growth to be expected in the number of severely cognitively impaired patients in the United States and Western Europe, the recent data disclose a decline in cognitive-survey impairment prevalence over the last 20 years.

The possible causes for this decline in severe cognitive impairment with aging may be due to the following:

- Continuing improvement in higher education level (see section entitled Learning and Longevity)
- Improved medical care and specific treatments (e.g., better prevention and management of stroke and heart disease and better medications to lower blood cholesterol) (Chapter 16) (108)
- Use of known steroid anti-inflammatory drugs (commonly utilized in the treatment of arthritis) associated with almost 50% reduction of AD (109,110)
- Hormone (estrogen) replacement therapy (Chapter 10)
- Better nutrition and improved physical activity (32) (Chapters 23,24).

The decline in severe cognitive, impairment prevalence has been associated with a parallel decline in chronic disabilities in the U.S. elderly population (111). Indeed, we can perhaps be more optimistic about future changes in the prevalence of severe cognitive impairment and other chronic disabilities than had been previously foreseen.

signs of Alzheimer's disease include morphologic, biochemical, and metabolic alterations as briefly identified in Chapter 6 and listed in Table 9.

Despite active research, the origin and nature of the characteristic lesions, tangles, plaques, and perivascular amyloid deposits, remain uncertain. One reason for the uncertainty is the available small number of animal or in vitro AD models; despite several promising transgenic models, none so far has proven entirely satisfactory. While AD clinical and neuropathogical hallmarks point to a well-defined syndrome, the cellular and molecular defects responsible for the disease are multiple, and their precise nature remains ambiguous. What seems to be generally accepted is that AD is characterized by a "common cascade" of pathologic events that may depend on an interaction between genetic defects and environmental influences (Chapter 3).

Among generally accepted mechanisms underlying AD pathology is the role of free radicals (Chapter 5). Another theory formulated first by Prusiner (113–117) suggests that dementia is caused by unconventional infectious agents, such as a virus or virus particles called prions (114,115). Spongiform encephalopathies, associated with dementia in animals (e.g., scrapie in sheep and mad-cow disease in cattle) and in humans (e.g., Creutzfeldt–Jakob disease) are characterized by structural lesions that resemble some of the AD brain lesions, although significant differences exist between the two conditions. The theory posits that prions would either participate in or induce the formation of the insoluble amyloid and of the abnormally

BOX 4 *A Capsule View of the Clinical Picture*

Of the myriad complaints we hear from aged patients and their family members and caretakers, none evokes (or should evoke) as much concern and anxiety as that of a change in mental function. ("I'm lost, lost somewhere in the corridors of my mind. . ." was the way an Alzheimer's victim, in one of his more lucid moments, explained it to his devoted wife.) Part of the tragedy of this terrible dementia is that it takes hold of fully developed, intelligent, dignified human beings, usually with family and friends, and slowly destroys them. It is a disease that kills the mind years before it takes the body.

Alzheimer's disease (AD) begins as simple forgetfulness, something that can be found in a large proportion of the normal healthy elderly. For most, this is the extent of the problem, but in roughly 10% of those over 65, there is progression to a confusional phase of mild-to-moderate dementia. Many, perhaps most, in this stage, eventually decline further to the point where they can no longer care for themselves and are, frankly, demented. By the time dementia has developed, the life expectancy is about 2.5 additional years. The clinical course of the disease averages about eight years.

Confusional states in the elderly are associated with a number of conditions that must be differentiated from dementia; indeed, many such conditions can be treated successfully. The most common conditions to be differentiated from primary dementia are listed in Table 8. Even when dementia has been diagnosed, AD must be differentiated from several other forms of dementia, the most frequent, in the elderly, is multi-infarct dementia.

TABLE 9 Selected Characteristics of Alzheimer's Dementia

Anatomohistology	Pathology	Metabolism
Brain atrophy, flattening of gyri, and widening of sulci and cerebral ventricles Loss of cholinergic neurons in nucleus of Meynert, hippocampus, and association cortices Loss of adrenergic neurons in locus ceruleus Denudation of neurons, stripping of dendrites, and damage to axons Increased microglia	Accumulation of cell inclusions: lipofuscin, Hirano and Lewy bodies, altered cytoskeletal Tau proteins, and ubiquitin Neurofibrillary tangles, neuritic plaques with amyloid, perivascular amyloid, distributed throughout the brain, but especially in frontal, prefrontal lobes, hippocampus, and association cortices	Decreased oxidative metabolism and slower enzyme activity Free radical accumulation Impaired iron homeostasis Other minerals, zinc, and aluminum Reduced level/ metabolism/ activity of neurotransmitters Increased amyloid β peptide with accumulation of amyloid proteins Increased prion protein Altered immune response

paired helical filaments and be responsible for the loss of cholinergic neurons in certain brain areas (117). Mutation in the prion protein may, in humans, lead to inherited familial "transmissible spongiform encephalopathies," such as the Creutzfeldt–Jakob's disease, with abnormal protein aggregates and dementia. The possible role of prions in AD etiopathology has called attention to the possibility that disorders of immune responses may cause or contribute to AD pathology (Chapter 14). The presence of abnormal brain proteins, their intra- and extracellular accumulation, and the resulting inflammation and proliferation of microglia, the immune cells of the brain, may activate immune responses that would further aggravate neural damage (118).

Still uncertain at present is whether AD lesions originate in the neurons and then spread to the extracellular and perivascular spaces or whether they are blood borne and are carried to the brain through a damaged blood–brain barrier. Most researchers in the field consider the amyloid-β fragment of the amyloid precursor protein (APP) to be the pathogenic molecule in AD (117). Nerve cell injury is viewed by others as the primary cause, and extracellular lesions, such as deposition of amyloid fibrils, are viewed as the secondary cause. Intracellular lesions may be consequent to or associated with alterations of cytoskeletal protein phosphorylation by protein kinases and accumulation of brain proteases or of toxic metals such as iron (118), zinc (119) and aluminum (120,121).

Biochemical analysis of perihelical filaments (PHFs) that accumulate intracellularly to form neurofibrillary tangles demonstrates that the principal PHF protein subunit is an altered form of the microtubule-associated tau protein (122–125). Tau protein is known to bind to tubulin and promote the assembly and stability of microtubules. With aging, tau proteins may become hype-phosphorylated (identified as A68 proteins and detected by the specific Alz 50 antibody) (124), perhaps due to increased activity of several kinases. As a consequence, these proteins would no longer be capable of stabilizing the microtubules, and, thus, PHFs and neurofibrillary tangles would form (108). By far the most popular but still controversial theory of AD pathogenesis today involves the overproduction of amyloid βprotein. The following is a synopsis of the major steps in the so-called "amyloid connection."

The Amyloid Connection

Amyloid degeneration and amyloidoses have been discussed briefly in Chapter 6. The term "amyloid" given to these deposits over 100 years ago implies erroneously that they are formed of a starch-like substance (Latin amylum for starch). Actually, the amyloid molecules are normal or mutated proteins or protein fragments that differ among the various amyloidoses they generate (Chapter 6).

As previously stated, in the brain, amyloidβ (Aβ) peptide, the major component of the neuritic plaque amyloid, is thought to be the pathogenic molecule in AD. What causes its significant accumulation in AD is not yet clearly known. The Aβ peptide, which contains 40 to 42 amino acids, derives from a larger molecule, the amyloid precursor protein (APP), a transmembrane molecule with 695 amino acids, which normally undergoes cleavage at multiple sites. APP itself does not harm the cells; it is only when Aβ peptide is clipped out of APP by protein-splitting enzymes (secretases) that the smaller molecule may lead to pathology (108). APP is embedded in the cell membrane vesicles (endosomes), while the Aβ molecule sits astride the membrane, where it cannot be reached by the protein-splitting enzymes (109).

During normal cellular processing, APP is split by the enzymes α-secretase, β-secretase, and γ-secretase. Processing of α-secretase occurs at or near the cell surface and, in AD, an underactivity of this enzyme precedes the formation of Aβ. Overactivity of the β-secretase may yield fragments that contain Aβ, which give rise to amyloid degeneration and accumulation (Chapter 6). This reaction occurs in the Golgi apparatus and lysosomes, the cell organelles that are usually the site of protein breakdown (109). Overactivity of the third proteolytic enzyme, γ-secretase, can also cause overactivity of the Aβ peptide. This occurs at the carboxyterminal segment. The question as to the identity of the factor(s) capable of causing this over- or underactivity of the secretases remains unanswered, but genetic mutations in the proteins, presenilin I and II are thought to be involved. Other theories regarding the role of the Aβ protein include

- alterations occurring during the early secretory trafficking of APP in the endoplasmic reticulum and Golgi apparatus or in endocytic recycling,
- presence of an APP mutation, or
- alterations in protein phosphorylation, and
- abnormalities of lysosomes (i.e., cell organelles containing hydrolytic enzymes involved in degradation of foreign materials engulfed by phagocytes).

Once Aβ-peptide has aggregated inside the lysosome, the cell has difficulty getting rid of it. Aβ-peptide accumulation would lead to cell damage and death; this is followed by amyloid accumulation in the extracellular spaces and formation of the neuritic plaque (with the remnants of the neurofibrillary tangles from the dead cell and other debris). At this point, microglial cells from the immune system would surround the plaque (Chapter 6, Figure 10). Some investigators emphasize that brain damage starts with the formation of amyloid by perivascular macrophages and microglial cells (110). The damage and loss of neurons would cause a selective loss of neurotransmitters, with alterations in synaptic signaling. The presence of an inflammatory component in the formation and accumulation of the neuritic plaques and amyloid deposits (110,111) provided the rationale for using anti-inflammatory drugs to prevent and treat and for attempts—thus far unsuccessful—to prepare a vaccine against the disease (126,127).

Aβ peptide or APP cleavage products are neurotoxic. The major factors held responsible for this toxicity include

- disruption of Ca^{2+} homeostasis by membrane damage and, thus, increased neuronal intracellular Ca^{2+},
- the increased intracellular Ca^{2+}, which would be responsible for the tau protein hyperphosphorylation and the formation of PHFs, which, in turn, form neurofibrillary tangles,
- the cascade of events leading to NFTs, which may be induced by elevated levels of Aβ (128).

Not all investigators support a central pathogenic role for Aβ protein in AD; their reluctance to accept the amyloid connection as the primary cause of AD is due, in part, to the lack of definitive evidence for a specific neurotoxic species of Aβ and of its effects on synaptic function in vivo. Rather, soluble oligomers (i.e., polymers consisting of a small number of units) formed within intracellular vesicles and, subsequently, secreted by the cells, would be responsible for the disruption of synaptic plasticity in AD. Moreover, prevention of the oligomer formation and cytotoxicity would restore synaptic plasticity (128–130).

Other studies support a normal (physiologic) rather than a pathologic role for Aβ; accordingly, symptoms of dementia may occur before significant plaque buildup and cell loss are evident. Other studies suggest a beneficial function of Aβ on the synapse. Thus, AD would be caused by synaptic failure rather than by neuronal death: neuronal activity would induce formation of Aβ and, in turn, the secreted Aβ would depress neuronal activity by a negative feedback function and reestablish synaptic balance, contribute to neuronal health, and regulate neuronal activity (130–133).

Therapeutic Strategies Based on the "Amyloid Connection"

Based on this amyloid hypothesis (Chapter 6), several therapeutic strategies have been proposed. Although therapeutic applications are still experimental, a list of these putative strategies may help to better understand the role of amyloid in AD. Therapeutic strategies intend

- to block delivery to the brain, by the bloodstream, of APP molecules ultimately responsible for the Aβ deposits;
- to inhibit the secretases that cleave APP to produce the Aβ peptide;
- to delay the formation of Aβ deposits by interfering with the formation of fibrillar, cytotoxic amyloid filaments;
- to interfere with activation of macrophages, microglia, and cytokine release that contribute to the inflammatory reaction surrounding the plaques; and
- to block the Aβ molecules on the surface of neurons to prevent their toxic action.

The Genetic Connection

Some patients with AD have a family history of autosomal-dominant inheritance; these cases are designated as "familial AD" (Table 10). The majority of AD patients, however, lack familial connections (difficult to ascertain with certainty because family histories are always retrograde) and is designated as affected by "sporadic" or "spontaneous" AD. In both familial and sporadic AD, the cause of the disease is currently unknown, although, for the familial type, a definite genetic connection is useful for early diagnosis and future treatment and possible prevention (134). As for sporadic AD, epidemiological studies would exclude the causal influence of animal contacts, smoking, pollutants, drinking, and dietary habits. Other observations suggest an association with elevated homocysteine levels in some AD patients, perhaps related to a decreased level of folate (Chapter 15), prior viral infections, exposure to chemicals, or aluminum, or any correlation with neoplasms. However, still other data associate AD with head trauma, cardiovascular disease, and a less convincing association with thyroid disease (Chapter 12). There is a definite association of AD with Down syndrome.

The cloning of the gene encoding the Aβ-peptide and its mapping to chromosome 21 have strengthened the amyloid connection hypothesis. Chromosome 21 is altered in Down syndrome: individuals with this trisomy are severely mentally impaired and develop clinical signs of AD at an early age (Chapter 3). The association of mental disability with AD pathology is strong evidence that overproduction of Aβ is the root cause of the neural degeneration underlying dementia (134,135). Mutation in the APP and the onset of early-onset AD in some patients has been traced to chromosome 21.

Early-onset AD associated with APP is also associated with mutations in the presenilins I and II (*PS1* and *PS2*) on chromosomes 14 and 10, respectively. *PS1* and *PS2* mutations lead to dysregulation of secretase activity in a way that would selectively enhance the proteolysis of APP toward the amyloidogenic β-secretase pathway (135–137). How presenilin mutations increase Aβ peptide accumulation is still unclear, but the effect may be due to the formation, in the endoplasmic reticulum or the Golgi apparatus, of complexes between βAPP and Aβ peptide, with perhaps the addition of other peptides.

The apolipoprotein E allele, E4 (APO E4) on chromosome 19 is not a cause, but rather a risk factor for the late-onset form of familial AD (138,139) (Chapters 3 and 16). Fifty percent of AD cases do not carry an APO E4 allele, suggesting that other risk factors exist. One of susceptibility locus for late-onset familial AD has been reported on chromosome 10 (140,141) and may act by modifying Aβ peptide metabolism and/or increasing its production.

The human brain is an expensive tool with a huge proportion (40% and higher) of human genes involved in constructing and functionally maintaining the CNS (142). The information from the human genome project will accelerate our knowledge of normal brain function as well as the identification of the genes involved in neurodegenerative diseases such as AD. A list of genes responsible for some forms of familial (inherited) AD or representing factors increasing susceptibility to AD are listed in Table 10.

The Estrogen Connection

The potential benefits or dangers of hormone replacement therapy on the nervous and cardiovascular systems of postmenopausal women are controversial. A discussion of the effects of estrogens on normal and abnormal aging of the brain is presented in Chapter 10, with the overview of the aging of the female reproductive system.

AD Management: Maintaining an Optimistic Outlook

The etiology of Alzheimer's dementia remains unknown despite the several theories briefly surveyed here (e.g., prions, Aβ peptide, specific genes, alterations of microtubular protein tau, abnormal proteins, inflammation, and actions of metals) and in Chapters 5, 12, and 14. Prevention, diagnosis, and effective treatment of AD must wait until we have identified its etiology, and from what we know presently, it is likely that there are multiple etiologies rather than a single one.

Various treatments have been proposed to prevent or at least slow AD progression or alleviate associated symptoms

TABLE 10 Genes Known to be Linked to Alzheimer's Disease[a]

Gene	Chromosome	Inheritance pattern	Age of onset	% cases familial	% cases all
Presenilin 2	1	ADIP	40–70 yr	20	2–3
Unknown	10	Risk factor	>60 yr	–	–
Presenilin 1	14	ADIP	30–60 yr	40–60	5–10
Apolipoprotein E4	19	Risk factor	>60 yr	–	40–50
APP Mutation (βAPP) Down syndrome trisomy	21	ADIP	45–60 yr	2–3	<1

[a] Given the rapid progress in genetics, additional genes may be related in the future to Alzheimer's disease.
Abbreviation: APP, amyloid precursor protein; ADIP, autosomal-dominant inheritance pattern.

(143). Many of these treatments are promising, but to date, none can provide a cure (Table 11). The basic medical workup for dementia consists of a complete and ongoing history and physical examination and a formal mental status exam, usually with the help of family or friends. Laboratory tests include, besides the usual blood tests, neuropsychological testing and brain imaging, at least initially and later in the progression, as indicated. PET may provide some useful information in terms of specific diagnosis in isolated cases, particularly with respect to eventual metabolic changes in specific brain areas such as the hippocampus (Fig. 4).

Once dementia has been diagnosed, a variety of therapeutic modalities are currently available to treat cognitive and behavioral symptoms and signs; but no intervention is available, as yet, to alter or reverse the deterioration. Besides the ACHE inhibitors and memantine and a wide variety of psychotropic medications to treat associated psychiatric symptoms, a wide range of drugs have been, or are being, explored that appear to be useful. Among the drugs used for AD treatment are

- antioxidants,
- antiviral and antiprion agents,
- calcium channel blockers,
- endorphin blockers, and
- hormones (e.g., estrogens).

Newer agents are continually being tested for potential benefit for AD patients. The lack of a specific cure for AD, however, should not discourage the physician from helping patients and their families. Some of the basic goals in AD management are outlined in Table 11. Many of these approaches will not be easy to implement and may not be successful, but the goal of optimizing treatment is worth the effort. Dementia is a condition that requires not only the attention of the physician but also the family and other caregivers as well as other branches of the health-care system (the "team") in order for the patient to be effectively managed.

TABLE 11 Basic Goals of Alzheimer's Disease Management

To maintain the patient's safety while allowing as much independence and dignity as possible
To optimize the patient's function by treating underlying medical conditions and avoiding the use of drugs with side effects on the nervous system
To prevent stressful situations that may cause or exacerbate catastrophic reactions
To identify and manage complications that may arise from agitation, depression, and incontinence
To provide medical and social information to the patient's family in addition to any needed counseling

■ Multi-Infarct Dementia and Other Dementias

Multi-infarct dementia (MID) is one of the vascular dementias. It may be classified by the underlying cerebral vascular disease or stroke, according to location, size, vascular distribution, severity, and etiology of the lesions. MID can be characterized by a history of abrupt onset and/or stepwise deterioration related to the episodes of hypoxia and strokes. MID results from recurrent infarcts (hence, its name) and vascular lesions of the brain following stroke or localized transient ischemia, that is, localized tissue anemia due to obstruction of blood flow (144): both stroke and ischemia are due to atherosclerosis of cerebral arteries (Chapter 15). As for the diagnosis of AD, the presence of these lesions can be detected by computerized X ray, computed tomography (CT) scan, or, preferably, by neuroimaging devices such as MRI and metabolism PET. All these neuroimaging procedures can be used as clinical diagnostic tools for AD (145). If the infarcts are too small to be visualized, a clinical diagnosis of MID can be made if the symptoms presented in Table 12 are present.

Unfortunately, as in AD, once the diagnosis has been made, therapeutic measures to cure the cognitive symptoms of the dementia are not currently available. However, based on postmortem studies, it is now known that vascular lesions may produce significant cholinergic dysfunction in brain, and use of ACHE inhibitors are now used routinely in these patients with improvements in both cognitive and functional symptoms (146). Preventing stroke, through control of hypertension, atherosclerosis, and some of the concomitant risk factors such as obesity, smoking, elevated cholesterol, and diabetes mellitus, continues to provide an important means to reduce the incidence and progression of this disease (Chapters 12, 15, and 16).

Other forms of chronic and progressive dementias include:

- Dementia with Lewy Bodies, with fluctuating cognitive deficits, motor deficits, and recurrent visual hallucinations
- Parkinson's disease with dementia (PDD) with cognitive and motor deficits
- Frontotemporal dementia, also known as Pick disease with emotional, language, motor, and cognitive deficits

The pathology of these forms of dementia is complex and the knowledge of their cause, diagnosis and treatment is still evolving. They will not be discussed here and the interested reader may wish to consult more specialized texts (147–149).

TABLE 12 Characteristics of Multi-Infarct Dementia

History of abrupt onset or stepwise deterioration
History of transient ischemic attack or stroke
Presence of hypertension or arrhythmia
Presence of any neurologic focal symptoms or signs

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8

Sensory Systems: Normal Aging, Disorders, and Treatments of Vision and Hearing in Humans

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INTRODUCTION

This chapter describes aging changes in the structure and function of the human visual (see section entitled Vision) and auditory (see section entitled Hearing) systems. Aging-associated visual (Tables 1 and 2) and auditory impairments, disorders, and diseases are also presented in the above-mentioned sections, and current efforts for their treatment and rehabilitation are reviewed (Boxes 1–6). The basics of the aging changes in the senses of taste, smell, and somatic sensation are not included in the present chapter, mainly due to lack of space, but can be found in the previous editions of this chapter (1). In this fourth edition, some discussion of the aging of taste and smell is presented in Chapter 19 along with aging of the gastrointestinal system.

Sensory impairments in vision and hearing occur so commonly with aging that they often tend to characterize the aged and the aging process.

These impairments

- are due to intrinsic aging processes occurring in the sense organs and their neural and brain components,
- may be caused by environmental effects, or
- represent manifestations of aging diseases.

The study of the aging process in the human sensory systems, in addition to its importance and applicability to geriatrics, also provides some of the most interesting and challenging cases of gerontological investigation.

The elements comprising the various senses and their aging portray the entire spectrum of cellular, tissue, organ, and system aging: The peripheral receptor cells of the ear's cochlea and the eye's retina are permanently established at birth, with no turnover and regeneration in later life, in part contributing to the functional decrements in vision and hearing. The aging changes in the eye's lens provide another interesting model system for the study of the aging process, as they begin so early in life and lend themselves to a wide variety of investigations ranging from molecular biology to physiological optics.

IMPACT OF SENSORY IMPAIRMENTS ON ELDERLY LIFE AND HEALTH

The incidence of sensory impairments increases markedly in people with aging (Fig. 1). More than 25% of the population 85 years or older suffers from visual abnormalities; twice as many suffer from hearing impairments. Impaired vision and hearing reduce the capacity for social communication, one of humans' cardinal needs and functions, resulting in social isolation and deprivation. The impact of hearing and visual impairments on elderly health and mortality has been reviewed by Guralnik (3). Age-related vision and hearing impairments have a greater impact on long-term health than previously thought. The results of a 10-year study of 5000 subjects aged 55 to 74 years show that measured (not subjective) visual impairment was predictive of 10-year mortality; similarly, both measured and subjective visual impairments were significant in predicting certain aspects of functional disability.

Measured combined visual and auditory impairments lead to the highest risk of functional impairment (3). The synergistic effect of co-occurring impairments (4) may lead to effective future treatments of visual and hearing disorders of the elderly (3). The basic aspects of the effects of reduced capacities in somatic, olfactory, and gustatory senses on aging and physical and mental health of the elderly have been reviewed by Meisami (1). The reduced sensory abilities may lead to depression; in those already suffering from depression, they may hinder the progress of recovery. Because sensory losses in old age are so common and their consequences so widespread, an understanding of these impairments is now essential in geriatrics and elderly care (3).

VISION

The eye, with a structure that indicates exquisite adaptation for optical and nervous functions, is the sensory organ for vision (Fig. 2). The cornea, lens, pupil, the aqueous humor, and vitreous gel participate in the optical functions of the eye, while the retina carries out neural visual functions. Both the optical and neural compartments undergo aging changes, although those of the optical compartment are better known. Some of these changes in the eye's optical apparatus, such as those in the lens, start early in life. The changes in the optical compartment are probably the primary causes of decline in the visual capacities of the elderly, while the degenerative changes in the retina are one of the leading causes of old age blindness (Table 1) (6–13). In addition to the eye, aging changes in the visual pathways and central visual structures, such as the lateral geniculate bodies and the visual cortical areas, which

TABLE 1 Summary of the Aging Changes in the Human Eye

Structural changes	
Cornea	Increased thickness; decreased curvature; transparency loss; pigment and lipid accumulation; loss of epithelial cells; reduced epithelial regeneration
Anterior chamber	Decreased volume and flow of aqueous humor
Iris	Decreased dilator muscle cell number, pigment, and activity; mild increase in density of collagen fibers in stroma
Lens	Increased anterior-posterior thickness; decreased curvature; increased pigment accumulation and opacity; decreased epithelial cell number; decreased new fiber formation and antioxidant levels; increased crossover in capsule collagens and lens crystallins; increased hardness in capsule and body
Vitreous body	Increased inclusion bodies; decreased water; lesser support to globe and retina
Ciliary body and muscles	Decreased number of smooth muscles (radial and circular); increased hyaline substance and fiber in ciliary process; decreased ciliary pigment epithelial cells
Retina	Decreased thickness in periphery; defects in rod outer segments, and regeneration of discs and rhodopsin; loss of rods and associated nerve cells; some cone loss; reduced cone pigment density; expansion of Muller cells; increased cyst formation; formation of Drusen-filled lesions; degeneration of macular region
Pigment epithelium	Loss of melanin; increased lipofuscin granules; atrophy and cell loss
Functional changes	
Corneal and lens functions	Decreased accommodation power (presbyopia); increased accommodation reflex latency; increased near point-of-vision; increased lenticular light scattering; decreased refraction; decreased lens elasticity
Retinal function	Decreased critical flicker frequency; decreased light sensitivity (increased light thresholds before and after dark adaptation); reduced color vision initially in yellow to blue range and later in the green range
General optic functions	Increased pupillary constriction (senile miosis); reduced visual acuity; presbyopia
Major pathologies	
Cornea	"Against the rule" astigmatism
Lens	Cataract; hardening and loss of elasticity
Retina	Senile macular degeneration; diabetes retinopathy
Glaucoma	Open angle and closed angle types

have been found to be quite extensive in humans, may also be responsible for some of the visual impairments of the elderly, but the knowledge of these aspects is only recently developing.

■ The Eye's Optical Components

Cornea

The cornea is the anterior portion of the eye, and its curved surface together with the watery layer of tears is responsible for most of the refraction of the light rays. During aging, the cornea becomes thicker and less curved, mainly due to an increase in the horizontal diameter of the eye. These changes alter the refractive properties of the cornea, leading to "against the rule" astigmatism, a condition characterized by defective corneal curvature and diffusion of light rays (8). The cornea is also highly sensitive to irritable stimuli, a protective function for the eye (8). Corneal sensitivity declines by nearly one half between youth and old age (8).

Other conditions in the cornea associated with aging are the arcus senilis (or senescent arc), the Hudson–Stahli line, and spheroidal degeneration. The arcus senilis increases in frequency and density with aging, particularly after 60 years. It is a yellowish-white ring around the cornea's outer edge, formed by cholesterol ester deposits derived from plasma lipoproteins (lipid arcus). In the dilated pupil, this ring would interfere with passage of light rays; however, because of partial pupillary constriction in the elderly, arcus senilis is not detrimental to visual function (7–10).

The Hudson–Stahli line is a horizontal brown line formed by iron deposits in corneal basal cells. Its frequency increases from 2% at 10 years to 14% at 30 years and 40% at 60 years but has no detrimental effect on vision. *Spheroidal degeneration* occurs in cornea's Bowman's layer. It is observed frequently in aged populations exposed to high levels of ultraviolet (UV) radiation or ambient light reflected from snow or sand (8).

The corneal endothelial cells number about one million per cornea at birth; this number declines to 70% by 20 years and to 50% and 30% by 60 and 80 years, respectively. Normally, the pumping action of corneal cells removes water and helps keep the cornea transparent. Because these cells do not divide after birth, their loss due to aging or injury after surgical treatments of the cornea or lens can lead to a decline in corneal transparency. Endothelial cells also secrete the cornea's basement membrane. With aging, warts (cornea guttata) appear in this membrane mainly in the cornea's periphery and cause marked increase in corneal permeability. Guttatas are observed with increasing frequency with aging: 20% in youth, 60% in the sixth decade, and nearly 100% in very old age (8).

Recent Studies on Corneal Aging: Biochemical and Structural Changes

Biochemical studies revealed a gradual decline in high-energy metabolism of the aging cornea as shown by linear decreases in phosphomonoesters, phosphocreatine, and ATP, accompanied by decreased inorganic orthophosphate (14). Corneal aging is accompanied by a linear loss of keratocytes paralleling loss in endothelial cell density (15). A three-dimensional expansion of collagen fibrils along the axial direction occurs in corneal stroma with aging. The expansion is due to decreased molecular tilting angle within fibrils. It reflects an increased number of fibrils due to expansion of intermolecular Bragg spacing caused by glycation-induced cross-linkages (16). Previously described morphological aging changes in cornea have been confirmed and better described. Human corneas become less symmetrical with age; increased pupillary dilation and wavefront aberration become more pronounced with aging (17). Steepening of corneal curvature with aging, as shown by a decrease in vortex radius and an increase in P-value, reflects a shift to a more spherical surface (18).

Corneal and Conjunctival Sensitivity

In a recent study, Acosta et al. (19) found that corneal thresholds to mechanical and chemical stimuli increased with age. Premenopausal women were more sensitive to corneal stimulation than men of similar ages, but overall differences in mechanical and chemical threshold between men and women were not significant. Also corneal mechanical threshold depended on age and iris color. Similar results were also noted by Roszkowska et al. (20) with regard to corneal sensitivity.

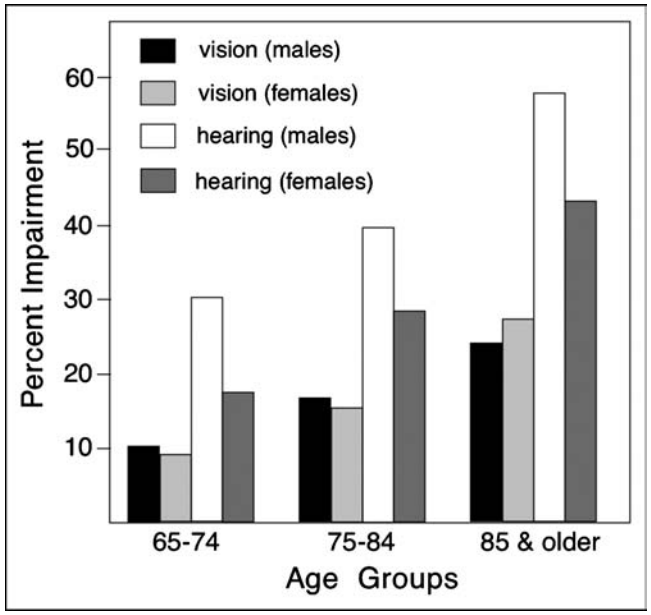

FIGURE 1 Increase in the incidence of visual and hearing impairments in males and females 65 years of age and older in the general U.S. population. Note the higher rate of sensory impairments in males, particularly for hearing. *Source*: From Ref. 2.

Asphericity and Cell Density

A flattening (more horizontal than vertical) of both anterior and posterior corneal surfaces of the cornea was reported in a recent study (21). With aging, the asphericity of both corneal surfaces change significantly, causing a peripheral thinning of the cornea. As a result, the astigmatism (i.e., defective curvature of the cornea resulting in blurred and imperfect image) of the posterior corneal surface compensates for that of the anterior one. According to another recent study by Roszkowska et al. (22), both central and peripheral endothelial cell densities decrease with age in the cornea, with higher peripheral decrements in older subjects. Old subjects showed significant differences in cell densities between corneal center and periphery but not the young adults. Similar cell density changes were noted with aging but differences were not found in corneal endothelial cell density values between emmetropic (i.e., normal accommodation and refraction), myopic (i.e., short-sighted), and hyperopic (i.e., far-sighted) subjects (23).

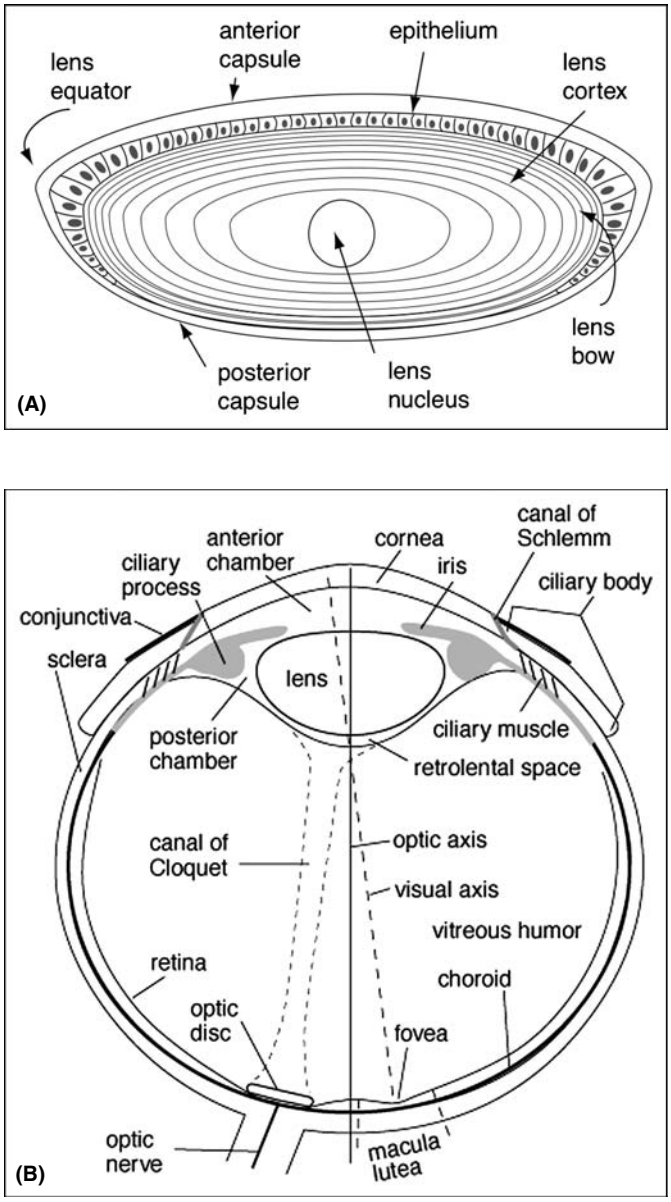

FIGURE 2 Schematic drawing of the human eye and lens. *Source*: From Refs. 5 (eye illustration **B**), 6 (lens illustration **A**).

The Lens

In the process of image formation, the crystalline lens of the eye performs two important functions, refraction and accommodation. For refraction, the lens requires an appropriate crystalline structure and transparency; while for accommodation, it needs to be elastic, amenable to changes in its curvature. The increase in the opacity (optical density) and the hardness (loss of elasticity) of the lens with aging are two of the best-known changes in the eye's optical properties that interfere with refraction and accommodation, respectively (6,24).

Knowledge of the structure and development of the lens is essential for understanding its aging. The biconvex lens is basically a fibrous and relatively acellular structure, consisting of a core surrounded by a capsule (Fig. 2A). Anteriorly, the capsular epithelial cells form the fibers and other lens proteins. The collagen fibers of the lens capsule facilitate changes in lens shape during accommodation. The lens core is packed with transparent protein fibers and consists of an inner nuclear zone surrounded by a cortex (Fig. 2A).

Structural Aspects of Lens Growth and Aging

The lens is formed during the embryonic period and is fairly spherical in the fetus and newborn. During postnatal development and throughout maturity, the lens continues to grow by addition of new layers of protein fibers laid down by the capsular epithelial cells. As new fibers form, older fibers are pushed into the lens core. This mode of growth results in increased horizontal thickness of the lens together with increased compaction of the fibers in the nuclear zone (8,24). The lens thickness increases from about 3.5 mm in infancy to 4.5 mm in middle age and to 5.5 mm in old age, growing at a steady, linear rate of 25 m per year (8,12,24). Underlying this process of growth are the capsular epithelial cells, which divide and differentiate,

losing their nucleus and organelles, and eventually transforming into an inert skeleton of fibrous proteins (24).

Recent Volumetric and Morphological Studies on Aging Lens

According to Koretz et al. (25), although total lens volume increases with age, the volume of lens nucleus and the shape of nuclear boundaries do not show any significant changes with aging. The lens center of mass and central clear region move anteriorly with aging (25). In addition to an increase in lens mass and volume with age, changes occur in point of insertion of the lens zonules (24). Also, the radius of the lens' anterior surface curvature decreases with aging. The increase in sagittal lens thickness with age is caused, in part, by the anterior movement of lens mass and shallowing of the anterior chamber (8,24).

Aging Changes in Lens Dimension

In a recent in vivo study, Dubbelman et al. (26) used densitometry and compared thickness of the lens cortex versus nucleus with age and found cortex thickness increases with age seven times more than the nucleus; also the anterior cortex was thicker than the posterior one. These increases were limited to zone C2 and did not involve zones C1 and C3. In vitro aging changes in human lens between the ages of 20 and 99 years were investigated: the lens dimensions and the anterior radius of curvature increased linearly with age while the posterior curvature remained constant (27). The ratio of anterior thickness to posterior thickness was constant at 0.70. It is suggested that in vivo forces alter the apparent location of the lens equator (27).

Increased Opacity of the Lens

Although many cytoskeletal proteins such as actin, tubulin, and vimentin are found in the lens core, the transparency of the lens is, in principle, due to a particular supramolecular arrangement of the specific lens proteins, α-, β-, and γ-crystallins, within an ion- and water-free environment (8,24). During aging, the lens opacity increases, leading to decreased transparency and increased refraction. Because the crystallin fibers in the lens interior are not regenerated during growth and aging, they undergo many posttranslational changes, including glycation, carboamination, and deamidation. These changes increase crossover and interdigitation among crystallins, making them less elastic, more dense, opaque, and yellowish (8,12,24,28,29).

Some of these aging changes in the lens proteins occur as a consequence of oxidative damage (Chapters 4 and 5) to the protein antioxidants, such as glutathione (GSH) and ascorbate, which diminish in concentrations in the aged lens, while yellow chromophores, particularly metabolites of tryptophan (β-OH-kynurenine, anthranilic acid, bityrosine), increase in frequency of occurrence and concentration. The net results of this increased oxidative damage are:

- a threefold increase in lens optical density (at 460 nm, blue) between 20 and 60 years (13),
- a resulting decreased transmission and increased light scattering, particularly in the blue and yellow range but much less so in the red range, and
- a decreased percentage of transmission of light by the eye from about 75% at 10 years to 20% at 80 years.

In addition to impairing transparency and refraction of light, these aging changes may also affect color perception. Excess lens opacity as a consequence of extensive accumulation of pigments may result in a pathological condition known as *cataract*, characterized by a cloudy lens (7–10,24). This condition may cause reduced vision or blindness (see also below). In normal aging, the accumulation of yellow chromophores and the increased refraction of blue light may protect the retina from the damaging effect of blue light, "blue-light-hazard" (8,24).

Recent Studies on Biochemical and Biophysical Changes in Lens with Aging

Biochemical changes in the human lens with aging include increased insolubility of nuclear-region crystallins, accompanied by formation of high–molecular weight aggregates that may underlie the deformity of the lens nucleus. Increased light scattering, spectral absorption, and lens fluorescence are likely causes of the decrease in light transmission with age. Accumulation of glutathione-β-hydroxykynurenine (GSH-βOHKyn) glycoside causes increased yellowing and fluorescence of the lens, and these may be responsible for accumulation of high–molecular weight aggregates (24). Aging changes were observed in some but not all crystallins, including increased truncation of N-termini, degradation of C-termini, and partial phosphorylation (29). Other studies show increased β-crystallin, but decreased γ-crystallin proportions during the postnatal period. A major portion of water-soluble proteins in adult lenses is truncated between β-B1 and β-A3/A1 crystallins, and all crystallins are susceptible to deamination with aging (24,30). According to a recent study by Wilmarth et al., deamidation in lens crystallins significantly increased with age, especially in the water-insoluble fractions, and methylated cysteines with other products of posttranslational modification were present at lower levels (31).

Phospholipid and Lipid Changes in the Lens Membranes

Phosphatidylcholine decreases with age in epithelial and fiber membranes, but the rate of decrease is higher in the epithelial membranes; both membranes show a steady increase with age in the percentage of sphingomyelin (32). Epithelial membranes contained about five times more phosphatidylcholine than age-matched fiber fractions (32). The distribution of 3-β-OH-cholesterol shows a decrease in the anterior region of the lens relative to the posterior region with aging (24). Lipid oxidation increases linearly with age (Chapter 16) (33). Ganglioside composition changes with age; ganglioseries gangliosides increase, with no significant accumulation of sialyl–Lewis X gangliosides; however Lewis X–containing neolacto-series glycolipids increase with age and cataract progression (34). Lens aging is accompanied by decreased transport of water and water-soluble low–molecular weight metabolites and antioxidants entering the lens nucleus via the epithelium and cortex; this may lead to progressive increase in oxidative damage (35).

Decline in Accommodation and Development of Presbyopia

With aging, the increased crossover and interdigitation and compaction of collagen fibers in the capsule and crystallins in the lens nucleus result in gradual hardening and reduced elasticity of the capsule and lens interior (6–8). These changes make the lens gradually less resilient to accommodate for near vision. Indeed, the point of near vision, that is, the minimum distance between the object and the eye for formation of a clear image, increases tenfold during human life, from 9 cm at the age of 10 years to 10 cm at 20 years, 20 cm at 45 years, and 84 cm at 60 years.

The loss of accommodation with aging can also be determined by studying the changes in the eye's refractive power, measured in diopters (reciprocal of the principal focal distance of the lens in meters). Thus, the newborn's lens, being

more spherical, shows the highest refractive power (about 60 diopters). As shown in Figure 3, the accommodation power of the human lens decreases to about 14 diopters at the age of 10, and 5 diopters at 40 years, reaching a minimum of 1 diopter by the sixth decade. At this age, the lens becomes hard and nonresilient, and is essentially unable to accommodate for near-vision tasks. This condition is known as presbyopia (Fig. 3) and is of major clinical significance, as practically everyone over 55 years needs corrective convex lenses or eyeglasses for reading and other near-vision tasks.

Environmental effects such as those of heat and temperature can increase the rate of aging changes in the lens fibers, accelerating presbyopia. People from warmer climates show earlier presbyopia (9,14). The aging changes in the suspensory ligaments, the ciliary muscles, and their parasympathetic nerve supply and the associated synapses may contribute to the decline in accommodation with aging. Latency of accommodation reflex decreases during aging.

Recent Studies on Aging of Accommodation Response and Its Causes

The accommodation response shows a decrease in magnitude of fluctuation as well as in amplitude and speed of accommodation with age, indicating a decrease in accommodation dynamics. In older subjects, lens shape contributes little to power, while lens position in the eye significantly influences the power spectrum (37). The time constant for far-to-near accommodation increases with age at a rate of 7 msec/yr, while that for near-to-far accommodation increases at 6 msec/yr, supporting a lenticular cause of presbyopia (37). The damping coefficient of the lens increases 20-fold between 15 and 55 years of age (37).

The static accommodation response with aging reveals a slow decline from youth to the age of 40, after which the curve slope shows a rapid decline (38). Also observed is a decrease in tonic accommodation and its amplitude, presumably caused by biomechanical factors (38). However, subjective depth of focus increases due to increased tolerance to defocus, which is related to onset of presbyopia (38). Based on analysis of aging changes in focal length, surface curvature, and resistance to physical deformation, carried out in isolated human lens, lens hardening appears to be the major cause of presbyopia, and the loss of accommodation cannot entirely explain presbyopia (39). A magnetic resonance imaging study shows that ciliary muscle contraction remains active during aging, but the accommodated ciliary muscle diameter decreases with increasing lens thickness, indicating that presbyopia may depend on the loss of the ability of the lens to disaccommodate due to increased lens thickness or inward movement of the ciliary ring, or both (40).

Iris and Senile Miosis

The iris is a smooth muscular ring forming the pupil of the eye (Fig. 2B). Contraction and dilation of the pupil during the light reflex changes the amount of light entering the eye and is also important in the accommodation reflex. In the elderly, the iris appears paler in the middle, mainly due to loss of pigmentation in the radial dilator muscles. With aging, there is a mild but constant increase in the density of collagen fibers in the iris stroma and noncellular perivascular zones.

A characteristic ocular impairment in the elderly is a persistent reduction in the pupil size (diameter), the so-called "senile miosis" (13). Senile miosis is particularly notable in the fully dark-adapted eye; the reduction in diameter occurs gradually with aging, decreasing from a mean of 8 mm in the third decade to 6 mm in the seventh decade and 5 mm in the tenth decade of life (Fig. 4) (6). Senile miosis results from a relatively higher rate of aging atrophy in the radial dilator muscles, which dilate the pupil, compared to the sphincter constrictor muscles, which constrict it. As a result, the sphincter is constantly dominant in the elderly, causing persistent constriction. Compared to youth, the reduced pupil aperture of the elderly results in a one-third reduction in the amount of light entering the eye (13).

Anterior Chamber and Vitreous Gel (Humor)

The anterior chamber and its fluid, the aqueous humor, occupy a space between the cornea and the lens (Fig. 2B). The size and volume of the eye's anterior chamber decrease with age, mainly due to thickening of the lens. This growth occasionally exerts pressure on the *canal of Schlemm* (Fig. 2B), an outflow channel at the junction between the iris and the cornea, causing decreased flow and increased pressure [intraocular pressure (IOP)] of the aqueous humor. In normal aging, the increase in IOP is small and steady. Severe obstruction of the canal of Schlemm caused by degenerative changes in the endothelial cells of the trabecular sheets and

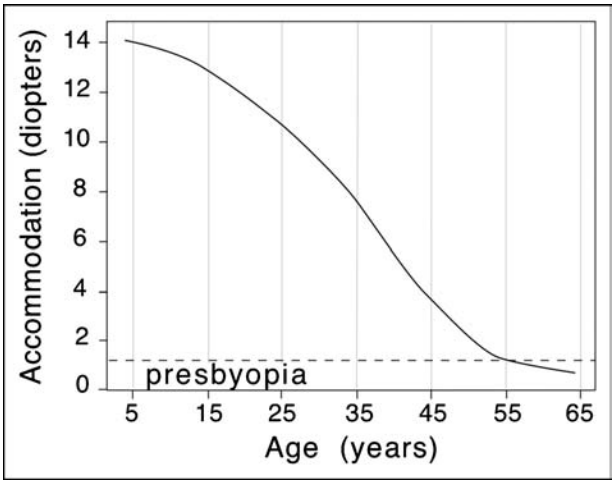

FIGURE 3 Changes in visual accommodation in humans with age. Note that the decline occurs throughout life, resulting in presbyopia in the early 50s. *Source*: From Ref. 36.

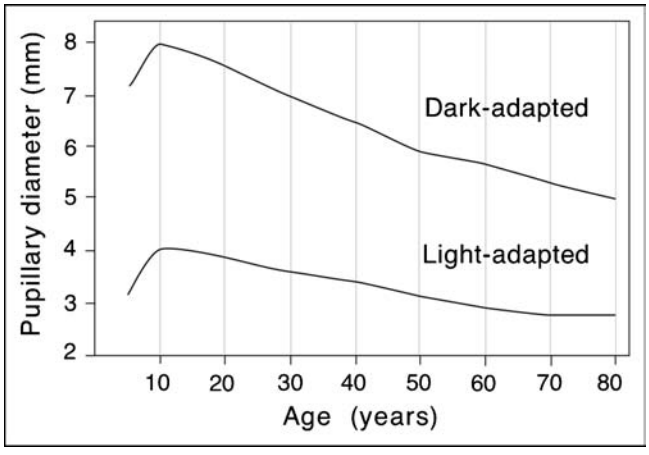

FIGURE 4 Changes with age in pupillary diameter and area, measured in light-adapted and dark-adapted individuals. Note the more pronounced effects in dark-adapted eyes. *Source*: From Ref. 13, based on the original data of Ref. 41.

meshwork leads to markedly elevated IOP (>22 mmHg) and the serious eye disease glaucoma (7,8,10) (see below).

The vitreous humor, also called vitreous gel, is a mass of gel-like substance filling the eye's posterior chamber. It gives the eye globe its shape and internal mechanical support (Fig. 2B). With age, the vitreous humor loses its gel-like structure and support, becoming more fluid and pigmented. The increasing inhomogeneity in its gel structure, a process called *syneresis,* can lead to vitreous collapse or its detachment from the retina; during this process, often *vitreous floaters (inclusion bodies)* are released in the process, which are responsible for occasional visual flashes. These physical changes in the vitreous humor may also be due to aging changes in its collagenous fibrous skeleton, which has attachments to the retina, particularly in the vitreous gel base near the periphery. These attachments change with age, moving posteriorly and decreasing in number (9,10,12).

Aging-Related Changes in the Retina

The human retina shows considerable age-related structural changes, particularly in its peripheral zones, although the macula and its fovea centralis are not spared. The aged retinal periphery is thinner (10–30 μm), containing a lesser number of rods and other nerve cell types. The aging-related loss of rods appears to be a slow process, beginning in the third and fourth decade, and may be related to accumulated damage due to physiological exposure to light (8–10). With aging rods, outer segments shorten and disengage from the microvilli of pigment epithelium, resulting in lesser amounts of membranous discs and their major constituent rhodopsin, the rods' photoreceptor molecule. These events may be related to changes in the turnover of rod discs with aging (8). Normally, the entire population of rod discs turns over every two weeks. This process and packing orderliness of discs slowly declines with aging, perhaps due to changes in the function of pigment epithelial cells that regulate the turnover of photoreceptor cells. The result is reduced efficiency of phototransduction (see below).

Aging of Retinal Neurons

Early Studies on Retinal Aging

Retinas of humans and monkeys lose cones at a rate of 3% per decade (8). The turnover rate of cones was believed to be about a year, making them susceptible to accumulated effects of light damage and posttranslational modification of their photoreceptor proteins. Cone pigment density decreases with aging, presumably as a result of cone cell loss (9). The remaining cones increase in average diameter. Because size and geometry of foveal cones are important in visual acuity, these changes may contribute to the observed losses in visual acuity with age (see below). Nerve cell loss during aging in other neuronal types of retina, i.e., bipolar, amacrine, and horizontal cells, is around 30%. The loss for the *ganglion cells* is believed to be higher, about 50%. *Mueller cells* (a type of glia cell in the retina) take over the space left by the lost neurons and form cysts, which are common in the aged retina (7,8,11). The degeneration of macula in advanced age is discussed in section titled Aging and Eye Diseases.

Recent Studies on Retinal Aging

Photoreceptor density decreases with age at a rate of 0.2% to 0.45% per year, with rods showing a more marked loss than cones (42). Cone mosaics are more organized in temporal regions compared to peripheral regions and show no change with aging, but aging distorts the regularity of the nasal peripheral cone mosaic (43). While foveal cone numbers remain stable with age, the parafoveal rods show a decrease of 30% by old age. This condition leads to greater loss of scotopic sensitivity compared to phototropic sensitivity (44). Short-, middle-, and long-wave–sensitive cones were analyzed for their sensitivity. A decrease in sensitivity, that is, an increase in threshold, was observed for all three types of cones (45).

In another recent study, Cavallotti et al. (46) studied numerous structural and biochemical changes in the human retinal tissues with aging. The study compared retinas from young adults and donors aged 60 and older, using scanning electron microscopy and biochemical methods. Highly significant changes in retinal thickness, numbers of ganglion cells, numbers of capillaries, synaptic bodies, and cellular processes as well as in intercellular connections were observed with aging. Also, tissue protein and cytoplasmic protein concentration were decreased significantly with age, but not structural protein concentrations.

Aging of the Optic Nerve

A study of the optic nerve head region in relation to open-angle glaucoma (OAG) revealed a decrease in neuroretinal rim area at a rate of 0.28% to 0.39% per year; vertical optic cup diameter and optic cup areas increased with age, and so too did mean cup/disc diameter ratio (0.1 from 30 to 70 years) (47). For more discussion of optic nerve changes with aging, see section titled Glaucoma.

Aging of Retinal Pigment Epithelium

Cell Number and Regional Distribution

Earlier studies indicated that retinal pigment cells do not divide after maturity and decrease in number in advanced age (8). Recent studies show that aging causes an increase in pigment cell area, peripherally, but a decrease centrally, while total cell number does not change markedly (48,49). The distribution of cells in the retinal pigment epithelium (RPE) of older retinas becomes more heterogeneous, and RPE cell density from fovea to mid-periphery and to peripheral fundus regions decreases with aging at a rate of 0.3% per year (50).

Recently Del Priore et al. (51) reported a significant increase in the proportion of apoptotic human RPE cells with age; these cells were confined mainly to the macula of older human eyes. Since the density of RPE cells remains unchanged in the macula and decreases in the periphery, it is possible that migration of peripheral RPE cells may compensate for the death of macular RPE cells.

Lipofuscin and Lysosomal Activity

RPE cells in the foveal region show increased accumulation of the aging pigment, lipofuscin, during aging (8). Increased lipofuscin formation and accumulation in postmitotic cells may be related to increased autophagocytosis and decreased intralysosomal degradation or exocytosis (Chapter 6). The RPE layer shows cell loss, pleomorphic changes, decreased content of intact melanin, and metabolic changes (52). Lysosomal activity in RPE increases, as shown by an increase in cathepsin-D and β-glucuronidase; but arylsulfatase-B and α-mannosidase show no change with age (49). In another study, the latter two and other glycosidases were found to decrease with aging (53).

■ Aging of Visual Functions

Eye Movements, Visual Thresholds, Critical Flicker Frequency, and Field and Spatial Vision

Smooth pursuit and saccadic movements

Smooth pursuit movement gains were decreased with aging, while saccadic reaction times increased (54). Saccadic reaction

times were slower in people older than 60 years, compared to young adults (55). Visual sensitivity to motion decreases with age, and the change is more pronounced in the central visual field compared to other areas (8).

Sensitivity and Visual Threshold

As discussed above, the decrease in pupillary aperture with aging results in lesser light input. The decline in the number of photoreceptor cells (rods) and other aging changes in the retina result in reduced availability and regeneration capacity of the photoreceptor pigment (rhodopsin), leading to reduced light utilization in the aged eye. As a result, the visual threshold, i.e., the minimum amount of light necessary to see an object, increases with aging. This is tested by measuring the change in visual threshold as a function of time spent in darkness (dark adaptation). It is known that the threshold for light detection decreases with increased duration of dark adaptation, because rhodopsin regeneration is enhanced in the dark. With advancing age, this regeneration is presumably deficient, resulting in higher light thresholds (i.e., lower sensitivity).

As illustrated in Figure 5, the enhanced light sensitivity after dark adaptation is markedly reduced with aging; in fact, the visual thresholds in the completely dark–adapted eyes of the aged group (80 years) is 100 times higher than that of the young group (20 years). However, as evident from the data, the pattern of change in sensitivity during dark adaptation is basically similar in the different age groups. This indicates that retinal function is quantitatively, but not qualitatively, impaired. The decline in threshold may be due to reduced oxygenation of the retina and the rods in the aged (7,8).

Recent Studies on Scotopic Sensitivity

Scotopic sensitivity shows a 0.5 log unit decrease with age; the loss is enhanced in the perimacular region (57). The area of scotopic spatial summation (Rico's area) was measured in adults in the age range of 20 to 85 years and was found to increase with age (58).

Critical Flicker Frequency

The rate at which consecutive visual stimuli can be presented and still be perceived as separate is called the critical flicker frequency (CFF). Determination of CFF in different age groups provides one way by which changes in visual function with age can be measured. These tests reveal a decline in CFF with aging, from a value of 40 Hz (cycles/sec) during the fifth decade to about 30 Hz in the eighth decade (59). The persistent miosis in the elderly must contribute to this decline, because the decline is less marked with fully dilated pupils. Foveal flicker frequency sensitivity in healthy aging eyes showed a decrease in foveal temporal contrast sensitivity coupled with losses in amplitude but not temporal resolution. The mean rate of loss was 0.78 decilog per decade after the age of 45 years (60).

Aging Changes in Visual Field and Spatial Vision

With aging, a loss in the size of visual field is observed, ranging from 3% to 3.5% in middle age to two and four times as much at 60 and >65 years, respectively. Disturbances in visuomotor performance are particularly observed when changes in "useful or effective visual field" are measured, in contrast to measures of visual field under standard clinical conditions (61). Visual acuity reflects the ability to detect details and contours of objects. It is defined as the minimum separable distance between objects (usually fine lines) and is one of the measures of the visual system's spatial discrimination ability. As shown in Figure 6, visual acuity declines, commencing at 50 years, particularly worsening during and after the eighth decade, when it becomes detrimental to vision (8,61).

Recent Studies on Spatial Vision and Visual Acuity

A recently devised measure of spatial visual ability is contrast sensitivity, where the test format and conditions more closely resemble real-world conditions (61). Aging studies in this category indicate that at low spatial frequencies, such as when grating of wide bars are presented, little aging changes are observed, while at high frequencies (fine bars), a marked decline in contrast sensitivity is observed with aging, beginning at 30 years. This deficit may underlie certain reading disorders in the elderly, such as reading of very small or very large repetitive characters, where the elderly show nearly 70% deficit (61). A recent analysis on visual acuity changes with aging did not reveal significant change in high visual acuity, but all aspects of spatial vision showed a decrease, particularly under conditions of decreased contrast, luminance, and glare. Also found was reduced stereopsis, poor color discrimination, and decreased peripheral vision, particularly when divided attention was required (63).

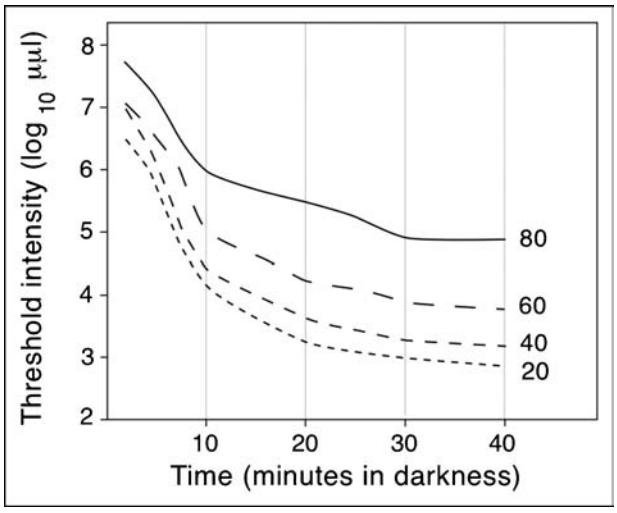

FIGURE 5 Decline in light sensitivity with age. Data show changes in visual threshold after dark adaptation in different age groups (20, 40, 60, 80 years). *Source*: From Ref. 28, based on the original data of Ref. 56.

FIGURE 6 Changes in visual acuity measured in Snellen index with age. Note that the aging decline begins after 60 years, resulting in serious deficits in acuity after 80 years. *Source*: From Ref. 62.

The decline in visual acuity and contrast sensitivity with age may be the result of alterations in the following optical and neural factors:

1. altered refraction of the light rays by the cornea and the lens
2. decline in accommodation
3. decreased light input due to constricted pupils (senile miosis)
4. decline in the density, number, and function of visual receptor cells, particularly in the fovea
5. aging changes in central neural structures of the visual system

It is important to note that elimination of glare and improved illumination can help enhance visual acuity substantially in the aged population.

Impaired Vision in Everyday Tasks as Shown by Changes in Useful Field of Vision

Early studies indicated that impaired visual capacity is a major factor in performance of the elderly in such everyday visual tasks as driving (61). Incidence of driving accidents was four times higher in the elderly with reduced "useful field of view," compared to the age-matched group with maximum useful field of view. Indeed, in the category of accidents occurring at intersections, the incidence rate was 16 times higher in individuals with reduced useful field of vision (61). Recent studies on the effects of aging on useful field of view by Sekuler et al. (64) confirmed earlier findings of a decrease in this parameter with age; the decrease begins at about the age of 20 years. The reduced ability is more pronounced when conditions require divided attention between central and peripheral tasks (64). A similar increase in number of errors for localization of peripheral targets indicates a decrease in the useful field of view (65).

Color Perception

Beginning with the fourth decade of life, color perception shows a progressive decline with age. Women are relatively less affected than men. During the fourth and fifth decades, the deficiency in color perception is mostly in the short wavelength range, i.e., yellow to blue. This is explained by the changes in the lens, as it becomes more yellow with age. In the later decades, after the sixties, a deficiency in the green range also becomes manifested, probably due to retinal or more central factors, as evident even after removal of the lens (28,61). Aging defects in color perception are exaggerated under reduced illumination.

Changes in Central Visual Pathways

With advancing age, considerable changes in the *optic* nerve (66) and visual cortex (67) have been reported. Changes in the visual cortex have included thinning and cell loss. Earlier electrophysiologic investigations indicated marked flattening of the *evoked potential* responses to light flashes in the different parts of the cortex. In a more recent study, it was found that the amplitude of some of the components of visual-evoked potentials (VEPs) is markedly reduced, while the latency of response is increased (68). The latter changes are significantly more marked in elderly men.

Recent Findings on Central Visual Impairments in Aging

Justino et al. (69) investigated visual electrophysiologic responses, such as electroretinograms (ERGs) and cortical VEPs, in healthy elderly subjects (>70 years), compared to young adults, using stimuli biased toward the functioning of the intensity-sensitive magnocellular and color-sensitive parvocellular subdivisions of the visual system. The elderly subjects showed a decrease of ERG and VEP amplitudes, as well as an increase in VEP latency. These age-related effects were magnified when stimulus conditions combined magnocellular and parvocellular pathways, indicating that normal aging affects the functioning of both visual pathways. A positron emission tomography (PET) study by Levine et al. (70), measuring regional blood flow in the brain, indicated that young adults and the elderly utilize different pathways for form perception. Older subjects activate occipital and frontal regions while perceiving forms, while younger people utilize the occipito-temporal pathway preferentially, indicating possible reorganization of visual processing during human aging. Another recent study by Tam et al. (71) focused on the effects of aging on the multifocal electroretinogram (mfERG). Age-related decreases in mfERG were found which were due to optical factors (decrease in retinal light levels and light scattering) before the age of 70 years, but neural factors affected mfERG topography after the age of 70.

■ Aging and Eye Diseases

Various degrees of vision loss and blindness, commonly caused by senile cataract, glaucoma, macular degeneration, and diabetic retinopathy, represent the extreme consequences of age-related ocular pathologies (Table 1). Diabetic retinopathy is discussed in Chapter 14. In the United States, for patients in the age range of 75 to 85 years, the prevalence of cataracts is 46%, macular degeneration 28%, and glaucoma 7.2% (8,11). Screening for these disorders includes testing visual acuity, ophthalmoscopic examination, and checking IOP. As a result of increased occurrence of eye diseases with age, the incidence of blindness shows a 25-fold increase with age, from about 0.1% in the middle-age group to 2.5% in the elderly (>75 years) (8).

Cataract

In some individuals, the accumulated normal aging changes in pigment and protein composition in the lens take pronounced and pathological dimensions, leading to a condition known as cataract (7,8,10). Among the causes of cataract is the glycation of lens proteins, such as the Na^+-K^+-ATPase, resulting in abnormal ion–water balance, swelling, and breakdown of crystallin lens proteins. Also, water-insoluble crystallins and yellow chromophores accumulate excessively, particularly in the nuclear type (8).

As a result of the above pathologies in senile cataract, the lens interior becomes cloudy and opaque, light refraction is greatly reduced, and light scattering is markedly increased. These effects lead to loss of visual acuity, reduced patterned vision, and eventually to functional blindness, with only a degree of light perception remaining. The occurrence of lens opacities markedly increases with age from about 4% in the middle-age group to 12% at 50 years to nearly 60% in the seventh decade (8). In the same age groups, the percentage of individuals showing visual loss as a result of cataract increases from about 1% to 3% and 30%, respectively. Cataract occurs more frequently in diabetic individuals, especially females. Severe cataract is the third leading cause of blindness in the Western world, after macular degeneration and glaucoma. In general, 50% of people over 65 years of age have cataract.

Cataracts may occur in the lens periphery (cortical cataract) or center (nuclear cataract). The incidence of the nuclear type is always higher than that of the cortical type (65% compared to 28% in the >75 years group). Cortical cataract is particularly detrimental to visual acuity, while nuclear cataract interferes more with

color perception. A mild cataract can be managed with periodic examination and use of eyeglasses. However, when the reduction in visual acuity interferes with the patient's daily activities, cataract surgery may be necessary (7–9) (see also below).

Genetic, Racial, and Environmental Factors Influencing Cataract Development

Genetic factors are important in the development of cataract. Incidence of cortical cataract is much higher among mono- or dizygotic twins compared to the general population (72). However no correlation between size at birth and development of age-related cataract has been found (70). A recent Japanese study, in support of genetic causes, shows that galactokinase deficiency, which occurs at a rate of 4% in Japanese with normal vision, is associated with 8% of bilateral cataract cases in Japan and 3% in Korea. No correlation is seen among white or black Americans (73).

According to a recent study by Congdon et al. (74), an estimated 20.5 million (17.2%) Americans older than 40 years have cataract in either eye, and 6.1 million (5.1%) have pseudophakia/aphakia. The total number of persons who have cataract is estimated to rise to 30.1 million by 2020. Cataract prevalence is higher in women than men, with no racial differences in prevalence rates for women. Among men, whites have higher prevalence rates than blacks. Therefore the number of Americans affected by cataract and needing cataract surgery will dramatically increase over the next 20 years as the U.S. population ages.

Other Risk Factors for Cataract

Among the risk factors for senile cataract are the following:

- Advanced age
- Heredity
- Female gender
- Excessive exposure to UV-B radiation
- Diabetes
- Poor education
- Lower socioeconomic status
- High- or low-body-mass index
- Heavy alcohol consumption
- Smoking

Smoking appears to provide an oxidative challenge associated with depletion of antioxidants as well as with enhanced risk for cataract formation (75). According to Robman and Taylor (73), age and heredity are the most important risk factors for cataract. Few risk factors satisfy the criteria for causal effect: smoking, which results in the increased risk of nuclear cataract, and diabetes increases the risk of cortical cataract, while steroids, diabetes, and ionizing radiation lead to the formation of posterior subcapsular opacity (76). Incidence of cataract is high among Hispanics of Mexican descent in the United States, as evidenced by rates of cataract and cataract surgery. Language and financial barriers in this population impede access to surgery (77). According to another recent study by Congdon et al. (78), the probability of development of nuclear cataract was significantly increased among individuals with a sibling with nuclear cataract, a conclusion consistent with a genetic effect for age-related nuclear (ARN) cataract, a common and clinically significant form of lens opacity.

Recent Studies on the Role of Oxidative Stress, Antioxidants, Glycation, Multilamellar Bodies, Nuclear Compaction, and Light Scattering in Cataract

Decreased uptake of protective antioxidative fat-soluble nutrients (carotenoids, retinal, and tocopherol) in the aged lens and also differential localization of nutrients to different regions of the lens may account for differential risk of developing cataract in a different lens region (79). Similarly, increased intake of vitamin C, an antioxidant component, has a protective effect against age-related cataract, acting possibly to prevent the lens tissue oxidative damage seen in cataracts. Sweeney and Truscott report that decreased GSH levels correlate with cataract development (80). Glutathiene (GSH) is the principal antioxidant, and older lenses show reduced levels in the central nuclear region. It is proposed that with aging, a barrier develops in the lens, preventing diffusion of GSH to central lens regions, thus allowing oxidation of nuclear proteins and cataract (80).

According to Zarina et al. (81), increased glycation end products occur in human senile and diabetic cataractous lenses. More glycation occurs in the nucleus than in the cortex of the lens, and the diabetic group showed still higher amounts compared to the normal senile group (81). Dark iris color increases the risk of age-related cataract due to an increase in lens optical density. Gilliland et al. (82) show that multilamellar bodies seen in cataractous lenses may be the light-scattering objects responsible for forward light-scattering seen in nuclear cataracts. Another explanation for this abnormality is nuclear compaction in cataractous lenses (83). Cataractous lenses also show higher levels of sodium, magnesium, and potassium ions compared to normal age-matched lenses (84). Telomerase activity in lens epithelial cells is increased in cataractous lenses; but the decline in cell number in the lens epithelium is not related to cataract development.

According to a recent study by Truscott (85), oxidation is the hallmark of ARN cataract. With age, sulfhydryl groups are reduced and the oxidation of methionine and cysteine residues increases progressively, resulting in increased cataract formation. But protein oxidation in the lens center is not observed with aging, even past age 80. Concentration of GSH in the lens nucleus can help preventing oxidation, as is the coupling of the metabolically active cortex, the source of antioxidants, to the quiescent nucleus. It appears that the lens cortex remains viable in old lenses, and even possibly in ARN cataract lenses. The authors hypothesize that the "lens barrier," which becomes apparent in middle age, acts to impede the flow of small molecules between the cortex and the nucleus. The barrier, rather than nuclear compaction, may contribute to the lowered concentration of GSH in the lens nucleus after middle age. This hypothesis emphasizes a primary role for oxidants generated within the lens [see also above (80)].

In a related study, Rachdan et al. (86) show that human lens GSH reductase activity, which is known to decrease with aging and cataract, could be revived by different reducing agents as well as by a molecular chaperone (α-crystallin). In addition to α-, β-, and γ-crystallins, which are the major structural proteins of mammalian lenses, the human lens also contains tryptophan-derived UV filters, which are known to spontaneously deaminate at physiological pH and covalently attach to lens proteins. 3-hydroxykynurenine (3OHKyn) is the third most abundant of the kynurenine UV filters in the lens, and previous studies have shown this compound to be unstable and to be oxidized under physiological conditions.

Recently Korlimbinis et al. reported that while normal-aged human lens showed no evidence of oxidation of α-crystallin, oxidation was detected at methionine-1 in both α-A- and β-B-crystallin from human cataractous lenses (87). ARN cataract is associated with coloration and insolubilization of lens proteins and extensive oxidation of cysteine and methionine residues. 3OHKyn can readily catalyze the oxidation of methionine residues in both α-B- and α-A-crystallin, and it has been reported that α-crystallin modified in this way is a

poorer chaperone. Thus, 3OHKyn promotes the oxidation and modification of crystallins and may contribute to oxidative stress in the human lens.

Although the use of antioxidant vitamins was thought earlier to decrease cataract (see above), a study by McNeil et al. found that vitamin E given for four years at a dose of 500 IU daily did not reduce the incidence of, or progression of, nuclear, cortical, or posterior subcapsular cataracts (88). Similarly, Meyer and Sekundo note that any effect of antioxidant vitamins on cataract development is likely to be very small and probably is of no clinical or public health significance, thus removing a major rationale for "anticataract" vitamin supplementation among health-conscious individuals (89).

Surgical Removal of Cataractous Lens

Although certain drug treatments can alleviate some of the symptoms of cataract in some patients, surgical removal of the cataractous lens has been the option of choice in recent years. In most cases, after lens removal, a plastic lens implant is placed instead of the old lens. In the United States, nearly a million cataract operations are performed per year, resulting in improved vision in 97% of the cases. All cataract operations involve removal of opacified lens via a corneal incision followed by fine suturing. In most cases, a plastic lens, called a "lens implant," is inserted in place of the removed lens (see below). The operation, usually performed on an "outpatient" basis, is done under local anesthesia and sedation. The postoperative patient requires means for optical corrections due to loss of lens. Various options available are summarized in Box 1.

Glaucoma

Another treatable eye disease is *glaucoma*, which is characterized by the following triad: the IOP increases (>21 mmHg); this leads to progressive excavation of the optic disc, the site where the optic nerve leaves the eye; and cupping of the disc. This leads to ischemic damage to the optic nerve fibers, which may result in blindness. Two types of glaucoma are known, chronic open-angle glaucoma (COAG), which is the frequently diagnosed type, and the closed-angle type, which is rare (7–10). The incidence of COAG in the population increases rapidly with age, from a low of 0.2% in the fifth decade to about 1% in the seventh decade, 3% in the eighth decade, and 10% in the ninth decade. OAG is characterized by an insidious and slow onset, initially asymptomatic and then gradually leading to blindness if unchecked. Closed-angle glaucoma is rare and is characterized by an acute attack of severe eye pain and marked loss in vision due to a rapid increase in IOP compressing the entire retina.

Recent Research on Glaucoma

Systemic blood pressure and hypertension correlate with IOP and high-tension glaucoma, but no link appears to exist between blood pressure and normal-tension glaucoma (NTG) (84). Prospects of genetic intervention in primary open-angle glaucoma (POAG) have been investigated, and five primary open-angle genes have been mapped. Understanding these genes and their products may help in finding better treatments (92). Collagen degradation appears to play a role in glaucoma (93). Imbalance in rates of extracellular matrix production and turnover may be important in OAG, and matrix metalloproteinases are likely to be used in its treatment (93).

Recent Studies on Risk Factors for Glaucoma

According to Bergen et al. (94), POAG is a group of multifactorial diseases that affects 1.5% of the population. Important risk factors for POAG are older age, elevated IOP, the presence of POAG in relatives, and still largely unknown molecular genetic factors. The three genes known to be involved in POAG are *MYOC*, *CYP1B1*, and *OPTN*, and they account for up to 18% of the POAG cases.

In a report based on the data of the "Rotterdam study," de Voogd et al. (95) reveal that diabetes mellitus in people 55 years or older is not a risk factor of OAG. Also neither atherosclerosis nor serum C-reactive protein level, which is an index of heart disease, was an important risk factor for OAG (96). A recent study by Friedman et al. (97) emphasized the higher incidence of OAG in older people and also finds that the incidence of this eye disorder is higher among older blacks than age-matched whites. Varma et al. (98) found that the prevalence of OAG and ocular hypertension is high among Latinos of Mexican ancestry, with no gender-related differences.

In relation to genes as risk factors, Tang et al. (99) has reported that the optineurin (*OPTN*) gene has been identified as a causative factor for NTG. Alterations in this gene were found in Caucasian families with NTG. While no glaucoma-specific mutations were found in the *OPTN* gene in Japanese glaucoma patients, some novel single-nucleotide polymorphisms (SNPs) in the exons and introns were reported (99). Fan et al. (100) identified disease-causing mutations in *MYOC* and *OPTN* in 1.75% and 1% of POAG Chinese patients, respectively. Common polymorphisms in *MYOC*, *OPTN*, and *APOE* might interactively contribute to POAG, indicating a polygenic etiology. Similarly, Sripriya et al. (101) report that SNPs rather than mutations in *OPTN* may play a role in POAG pathology in the Indian population.

Glaucoma Treatment

Whereas cataracts are usually treated surgically, glaucoma is often treated medically with eye drops containing various drugs. However, for patients who do not respond to anti-glaucoma medications, surgery and, more commonly today, laser therapy are used to lower IOP (Box 2).

Age-Related (Senile) Macular Degeneration

An important cause of visual impairment in the elderly, often leading to legal blindness within five years after onset, is senile macular degeneration or age-related macular degeneration (ARMD or AMD) (7–10,107,108). The disease accounts for nearly half of the registered (legal) cases of blindness in the United States and England. The incidence of ARMD increases with increasing age, from about 4% in the 66 to 74 years age group, to 17% in the 75 to 84 years age group, and 22% in the >84 years age group.

Aging Changes in the Macula

The *macula lutea* is an area of retina 6 mm in diameter that is located at the posterior end of the eye's visual axis (Fig. 2B). Through its high density of cones and involvement in day and color vision, the macula, and in particular its central zone, the fovea, provides the structural basis for high visual acuity. Hence, macular degeneration, more than any other eye disease, affects visual acuity and central vision. This disease occurs generally in both eyes and more often (50%) in women. It is believed to be a hereditary disorder, not caused by simple aging of the retinal nerve cells, but largely related to manifestation of inherited pathologies in the nonneural retinal elements such as the pigment epithelium. The patients also show an increased incidence of hyperopia (farsightedness). The disease may result from disturbances in the walls of subretinal capillaries or in the thickness of subretinal membrane or the RPE (7–12). For a recent review of the pathogenesis of ARMD, see Ref. 109.

BOX 1 *Options Available for Visual Corrections After Removal of Cataractous Lens*

1. *Eyeglasses:* These are thick and heavy, and increase object size by 25%; they induce optical distortions and interfere with peripheral vision; although they provide good central vision, they cannot be used after surgery, if the other eye is normal.
2. *Contact lenses:* Hard or soft extended-wear contact lenses have been used; they are more difficult to use, and eyeglasses are required for reading; however, they correct central and peripheral vision, increase image size by only 6%, and can be used after surgery on one or both eyes.
3. *Intraocular implant lens:* This is surgically placed in front of or behind the iris at the time of cataract surgery; it requires the use of bifocal eyeglasses and has a higher incidence of surgical and postsurgical complications; however, it increases image size by only 1%, corrects central and peripheral vision, and can be used on one or both eyes; lens implants are made of silicone, acrylic, or hydrogel materials; the lens implants are placed either in front of the iris (intracapsular) or behind the iris (extracapsular).
4. *Refractive keratoplasty*: In this method, the cornea is cut and reshaped by making surgical or laser incisions; this method has become effective and popular in recent years in correcting for far- and near-sightedness and astigmatism, but its applications for correcting for presbyopia or cataract are still in the experimental stage. Recently, newer methods have been developed, namely penetrating keratoplasty (PK) and deep lamellar keratoplasty (DLK). DLK is a better surgical choice because it involves less endothelial cell rejection and offers better-corrected visual acuity (BCVA); it is also safer in the short term and long term (90,91).

Recent Biochemical and Pathological Findings in ARMD

Tissue inhibitors of metalloproteinases (TIMP-3) have been implicated in aging and in ARMD. TIMP-3 levels in Bruch's membrane in macula increase with age, and these levels are higher in ARMD subjects compared to normal age-matched individuals (110). Gelatinase-A (MMP-2) levels are highest in the interphotoreceptor matrix and vitreous gel and do not increase with age; however, levels are twice higher in the matrix of RPE-associated cases (111). A summary of the pathological hallmarks and possible sequence of events leading to ARMD is listed in Table 2.

A recent study by Bailey et al. (112) suggests that TIMP-3 expression does not alter significantly with age, so that TIMP-3 protein accumulation with age in the retina must occur by a mechanism other than increased expression. Bailey et al. (112) confirm that TIMP-3 protein levels may still prove to contribute significantly to events associated with macular aging, such as matrix remodeling in Bruch's membrane. Another study by An et al. (113) found that RPE cells secrete a variety of extracellular matrix proteins, complement factors, and protease inhibitors that have been reported to be major constituents of "drusen" (hallmark deposits in ARMD). Interestingly, RPE cells from ARMD donors secreted two- to threefold more galectin-3–binding protein, fibronectin, clusterin, matrix metalloproteinase-2, and pigment-epithelium-derived factor than RPE cells from age-matched healthy donors. Conversely, compared to healthy subjects, "secreted protein acidic and rich in cysteine" was reduced by twofold in RPE cells from ARMD donors. Overall, data strongly suggest that RPE cells are involved in the biogenesis of drusen and the pathology of ARMD.

Risk factors for and treatment of ARMD are less well known than those for cataract and glaucoma. They are summarized in Box 3. Information on visual dysfunctions in Alzheimer's disease (AD) is presented in Box 4.

■ HEARING

Changes in auditory functions with age provide some of the classic and important case studies in gerontology and the physiology of aging. Incidence of hearing disorders rapidly increases with aging, afflicting nearly a third of people over 65 and one-half of those over 85 years (Fig. 1). Hearing impairments are 20% more frequent in elderly men than women. Hearing disorders interfere with the perception of one's own speech and that of others, creating behavioral and social disabilities. These conditions may lead to social withdrawal and isolation, particularly under the extreme condition of deafness (3). As in the visual system, the age-related problems of the auditory system may stem from structural and functional disorders of the

TABLE 2 Summary of Pathology and Sequence of Events Leading to ARMD

Pathological hallmarks

Senile macular degeneration may be accompanied by any of the following changes depending on the stage and extent of the disease (107):

1. White excrescences in the subretinal membrane, called Drusen (nodules), hyaline deposits ranging in size from punctuate lesions to dome-shaped structures 0.5 mm in diameter, often observed during the early stages but also found dispersed throughout the fundus
2. Atrophy of RPE
3. Serous detachment of RPE
4. Subretinal neovascularization
5. Disciform scars, due to RPE detachment

Proposed sequence for pathogenesis of ARMD

1. ARMD involves aging changes plus additional pathological changes (i.e., ARMD is not just an aging change)
2. In aging and ARMD, oxidative stress causes RPE and, possibly, choriocapillaris injury
3. In ARMD (and perhaps in aging), RPE and, possibly, choriocapillaris injury results in a chronic inflammatory response within the Bruch membrane and choroids
4. In ARMD, RPE and, possibly, choriocapillaris injury and inflammation lead to the formation of an abnormal ECM, which causes altered diffusion of nutrients to the retina and RPE, possibly precipitating further RPE and retinal damage
5. The abnormal ECM results in altered RPE–choriocapillaris behavior, leading ultimately to atrophy of the retina, RPE, and choriocapillaris and/or choroidal new vessel growth

Note: In this sequence of events, both the environment and multiple genes can alter a patient's susceptibility to ARMD.
Abbreviations: ARMD, age-related macular degeneration; ECM, extracellular matrix; RPE, retinal pigment epithelium.

BOX 2 *Treatment of Glaucoma*

■ PHARMACOLOGICAL TREATMENT

Antiaglaucoma medications include the following:

- Miotics (substances that constrict the pupil, such as pilocarpine) are most used.
- β-Blockers (e.g., timolol blocks specific sympathetic innervation) are used with caution because of possible systemic side effects (e.g., heart failure).
- Sometimes systemic medications are used (e.g., carbonic anhydrase inhibitors).

Modern pharmacological therapy for glaucoma may be pursued in three stages (102).

First-line treatments are ocular hypotensive agents [e.g., β-blockers (timolol)]. If β-blockers are ineffective, prostaglandin and carbonic anhydrase inhibitors and α-2-adrenergic agonists (e.g., epinephrine) are used. Miotics (pilocarpine) represent second or third lines of treatment; along the third line of treatments are the cholinesterase inhibitors, with fewer side effects. Therapy is often enhanced when drugs are used in combination (102).

Dorzolamide (dorzolamide hydrochloride), a topical carbonic anhydrase inhibitor, is highly effective in the management of glaucoma and ocular hypertension. It reduces intraocular pressure by decreasing aqueous humor formation. Effects are additive when used with topical α-adrenergic antagonists. Side effects are bitter taste, transient local burning, and a stinging sensation. The prostaglandin analogs latanoprost and unoprostone, which act as hypotensive drugs, are new and commonly used therapeutic agents (103), often in conjunction with other glaucoma medications. Latanoprost is preferred for its efficient hypotensive action and minor side effects.

Conventional first-line treatment of glaucoma usually begins with a topical selective or nonselective β-blocker or a topical prostaglandin analog (104). Second-line drugs of choice include α-agonists and topical carbonic anhydrase inhibitors. Parasympathomimetic agents (pilocarpine) are considered third-line treatment options. For patients who do not respond to antiglaucoma medications or in case of an acute attack of closed-angle glaucoma, laser therapy and incisional surgery can be used to lower intraocular pressure. The latter techniques work by increasing outflow of aqueous humor through the trabecular meshwork. Other surgical options are glaucoma drainage tube implantation and ciliary body cyclodestruction (105).

■ SURGERY AND LASER THERAPY

Argon laser trabeculoplasty (ALT) has been increasingly used instead of surgery for treating open-angle glaucoma that is unresponsive to drugs. The treatment consists of tiny laser burns evenly spaced around the trabecular meshwork; it can also be used as a preventive measure, while pharmacological treatment continues.

ALT is being increasingly replaced by diode laser with continuous wave, and in recent years by "Q-Switched Nd:YAG laser," second harmonic 532 nm (106). This laser allows for selective photoablation of pigmented cells in trabeculum without a thermal effect. These new lasers enable safer, quicker, more effective antiglaucoma operations, increase efficacy of laser surgery, and allow for bypassing hospitalization, with less treatment cost.

peripheral auditory components, the central neural aspects, or both. Here, too, more is known about the aging of the ear than the central auditory system (Table 3).

■ Age-Related Hearing Loss (Presbycusis)

Age-related hearing loss (ARHL) is called presbycusis. Numerous studies have reported loss of hearing with age, particularly for sounds in the high-frequency range (28,122,123). Presbycusis occurs in both ears but not necessarily at the same time. To assess auditory loss with age, pure-tone audiograms of subjects in different age groups are determined. That is, the hearing threshold in decibels (dB) (unit of sound intensity) for sounds of increasing frequency is determined, and the results are presented as relative loss of decibels at different frequencies.

Progressive Hearing Loss with Aging in the High-Frequency Range

In the low-frequency range (0.125–1 kHz), young subjects have essentially no hearing deficits, while old subjects show deficits of about 10 to 15 dB. In the high-frequency range, hearing loss for the young group is mild, while in the old group, it becomes progressively worse with increasing sound frequency (Fig. 7) (28,123). Typical magnitudes of hearing loss are 30 dB at 2 kHz, increasing by about 10 dB for each additional kHz. In octogenarian men living in urban areas, the hearing loss may be as much as 80 dB. Another way of determining ARHL is by measuring the maximum frequency of sound capable of being heard. This frequency is 20 kHz for children (10 years), which decreases to only 4 kHz for the elderly (80 years); the decline is steady and linear, occurring at the rate of about 2.3 kHz per decade (Fig. 7).

Pure-Tone Audiograms and Effects of Age, Sex, and Environment

Typical pure-tone audiograms for normal men and women at different age groups are shown in Figure 7. Hearing loss is higher in men than women; this sex difference, which begins after the age of 35 years, possibly reflects the effects of higher exposure of men to work-related and environmental noise.

BOX 3 *Risk Factors for and Treatment of Aging-Related Macular Degeneration*

The higher incidence of age-related macular degeneration (ARMD) in monozygotic, compared to dizygotic, twins suggests the importance of genetic factors (114). Alcohol consumption does not increase the risk of ARMD (115), but cigarette smoking poses a significant risk (116). Treatment of macular degeneration is not nearly as successful as that of cataract and glaucoma (108). Although a protective role for antioxidants and trace minerals against ARMD development is widely believed, the use of antioxidants for ARMD treatment remains controversial (117).

Laser therapy in ARMD. The only realistic goal of treatment for ARMD is to prevent subretinal detachment and hemorrhage, disorders that lead to an acute loss of vision. To accomplish this, the sites of subretinal neovascular formation are located using fluorescin angiography followed by laser photocoagulation. According to Ciulla et al. (118), laser photocoagulation of choroidal neovascular membranes (CNVMs) is currently the only well-studied and widely accepted treatment. New treatments may be divided into four major categories: (*i*) photodynamic therapy, (*ii*) pharmacologic inhibition of CNVM formation with antiangiogenic agents, (*iii*) surgical intervention, and (*iv*) radiation therapy.

Rosen et al. (125) have also shown that the elderly people from noise-free rural areas of Sudan show lesser hearing loss compared to those in the urban environment, suggesting that urban environmental noise is one of the determinants of presbycusis [see, however, an Italian population study by Megighian et al. (126) discussed below]. In a recent study, Lee et al. (127) found an average increase of 1 dB per year for subjects 60 years and over. Male and female subjects showed faster rates of change at different specific frequencies than younger, gender-matched controls. Also, older males and females showed increased rates of change in pure-tone threshold at different frequencies (males had a faster rate of change at 1 kHz, and a slower rate of change at 6 and 12 kHz, compared to age-matched females). Noise history did not play a role in these parameters.

Atherosclerosis and Role of Blood Lipids in Presbycusis

Another cause of presbycusis may be vascular, such as atherosclerosis and similar disorders related to elevated blood lipids (hyperlipoproteinemia) (Chapter 16). Incidence of hearing loss and inner-ear diseases may be high in patients with elevated cholesterol levels (128). With regard to the role of lipids in presbycusis, recent animal studies suggest that *statin* drugs that are commonly used to treat coronary artery disease and hypercholesterolemia may slow down presbycusis in humans, possibly by reducing vascular endothelial inflammation. In a mouse strain with accelerated aging (C57BL/6J mice), which is a model for presbycusis in humans, Syka et al. (129) found that atorvastatin (a statin drug) slows down the deterioration of inner-ear function with age. It was found that more outer hair cells survived in mice treated over a two-month period with atorvastatin versus control mice of the same strain, as measured by distortion-product otoacoustic emissions (DPOAE) amplitude (greater in treated mice).

Presbycusis Related to Ear Structure

The major structures of the ear include the following: (*i*) The external ear (pinna, external auditory canal, and tympanic membrane); (*ii*) the middle ear (the ossicular chain); and (*iii*) the

BOX 4 *Visual Dysfunctions in Alzheimer's Disease*

The Alzheimer's disease (AD) patients perform significantly worse than subjects without AD on tests measuring static spatial contrast sensitivity, visual attention, shape-form-motion, color, visuospatial construction, and visual memory (119–122). Correlation analyses showed strong relationships between visual and cognitive scores. Thus, effects of AD on multiple visual neural pathways and regions are compatible with the hypothesis that visual dysfunction in AD may contribute to performance decrements in other cognitive domains.

A better understanding of vision-related deficits can lead to better diagnosis and interventions that may, in turn, help improve functional capacity in patients with dementia and AD. Decreased attention may severely limit cognitive performance in the elderly. Deficits in sustained, divided, and selective attention, and in visual processing speed, occur early in AD, and can be significantly correlated with diminished overall cognitive function (120). These findings indicate that assessment of visual attentional ability may prove useful for AD diagnosis and lead to more precise measures of useful perception in AD patients with normal visual fields and acuity.

In mild to moderate cases of AD, a significant effect on perception of structure from motion occurs with relative sparing of motion direction discrimination. This problem is likely to have a cerebral basis and can potentially affect navigation and the recognition of objects in relative motion, as encountered during walking or automobile driving (119,120).

Certain atypical AD patients show characteristic visual abnormalities. Visual association pathways are usually not as disrupted in the more common form of AD (121). These visual symptoms were the most identifiable signs of this particular form of AD. In these patients, visual association areas in the occipito-temporo-parietal junction and posterior cingulate cortex as well as primary visual cortical areas demonstrated high concentrations of lesions, while the prefrontal cortical regions had fewer lesions than found typically in AD patients (121).

TABLE 3 Summary of the Normal Aging Changes in the Human Ear

Structural changes	
Hair cell degeneration	
Basal cochlea	Frequent, especially in the first quadrant; diffuse and patchy; main cause of sensory presbycusis
Apical cochlea	Infrequent
Nerve cell degeneration	
Observed in spiral ganglia often with basal cochlear hair cell loss but not with apical cases (involved in neural presbycusis); is accompanied by loss of myelinated auditory nerve fibers	
Atrophic changes	
Generally occur in nonneural components (vascular and connective tissue) of cochlea and lead to strial or conductive types of presbycusis	
In stria vascularis	Frequent in the middle and apical turns of cochlea
In spiral ligaments	Accompanied with devascularization in inner and outer spiral vessels
In Reissner's membrane	Due to vacuolization in basilar membrane, leading to mechanical damage
Central neural changes	
Little neuronal loss in lower auditory centers; heavy loss in conical auditory centers; dendritic degeneration of cortical pyramidal neurons	
Increased latency and decreased amplitude of auditory-evoked potentials; effects more marked in elderly males than in females	
Functional changes	
Pure-tone hearing	Loss of hearing in the high-frequency range (presbycusis): loss progressively worsens with age; effects more pronounced in males; noise exposure enhances loss
	Decline in maximum sound frequency capable of being heard, from 20 kHz at 10 yrs to 4 kHz at 80 yrs
Speech perception	Diminished ability to hear consonants; speech is heard but unintelligible
Sound localization	Diminished ability to localize sound source, particularly at high frequencies

inner ear (cochlea and the organ of Corti). The organ of Corti contains the hair cells that are the auditory receptors and the mechanoelectrical transducing organs (Fig. 8A). The cell bodies of the primary auditory neurons are in the cochlea's spiral ganglia, and the axons comprising the auditory nerve enter the medulla to synapse with central auditory neurons.

Auditory pathways in the brain include the medullary and midbrain centers for signal transmission and auditory reflexes, the inferior colliculi, and the auditory cortex in the temporal lobe of the cerebral cortex. The selective nature of presbycusis indicates that it is probably not associated with aging changes in the outer or middle ear (tympanic membrane and ossicles) but is more likely due to changes in the inner ear (cochlea) or the central auditory system (122,123,130).

Types of Presbycusis

Presbycusis may occur due to damage to different parts of the auditory systems. Based on the source of damage, four types of presbycusis are recognized: sensory, neural, metabolic (or strial), and cochlear conductive (123,130). The onset of presbycusis may be any time from the third to sixth decade of life, depending on type. Individuals suffering from these disturbances show distinct and differing audiograms (Fig. 8), which are clinically used to diagnose types of impairment. More complicated audiograms are produced when the pathology involves a combination of these disorders (Fig. 8). The standard type of presbycusis with hearing loss at high frequency is often associated with neural or sensory presbycusis (Fig. 8B,C).

Sensory Presbycusis

Individuals with sensory presbycusis show a major and sudden loss of hearing in the high-frequency range (4 kHz), indicating a selective deficit in transduction mechanisms of high-frequency sounds (Fig. 8B). Speech discrimination is normal. Although the hearing deficit is observed from middle age, the histopathological problems believed to be mainly associated with the cochlear hair cells may start much earlier. Cochleas of humans with sensory presbycusis typically show loss of outer hair cells and less often of the inner hair cells of the organ of Corti (Fig. 8A) (123,130). The loss is diffuse or patchy and is mainly limited to the first quadrant of the cochlea's lower basal turn. This part of the cochlea is specialized for detection of high-frequency sounds. The affected sensory hair cells and other supporting cells (Hensen's and Claudius' cells) show accumulation of the aging pigment lipofuscin, the amount of which corresponds with the degree of sensory deficits.

In a recent animal study in mice, Someya et al. (131) have shown that caloric restriction suppresses apoptotic cell death in the spiral ganglia of the mammalian cochlea and leads to prevention late-onset presbycusis. Calorie-restricted mice, maintained at the same weight as young controls, retained normal hearing and showed no cochlear degeneration. This was found to be the result of downregulation of genes responsible for apoptosis. (See also Box 8 for other animal studies on causes and models of presbycusis.)

Neural Presbycusis

In this disorder, hearing of pure tones for all frequencies are affected, but the extent of hearing loss increases with increasing frequency of sound, the deficit being about 40 dB at 1 kHz and nearly 100 dB for high frequencies (>8 kHz) (Fig. 8C). As a result, speech discrimination is reduced to 60% of the normal level. In the aging auditory system, the first-order sensory neurons are adversely affected. This damage ranges from synaptic structures between the hair cells and the dendrites of the auditory nerve fibers, accumulation of lipofuscin, to signs of degeneration in the cell bodies of the spiral ganglion neurons. Disruption of the myelin sheath of the auditory nerve fibers can cause disordered transmission, even if the nerve cells were present (123,132).

Metabolic Presbycusis

In this case, also called the *strial* type, the audiogram is flat (Fig. 8D), indicating a loss of about 30 to 40 dB at all frequencies. This type is believed to be associated with atrophic changes in

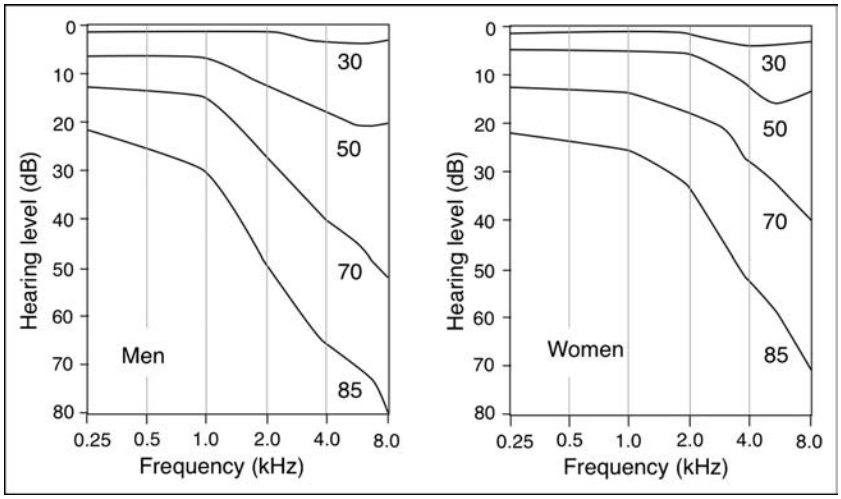

FIGURE 7 Amount of hearing loss in decibels for different pure tones in various age groups of men and women. Note a greater loss in higher frequencies and older ages and a more pronounced hearing loss in men. *Source*: From Ref. 28, based on the original data of Ref. 124.

the vascular supply to the cochlea (stria vascularis) (122,123, 130). The extent of hearing loss is correlated to the degree of degeneration in stria vascularis (123).

Cochlear Conductive Presbycusis

In this type (also called mechanical presbycusis), the audiogram indicates some hearing loss at all frequencies, with the loss magnitude increasing linearly with increasing tone frequency (see curve #2 in Fig. 8E). The magnitude of hearing loss is about half of that seen in the neural type, and speech discrimination is only slightly affected in most cases (96% of normal). This type of presbycusis is believed to be due to changes in the mechanical properties of cochlea's basilar membrane, hence its other name, "mechanical presbycusis." The basilar membrane is markedly thickened, especially at the basal cochlea, and shows calcified, hyaline, or fatty deposits. There is usually no change in the hair cells and sensory neurons (130).

Indeterminate Presbycusis

In a recent study, Chen et al. (133) reassessed the cases of "indeterminate presbycusis" as classified by Schuknecht (122,132) originally, i.e., those not due to changes in basement membrane thickness. In their retrospective quantitative analysis of neuritis, neurons, peripheral hair cells, and the stria vascularis in people who had presbycusis, they found that loss of peripheral dendrites (neurites) of the spiral ganglion cells corresponded better to a more gradual downward-sloping audiogram than presbycusis with hair cell loss (sensory presbycusis). This phenomenon was termed "neuritic presbycusis" (133).

Presbycusis Studies in Korean, Italian, and African-American Populations

A 20-year-long study of pure-tone audiometry by Kim et al. (134) on 6000 Koreans, aged 65 years and older, found a high incidence (~40%) of presbycusis as well as a significant difference in hearing threshold between men and women (males > females). Megighian et al. (126) analyzed the presbycusis data of over 13,000 Italian males and females, aged 60 and over, living in either city or rural environments in the Veneto region, and found typical trends of the audiometric curve in presbycusis. Hearing loss was less severe in females than males, especially at higher auditory frequencies. No significant differences emerged between subjects from the urban or the rural environments.

According to Bazargan et al. (135), poor hearing among aged African Americans was associated with a decreased level of psychological well-being, but this relationship was mediated by the effects of hearing loss on ability to function. Poor vision, on the other hand, is independently associated with a decreased evaluative level of well-being.

■ Sound Localization

Tests of sound localization indicate a decline in this ability with aging, beginning in the fourth decade (28). It is known that localization of low-frequency sounds depends on *temporal* discrimination (i.e., time of sound arrival) between the two ears, while in the high-frequency range, localization depends on discrimination of sound *intensity* between the two ears. Aging changes occur in both ears, but the rate of aging may be different in the two ears (122,130). Thus, deficits in localization of sound will be apparent for all frequencies but may be more marked for sounds of higher frequency, as the perception of these are particularly impaired in the old age (see above).

■ Other Aging-Associated Auditory Functional Changes

Middle-ear resonant frequencies were measured in ~500 adults 48 to 90 years of age, and no significant age-group trends were found; older women, however, had a slight but significantly greater middle-ear resonant frequency than older men (136). According to Oeken et al. (137), a major and significant decrease in DPOAE amplitude occurs with aging, caused by a deterioration in pure-tone threshold. It is suggested that delineation of the effects of presbycusis on DPOAE is important if they are to be used in the diagnosis of inner-ear disorders.

■ Peripheral-Aging Changes in Auditory Structures

Cochlear Ultrastructural Changes

Electron microscopic studies on extracted human temporal bones from individuals 53- to 67-years old, with high-tone

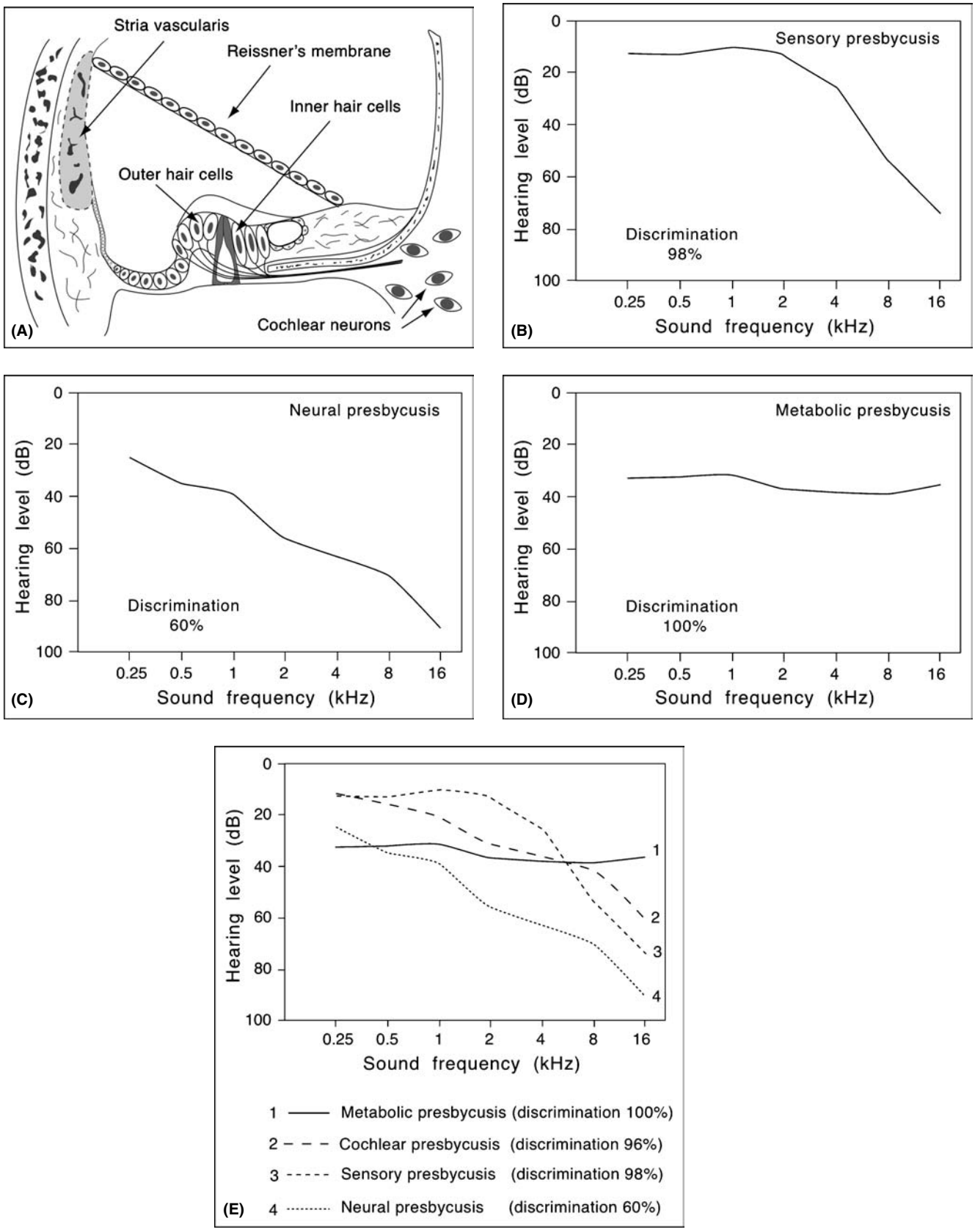

FIGURE 8 (**A**) Schematic cross section of human cochlea; (**B**–**E**) typical pure-tone audiograms of aged individuals suffering from different types of presbycusis (sensory, neural, cochlear, conductive, and strial). Each type of presbycusis reflects damage to a different component of the peripheral auditory system: sensory, to hair cells; neural, to primary auditory neurons; cochlear, to basilar membrane; and strial, to cells of the stria vascularis. *Source*: From Ref. 123.

hearing loss of unknown cause, revealed changes to the ultrastructure of the stereocilia, pillar cells, stria vascularis, spiral ligament, and the cuticular plate, the latter showing the greatest structural degradation (138). Thus, cuticular plate deformation may play a role in hearing loss, although so far, this has only been proven in guinea pigs (139). How age relates to otopathic function of the inner ear is still undetermined.

Spiral Ganglion and Other Neural Pathways

A quantitative study by Felder et al. (140) of neuron and fiber number in the auditory pathways of aged individuals with presbycusis showed clear evidence of cell and fiber loss, with more loss in peripheral structures than in central ones; normal controls showed no significant loss. Retrograde degeneration from the periphery to the spiral ganglion may be happening in presbycusis. Because interneuronal connections occur in human spiral ganglions, a trophic supply from other neurons at the spiral ganglion level may slow central axon degeneration in these cases. (See Box 8 for effects of neurotrophins.)

Aging Changes in the Cochlear Nucleus

In a recent review of experimental animal studies on age-related structural and functional changes in the cochlear nucleus, Frisina and Walton (141) concluded that cochlear (i.e., peripheral) age-related hearing loss (ARHL) changes are the primary inducing force of age-related alterations in cochlear nucleus. Also age-related auditory perceptual deficiencies such as a decline in complex auditory processing (isolating individual voice in room of people) may be related to the following physiological changes in the cochlear nucleus: (*i*) alterations in synaptic processing; (*ii*) a decline in the inhibitory effects of the neurotransmitter glycine with age; (*iii*) disruptions of temporal processing of information (141).

Possible Hormonal Effects on Presbycusis

Other physiological changes affecting presbycusis may include hormones such as aldosterone, which is known to influence salt transport in the kidney. In a recent study, Tadros et al. (142) found that elderly humans with presbycusis also had significantly lower aldosterone levels, suggesting that normal or high aldosterone level may be protective against presbycusis. Aldosterone may exert this effect by controlling K^+ and Cl^- transport in the cochlea by regulating the expression of their transporters as well as that of Na^+-K^+-ATPase. The authors note that the intracellular pathway of K^+ recycling in the human cochlea from the organ of Corti back to the stria vascularis is essential for maintaining viable hearing. Also type II, type IV, and type V fibroblasts are central to this recycling and are located in the stria vascularis, among other cochlear locations such as the nerve endings of the inner and outer hair cells.

■ Treatment of ARHL

Conventional Hearing Aids vs. Cochlear Implants

Currently, a satisfactory plan for rehabilitation and fitting of hearing aids does not exist. Further research and efforts are needed to develop better hearing aids that satisfy more precisely the needs of the elderly (143). Older adults and their immediate family can benefit from information and advice about the serious consequences of presbycusis.

For older patients, hearing aids remove many of the sensory, speech, perceptual, and psychological handicaps of hearing impairment (Boxes 5 and 6). Few older adults with hearing loss actually use hearing aids. Intervention and screening programs need to be improved to better identify older adults in need of amplification, whose quality of life would be improved with such treatment (144). Nursing-home patients have been found to consistently use their hearing aids. Amplification alone, however, does not serve all of the communication needs of the nursing-home residents. Environmental modification as well as improved assistive listening devices (Boxes 5 and 6 as well as Chapter 25) would increase quality of life and the ability to communicate for these patients (145).

BOX 5 *Cochlear Implants*

These novel neural prosthetic devices involve direct electrical stimulation of the auditory nerve, bypassing the sensory hair cells altogether. Through cochlear implants, elderly patients who are deaf can hear sound and understand speech, thereby improving their quality-of-life significantly (146,147). A holistic method has been developed to determine the appropriate treatment to improve hearing in people with varying degrees of audiological deficits, based on their communicative, physical, social and psychological profiles; a diagnostic flowchart is used to best match high-tech devices with appropriate candidates (148).

Cochlear implants are used to restore hearing in people with profound deafness; these neural prosthetic devices were designed initially for aging people but are increasingly used in all age groups. Bypassing the inner ear, cochlear implants directly stimulate the auditory nerve, through tiny wires inserted into the cochlea. Sounds are received by an external microphone placed behind the ear and are processed by a pocket speech processor, which converts sounds to electrical impulses, which are then transmitted (via FM signals) by a coil placed behind the ear, to an implanted receiver/stimulator. This receiver stimulates the appropriate electrodes, which in turn stimulate multiple populations of auditory nerve fibers, as in the case of multichannel cochlear implants. Multichannel stimulation allows for pitch perception, important for speech recognition. A typical 22-channel cochlear implant transmits in theory up to 22 unique pitch signals to the inner ear, but in practice only six to eight channels are usable concurrently. To improve the performances of the cochlear implants further, it is essential to increase the number of usable channels. Various approaches are being tried, including novel engineering designs, as well as designing "biomolecular cochlear implants," toward achieving this goal (145,146,148–149).

BOX 6 *Next Generation of Hearing Aids for Improving Speech Recognition*

Speech recognition is difficult for the elderly in the presence of background sounds. As described previously, conventional hearing aids offer little help in this regard. A new system being developed by Professor Albert Feng and collaborators at the Beckman Institute of the University of Illinois, Urbana-Champaign, using biaural microphones and sophisticated digital devices, offers to enhance speech recognition by removing multiple competing noise in the background (150).

Age-related defects in the auditory system such as hair cell degeneration (leading to high-frequency hearing loss), problems with sound localization, and temporal gap detection in speech contribute to the compromise in speech recognition. Conventional hearing aids offer little help in this regard and cannot group all the sounds from the source of interest into one coherent auditory perception. Audiological tests with a real-time system show marked improvement in speech recognition in many difficult listening conditions (150,151).

A variant of this system utilizes two microphones to process a sound signal biaurally, as occurs in biological systems, which use interaural time differences to localize sound sources (151). Localization of the source azimuths is accomplished by "integration of the locations of temporal coincidence across the broadband of frequencies in speech signals." Once the sound sources are localized, a noise, cancellation scheme is used to suppress all unwanted noise, keeping the gain of the desired signal unchanged. As a result, the device can effectively extract the signal from one particular talker in the presence of four to six competing talkers (150,151).

Increased Use of Assistive Hearing and Amplification Devices, Cochlear Implants, and Other Digital Aids

An important development in the future would be support for the open use of amplification devices, which comes with greater acceptance of the realities of hearing loss (Boxes 5 and 6 and also Chapter 25) (145). A strong preference for conventional hearing-aid use among the elderly shows that even though the sound quality of modern assistive listening devices is preferred over conventional aids, subjects usually are unwilling to endure all of the difficulties associated with the use of remote-microphone devices (145,148).

In a recent report based on interviews with people with presbycusis to analyze individual experiences with usage of hearing-aid technologies, Southall et al. (149) established four "landmarks" for successful use of technologies to treat hearing impairments:

- Recognizing problems with hearing
- Knowledge that the beneficial technology exists
- Assistance in determining which device to use and acquiring the devices
- Altering behavior to include usage of the device

The authors recommend that people with hearing problems need to move through these steps; achievement or failure at each step is likely to lead to either successful use of devices or failure to use devices effectively. Hearing therapists should use these guidelines as a framework to obtain a proper device for the patients, and encourage them to use it (149).

BOX 7 *Genes, Chromosomes, and Presbycusis*

MHC genes and presbycusis. Gene haplotypes in the MHC domain may be associated with the pathogenesis of certain types of hearing loss, including presbycusis. An extended MHC haplotype was identified for unrelated people in a cohort study with strial presbycusis and other types of hearing disorders. In this investigation, 44% of subjects expressed this MHC haplotype, as opposed to 7% of the general population (152).

DFNA25. Linkage analysis by Greene et al. (153) has found a novel, dominant locus, DFNA25, for delayed-onset, progressive, high-frequency, nonsyndromic sensorineural hearing loss of many generations of a U.S. family of Swiss descent. The 20 cM region of chromosome 12q21-24 with possible candidate genes [*ATP2A* (yeast-like FOFI-ATPase α subunit), *ATP2BI* (ATPase, Ca^{2+} transporting, plasma membrane 1 gene), *UBE3B* (a member of the E3 ubiquitin ligase family), or *VR-OAC* (vanilloid receptor–related osmotically activated channel, a candidate vertebrate osmoreceptor)] are implicated.

DFNA5. According to Van Laer et al. (154), over 40 loci for nonsyndromic hearing loss have been mapped to the human genome, but only a small number of these genes have been identified. One mutation found in an extended Dutch family is linked to chromosome 7p15 (DFNA5). This mutation has been defined as an insertion or deletion at intron 7 that removes five G triplets from the 3-prime end of the intron while not affecting intron–exon boundaries. This mutation, which is associated with deafness in this family, causes a skip of intron 8, with consequent termination of the open reading frame, and is expressed in the cochlea, but the physiological function is still unknown.

mtDNA4977 deletion. Studies of mitochondrial DNA (mtDNA) by Ueda et al. from cochlear sections of temporal bones of control subjects and those with presbycusis found a specific mtDNA4977 deletion in ~80% of those with presbycusis, compared to half as much in the cochlea of controls (155). Therefore, some of the advanced sensorineural hearing loss cases of presbycusis should be categorized as mitochondrial oxidative phosphorylation diseases, thus offering novel possibilities for treatment and prevention of sensorineural hearing loss, including presbycusis (155).

BOX 8 *Recent Models and Animal Studies on Presbycusis*

■ NEUROTROPHIN EXPRESSION AND PRESBYCUSIS (175)

1. Two known presbycusis animal models, Fisher 344 rats and Mongolian gerbils, show decreased amounts of brain derived neurotrophic factor (BDNF) mRNA in aging high-frequency cochlear neurons.
2. A reduction in BDNF signaling leads to a decrease in innervation of the outer hair cells of the basal region of cochlea, accompanied with significant hearing loss.
3. Loss of BDNF transcript occurs along a gradient of the cochlea's tonotopic axis, with the highest concentration at the basal, high-frequency end. Loss also occurs in dendrites going toward the brainstem, as well as central cochlear dendrites.

Conclusion: Neurotrophin (e.g., BDNF) are implicated in maintaining the integrity of normally functioning outer hair cells and/or brainstem synapses, and their loss during aging may be related to the presbycusis.

■ DOWNREGULATION OF β-SUBUNIT OF ACETYLCHOLINE RECEPTOR (ACHR) AND NEURONAL LOSS IN SPIRAL GANGLION IS IMPLICATED IN PRESBYCUSIS (176)

1. Nicotinic acetylcholine receptor (nAChR) subunit β2 is required for maintenance of spiral ganglion neurons (primary sensory relays in auditory system) during aging.
2. Hearing loss in β6 β2-/- mice is dramatic, with elevated hearing thresholds across the full range of frequencies.
3. In the spiral ganglion neurons of the auditory system of β2-/- mice, expression of the β2 subunit of AChR is reduced significantly; also, the number of neurons in this ganglion is reduced by 75% in aging, compared to controls.

Conclusion: β2 subunit expression is required for maintenance of spiral ganglion neurons during aging, and its loss may contribute to known presbycusis in this animal model.

■ DELETION OF ANTIOXIDANT ENZYME CU/ZN SUPER OXIDE DISMUTASE CONTRIBUTES TO AUDITORY AGING AND PRESBYCUSIS IN MICE (177,178)

1. At 12 months of age, Sod1 -/- mice have significantly increased thresholds at all frequencies investigated; thresholds at high frequency were 20 dB greater than even those observed in the B6 strain mice, known for their presbycusis.
2. Sod1 mice show fewer ganglion cells at all ages investigated; loss occurs more rapidly than age-matched control animals.
3. Hair cells and stria vascularis also are diminished relative to controls.
4. Overexpression of Sod1 did not protect against the aging effects.

Conclusion: Expression of Sod1 enzyme in at least 50% of optimal level is necessary for survival of cochlear neurons and the stria vascularis and to prevent age-related hearing loss.

■ CALORIC RESTRICTION RETARDS NEURONAL CELL DEATH IN SPIRAL GANGLION PRESBYCUSIS (131)

Caloric restriction (CR) suppresses apoptotic cell death in the spiral ganglia of the mammalian cochlea and prevents late-onset presbycusis. CR mice, maintained at the same weight as young controls, retained normal hearing and showed no cochlear degeneration. Results are thought to be due to downregulation of genes responsible for apoptosis.

■ RECENT REVIEW OF ANIMAL STUDIES (179)

Animal models have proven invaluable in studying genetic as well as environmental causes of aging of the auditory system and presbycusis. Recent animal models of age-related and noise-induced hearing loss have been amply reviewed by Ohlemiller (179).

Assistive Hearing and Amplification Devices

For aging people with mild-to-moderate hearing sensorineural hearing loss (SNHL), conventional hearing aids that basically are amplification devices designed to overcome the high-frequency loss are effective in assisting them to regain hearing ability in a quiet ambience. The efficacy of these aids in many everyday environments, however, is more limited, because the devices typically amplify all sounds in the room, the desired as well as the unwanted sounds. Additionally, these devices cannot restore the hearing ability in people with profound deafness. Modern devices have been developed to specifically address these needs. Among the latest devices, cochlear implants (146) and a new generation of "intelligent hearing aids" can be named (Boxes 5 and 6) (150,151).

■ Genetic Aspects of Presbycusis

Since the 1990s, many studies have focused on genetic aspects of presbycusis. Specifically, several DFNA loci have been determined to exist in relation to inherited deafness and presbycusis, as well as several mitochondrial associated mutations such as mtDNA4977 deletion (Box 7).

General Inheritance of Presbycusis

In a cohort study by Gates et al. (156) to find the prevalence of ARHL inheritance, genetically unrelated (spouses) and genetically related (siblings/parent–child) pairs were compared in regard to patterns of hearing-level groupings. A familial aggregation was definitely found to exist for sensory and strial presbycusis, as well as for those with normal hearing ability. Women showed a stronger aggregation than men, and the strial heritability estimate was greater than the sensory phenotype (156).

Mitochondrial Genetics and SNHL Presbycusis

Acquired mitochondrial defects have been postulated as being key to aging, especially aging of neuromuscular tissues. Mutations in the mitochondrial cytochrome oxidase II gene were found to be common in the spiral ganglion and membranous labyrinth of archival temporal bones of five patients with presbycusis, implicating mitochondrial mutations in at least a subgroup of presbycusis (157). Keithley et al. (158) point out, however, that the cytochrome oxidase defect cannot entirely account for presbycusis. In addition, an association between mitochondrial DNA and presbycusis has been found (155). Patients with SNHL had an increased prevalence for mitochondrial DNA deletion mtDNA4977 (75%) versus 30% in controls. It is proposed that certain SNHL subtypes should be categorized as diseases of mitochondrial oxidative phosphorylation (Box 7) (155).

Recent Genetic Studies on Hearing Disorders

In a recent review, McHugh and Friedman (159) presented a novel concept that many hearing loss–related genes (including those implicated in presbycusis) are not expressed in a Mendelian pattern but instead are influenced by allelism and modifier genes. Analyses of genes and the vast phenotypic spectrum involved in Usher syndrome as well as Wolfram syndrome indicate that modifier genes are likely playing key roles in the heterogeneity of syndromes involving hearing impairments. Modifier genes, which were previously ignored, appear to be keys to understanding many types of hearing loss including presbycusis.

Garringer et al. (160) recently carried out a linkage analysis of 400 genomic markers of a cohort of 50 pairs of aging fraternal twins (mean age, 73 years) with hearing loss in one or both ears. They found strong evidence for a hearing impairment locus near chromosomal location 3q22, near the recently discovered DFNA18 locus. The latter locus has been implicated in a type of hereditary deafness with progressive ARHL. Results suggest a genetic ink to ARHL in the population.

In a recent case–control study of elderly patients with and without presbycusis in Turkey, Unal et al. (161) noted that the different genotypes of the enzyme *N*-acetyltransferase 2 (NAT2), which plays key roles in detoxification of cytotoxic, carcinogenic compounds and reactive oxygen species, may be associated with presbycusis; specifically, they found a 15-fold greater risk of presbycusis in patients with NAT2*6A polymorphism.

■ Sound Localization and Speech Perception

Sound Localization

Tests of sound localization indicate a decline in this ability with aging, beginning in the fourth decade (28). It is known that localization of low-frequency sounds depends on temporal discrimination (i.e., time of sound arrival) between the two ears, while in the high-frequency range, localization depends on discrimination of sound intensity between the two ears. Aging changes occur in both ears, though the rate of aging may be different in the two ears (122,130). Thus, deficits in localization of sound will be apparent for all frequencies, but may be more marked for sounds of higher frequency, as the perception of these are particularly impaired in old age (see above).

Early Studies on Hearing Deficits and Speech Perception

Hearing of consonants vs. vowels

The marked hearing deficits in the high-frequency range have an important bearing on impaired speech perception in the elderly (123,162). Thus, *vowels,* generated by low-frequency sounds, are heard better than the *consonants* produced by high-frequency sounds. Similarly, voices of men are heard better than those of women and children, which have a characteristic high pitch. Because consonants make speech intelligible, while vowels make it more audible, a common complaint of the elderly is that they cannot understand spoken words, although they can hear them (28). Fortunately, lip reading, which is associated more with the expression of consonants, can greatly help with this deficiency.

Speech Speed and Comprehension

As indicated in the data of Figure 9, if speech is presented to the aged subjects too fast or with reverberations (i.e., echoing or booming), its comprehension declines markedly. The greatest decline in comprehension is observed if speech is presented with repeated interruptions, such as eight times per second, as is the case with many modem telephone systems (28). This presents an unfortunate situation for the elderly, who rely so much on the telephone for their communication with the outside world.

Sound Masking and Speech Comprehension

The ability to mask sounds, important for speech comprehension in a crowd of talking people, is considerably diminished in

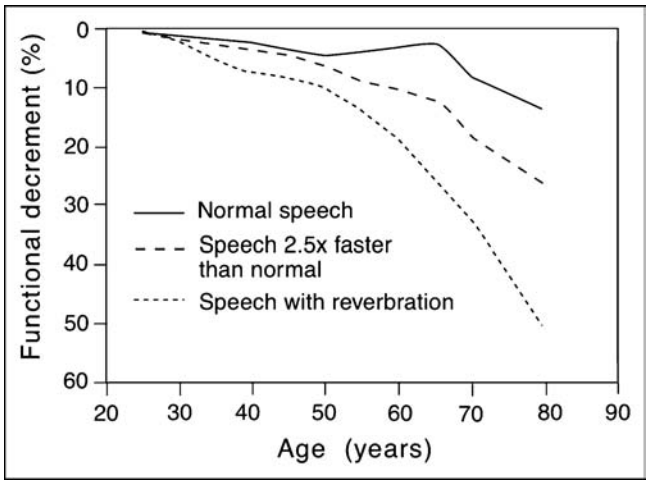

FIGURE 9 Deficits in speech comprehension at different ages. Note that normal aging deficits are exaggerated when speech is presented faster or with reverberations. *Source*: From Ref. 28, based on the original data of Ref. 163.

the elderly. Indeed, tests of hearing loss for pure tones provide usually conservative estimates of hearing deficits, because the tests are usually performed under quiet laboratory conditions, eliminating the need for masking. One example of loss of masking ability with age is the increase, with advancing age, in reporting the incidence of ringing in the ear *(tinnitus),* even though this problem is manifested at all ages. Perhaps the elderly cannot mask these unusual sounds, while the young can (28).

Recent Studies on Hearing Loss and Speech Recognition

According to Humes et al. (164), hearing loss is the most significant factor related to individual differences in speech recognition in the elderly, being responsible for 70% to 75% of speech recognition performance variance. In contrast, auditory processing and cognitive function measures account for little, if any, variance in speech recognition.

Identifying and Recalling Speech in Babble

According to Pichora-Fuller et al. (165), reallocatable processing resources are used to aid in auditory processing when listening becomes difficult due to noise- or age-related auditory deterioration. Thus, fewer resources are available for storage and retrieval aspects of working memory; therefore, "upstream" processing of auditory information is negatively impacted (165).

Speech Recognition in Presbycusis

Frisina and Frisina (166) show that peripheral auditory defects, as measured by decreased sensitivity for speech, pure tones, and substance, contribute to increased thresholds for speech recognition in a quiet environment as well as reduced speech recognition through noise. Cortical portions of the speech and language perception system are not a part of speech-understanding problems in the elderly who are able to use supporting context in speech perception as well as the young. Dysfunction of speech-recognition in noise still exists in the elderly, even when cognitive and peripheral audition are not affected. However, auditory brainstem or cortical temporal-resolution defects account for the loss of speech recognition in noisy environments.

Effects of Aging and Hearing Loss in Comprehension of Complex Speech

In a recent study of the effects of aging and hearing loss on comprehension of rapid speech varying in syntactic complexity, Wingfield et al. (167) compared young and old adults with or without mild-to-moderate presbycusis in their ability to comprehend short spoken sentences which varied in complexity of syntax. They found that age, hearing loss, and increased complexity of syntax were all associated with poor comprehension. Thus, age by itself correlates with decreased comprehension of complex syntax, a factor that should be taken into account when treating persons with presbycusis.

Effects of Sex Hormones in Speech Comprehension

Hormones can also influence speech comprehension in the elderly. Results of a cross-sectional study by Helfer (168) of 38 women aged 60 to 74 years using estrogen replacement therapy (ERT) versus those not using ERT indicated that elderly women on ERT were better able to understand distorted speech than nonusers of ERT (168). The study focused on the women's ability to understand speech, using a battery of speech tests.

Sound lateralization, speech gap, and adaptation effects

Sound lateralization is affected more than speech discrimination ability in presbycusis (169). Schneider and Hamstra (170) have shown that elderly subjects with early presbycusis have a decreased ability in detecting gap durations characteristic of human speech sounds, compared to young adults. Also, these elderly experience differential adaptation effects.

■ Aging Changes in Central Auditory Pathways

In addition to the frequently encountered degenerative aging changes in the neurons of spiral ganglia discussed above (130,132), varying degrees of aging changes in the central auditory structures have been reported (171). These include loss of myelin and neuropil (dendrites and synapses) and degeneration and loss of neurons and lipofuscin accumulation in auditory structures such as the cochlear nuclei and superior olives of medulla, medial geniculate of thalamus, and the inferior colliculi of the midbrain (122).

Auditory Cortex Changes

In contrast to the lower auditory centers, the human auditory cortex shows very marked and clear degenerative aging changes, consisting of a general thinning of the cortex and disrupted organization of the vertical columns; this is caused by heavy neuronal loss and degeneration. In his well-known study of human cortex aging, Brody (172) found the highest degree of cell loss to occur in the superior temporal gyrus, the anatomical locus of the auditory association cortex; the loss, amounting to about 50% between the ages of 20 to 90, occurred across the cortical thickness. Golgi studies of pyramidal neurons of the auditory cortex by Scheibel et al. (173) revealed marked degenerative changes in the basal dendrites of layer III and layer V neurons in old subjects (eighth to tenth decades).

Cortical Correlates of Presbycusis in Experimental Animals

To explore cerebro–cortical correlates of presbycusis in experimental animals, Turner et al. (174) examined response properties of auditory cortex layer 5 output neurons via in vivo single-unit extracellular recordings. They found that in aged rats, layer 5 neurons demonstrate decreased signal-to-noise coding compared to younger rats. One interpretation of these results is that GABAergic neurotransmission in the auditory cortex is diminished with aging. (See Box 8 for more on animal models of hearing disorders.)

Auditory-Evoked Potentials

Allison et al. (68) initially described significant changes in the auditory-evoked potentials in the elderly, including increases in the latency and decreases in the amplitude of the response. The changes are more marked in males than females and occur generally after 60 years. Undoubtedly, these degenerative changes contribute to the observed disorders of hearing and speech comprehension in the elderly, although an exact cause-and-effect relationship is still not established. In a more recent study by Oku and Hasegewa (180), the amplitude of auditory brainstem evoked potentials (Wave I), measured with electroencephalography, was found to decrease in subjects with presbycusis compared to age-matched controls. In addition, the lengthening and shortening of other waves' interpeak intervals was thought to reflect aging-related electrophysiological changes in the central auditory pathways.

Aging Changes in Central Auditory Processes

Event-Related Potentials

In a recent study aimed at exploring central auditory processes in aging subjects as well those with hearing loss, Bertoli et al. (181) used event-related potentials (ERPs) to see how central auditory signal processing is affected by distracting noise such as those of a cafeteria environment. Psychoacoustic frequency discrimination thresholds for 1000 Hz pure tone were measured in quiet as well as in "cafeteria" noise. To get ERPs, small frequency contrasts were tested with and without noise in attended as well as unattended conditions. It was found that elderly with or without presbycusis demonstrated a decrease in inhibiting irrelevant stimuli, compared to young people.

These cognitive differences between young and elderly subjects may be a factor in the increasing severity of hearing loss with age. Similarly, in a study of cortical cognitive potentials in elderly persons, Stenklev and Laukli (182) analyzed changes with age in 230 patients aged 60 years and over who were subjected to a battery of audiological tests. The results showed that speed of central auditory processing is reduced with increased age.

Role of Cholinergic System

A recent study by Pekkonen et al. (183) found that the cholinergic system is involved in processing of cognitive auditory signals (preattentive events). Auditory ERP components P50 and N100 index preattentive auditory aspects including stimulus detection, as well as the subsequent component, mismatch negativity, which reflects "comparison of incoming stimuli to memory trace of preceding sounds." These events comprise preattentive auditory processing. Combined electroencephalogram (EEG) and magnetoencephalography (MEG) recordings were obtained from nine elderly subjects treated with muscarinic antagonist or a peripherally acting antagonist (in a double-blind protocol). In the elderly, scopolamine, a centrally acting muscarinic cholinergic receptor antagonist, selectively delayed P50, indicating not only that the cholinergic system of the brain is involved in auditory stimulus detection, but also that defects in this system may underlie auditory deficits in the elderly.

■ Cognitive Aspects of Auditory Aging

Interaural Asymmetry of ERPs

According to Jerger et al. (184), upon presentation of syntactically and semantically anomalous words in continuous speech, an auditory ERP is evoked, characterized by a positive peak, 600 to 1000 msec in latency. In a dichotic listening task for normal children and young adult subjects, the target word is usually isolated to each side (left and right ear) with equal frequency. Also, amplitude and latency measures of this task were equal for the target-left and target-right conditions. Elderly subjects with presbycusis showed significantly greater latency in the target-left than the target-right tasks; similarly, maximal positivity was significantly higher in the left-attended condition. These findings support past findings of interaural asymmetry in dichotic listening tasks in the elderly (184).

BOX 9 *Alzheimer's Disease, Depression, and the Auditory System*

Alzheimer's disease (AD) lesions in central auditory nuclei: Individuals with AD show a specific and consistent degeneration, including senile plaques and neurofibrillary tangles in such auditory structures as the central nucleus of the inferior colliculus and the ventral nucleus of the medial geniculate body, but not in cochlear nuclei; no such changes occur in the age-matched controls (see Ref. 187 for details). The AD-associated degenerative changes suggest that neuronal loss could include all frequency ranges, causing sensory-neural presbycusis. This is in contrast with typical clinical presbycusis, which affects the high-frequency range, due to peripheral auditory lesions.

Lesions and aging changes in the spiral ganglion: According to earlier studies, lack of degeneration in the entirety of the cochlea, of neurofibrillary tangles, and of neuritic plaques in the peripheral auditory systems of AD subjects further confirms that the peripheral auditory system is not involved in AD lesions. In 1998, Sinha et al. (187) compared temporal bones from patients with and without AD and found significant differences in the number of hair cells remaining, spiral ganglion cells, and their peripheral processes in the basal cochlear turn only. However, it is possible these changes are due to presbycusis and not AD.

Auditory memory changes: AD patients present changes in auditory memory (188). Automatic stimulus discrimination in the auditory system is represented by the auditory mismatch negativity (MMN) type of the event-related potential (ERP). MMN is utilized as a measure of auditory sensory memory by changing the interstimulus intervals (ISIs). Auditory aging is associated with reduced amplitude of MMN to duration deviance at short ISI, while MMN to frequency deviance attenuation at long ISI is caused by age-related memory trace decay. This conclusion is supported by the finding that automatic discrimination for frequency change was not affected in the early stages of AD, while the memory trace decayed faster in AD patients (188).

Music memory in AD patients: Inspired by past suggestive clinical observations, Cuddy and Duffin (189) studied an 84-year-old woman with severe AD-like cognitive impairments, and a minimental status score of 8 out of 30 and severe pathology affecting memory, language, and cognition. A test of music recognition (based on positive or negative responses to melodies) indicated that the patient's ability to recognize familiar melodies and detect distortions of familiar melodies was similar to age-matched controls and suggests that musical memory may be spared in AD.

Depression: Patients with late-onset depression demonstrated more hearing deficits than early-onset depressives. Also, age at onset of depression had a significant effect on pure-tone thresholds for the 0.5 to 4 kHz range and on word recognition in a noisy environment in the subject's better ear (190).

In a recent study, Tremblay et al. (185) investigated the effects of age and ARHL on the neural representation of speech cues and found significant age-related deficiencies in discriminating similar-sounding speech fragments in elderly people with and without presbycusis. The tests examined central processing ability of time-limited speech cues in the elderly compared to younger subjects. It was concluded that aged subjects even without presbycusis have diminished response and abnormal EEG patterns. Such results may explain why some patients with hearing aids report that speech is louder, but not necessarily more understandable, with hearing aids.

Gap Detection Thresholds

Normal adults and elderly people in the early stages of presbycusis were asked to determine the presence or absence of a gap between two tones of equal duration. Older adults in the early stages of presbycusis were less able to detect a gap between short tonal markers (with durations characteristic of speech sounds) of less than 250 msec. These results point to differential adaptation effects between young and older adults (170).

Sound Lateralization and Speech Discrimination in Sensorineural Presbycusis

Sound lateralization and speech discrimination are linked to central auditory system functionality. Sound lateralization may be affected more than speech discrimination in patients with presbycusis and SNHL of unknown etiology (169).

Influence of Aging, Alzheiner's Disease, and Depression on Measures of Hearing Disability

A statistically significant interaction was found between age and hearing (186). Elderly with mild-to-moderate hearing loss reported less handicapping effects of loss of hearing than younger subjects with the same degree of hearing (186). Changes in various measures of hearing also occurred in AD and depression (Box 9).

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9

The Adrenals and Pituitary—Stress, Adaptation, and Longevity

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INTRODUCTION

The endocrine system, like the nervous system, coordinates physiologic responses to environmental signals to enhance individual survival and reproduction. Since aging often brings about a decline in physiologic function, it is not surprising that, in regard to the endocrine system, old age engenders

- a diminished capacity to adapt to internal and external demands, especially under stress conditions and
- a deterioration of reproductive function in men and a cessation of reproduction in women.

Both in early and late stages of the life span, development and aging are associated with changes in endocrine function, and endocrine function is known to affect hormonal levels. It is clear that cellular and molecular changes occur with senescence, but it is not certain that these changes are responsible for senescence and ultimately death. Nevertheless, it is strongly suspected that hormonal changes influence functional decrements, disabilities, diseases of old age, and the length of the life span (1–4).

In recent years, various tools have been added to the classical, clinical, physiological, and biochemical measurements of endocrine function. New techniques, adopted from the fields of genetic engineering and molecular and structural biology, have provided new advances in the study of mutations in humans and in the use of genetic disruption in transgenic or knockout animals (5,6). These new tools and techniques can fruitfully be applied to the study of physiologic systems, such as the hypothalamo-pituitary-adrenal (HPA) axis discussed in this chapter. The HPA axis includes the cortical and medullary components of the Adrenal Gland: HPA may refer specifically to the HPA Adrenal cortical component and its steroid hormones, and/or to the HPA Adrenal Medullary and its catecholamine neurotransmitter, part of the sympathetic nervous system. The HPA axis is primarily regulated by feed back mechanisms. Also, new technologies for detection of hormonal signaling are being used in the study of growth, development, and reproduction. Thus, mimicking human endocrine pathology through animal gene mutations is providing new insights into endocrine aging.

The section following this introduction discusses the assessment and measurement of endocrine function. The subsequent sections discuss the structure and functions of the adrenal cortex, the adrenal medulla, and the pituitary gland. The last section, entitled Stress and Adaptation, discusses the stress response and its consequences, highlighting the key role of the HPA axis and the mechanisms that regulate adaptation and contribute to survival and reproduction.

ENDOCRINE GLANDS, HORMONES, AND CHEMICAL MESSENGERS

The wide distribution, multiplicity, and diversity of hormones acting as chemical mediators in the body partly testifies to the critical role of endocrine regulation of bodily functions (Box 1). This important role certainly applies to the hormones of the HPA axis, which is discussed in this chapter. HPA hormones are responsible for communication among cells within the same organism and between the organism and its surrounding environment. The *hypothalamus*, situated in the midbrain, plays key roles in the regulation of several complex behaviors, such as endocrine, autonomic, and metabolic functions, and circadian rhythms. The *pituitary* (or hypophysis) secretes several hormones that stimulate peripheral targets, either other endocrine glands or specific tissues and organs. The location of the pituitary—in the close vicinity of the hypothalamus with which it articulates through a vascular net, the portal system, and through direct neuronal connections—makes it an intermediary between the nervous and endocrine systems. Among the endocrine glands, the *adrenals* regulate certain aspects of metabolism, behavior, and nervous and immune functions and, thus, play a key role in homeostasis. As with all endocrine glands, the adrenals and pituitary do not act in isolation. They are dependent for their function on neuroendocrine signals, usually initiated in or relayed through the hypothalamus (7–9). The endocrine glands are also dependent on the functional status of the target cells. With aging, changes in endocrine function may depend on changes in the following:

- A single endocrine gland
- Several endocrine glands simultaneously
- Other bodily systems (e.g., nervous, immune, cardio-vascular)
- Body metabolism and composition
- Cellular and molecular responses of target cells and tissues

In many cases, the assessment of a biological construct, such as aging, is sensitive to different experimental designs. These experimental designs may be modified in different ways to suit research needs. Thus, manipulation in experimental animals and diseases in humans, must be carefully considered by the researcher as they may challenge the validity of the results. Among the variables the most frequently included are:

- The influence of stress, disease, medications, and drugs
- The influence of heredity and environment
- The influence of diet and exercise

Assessment of Endocrine Function

It is difficult to evaluate endocrine function, and the reasons for this include the following:

BOX 1 *Intercellular Communication by Chemical Mediators*

Endocrine communication is mediated through *hormones secreted by endocrine glands. Secreted hormones are released into the blood circulation and act on distant target cells*. Well-recognized endocrine glands include the pituitary, adrenals, thyroid, parathyroids, pancreas, testes, and ovaries.

Other cells or groups of cells act by paracrine communication. These cells, interspersed among other cells, secrete, in the extracellular fluid, hormones that affect neighboring cells. Examples of paracrine hormone-producing cells are those of the pancreas (with both endocrine and paracrine secretions), the intestinal mucosa, and those producing prostaglandins. Secretory cells may also act by autocrine communication, that is, they secrete chemical messengers that bind to receptors on the very same cell that secreted the messenger. Yet, other cells act by *juxtacrine communication* in which the cells act directly on the neighboring cells.

Some neurotransmitters, such as epinephrine and norepinephrine, are also considered chemical messengers (Chapter 6). Other important messengers such as cytokines, thymic hormones, membrane receptors, and growth or apoptotic factors regulate immune and hematopoietic functions (Chapters 14 and 17). Chemical messengers are for the most part amines, amino acids, steroids, polypeptides, proteins, and, in a few instances, other substances. In different parts of the body, the same chemical messenger can function as a neurotransmitter, a paracrine mediator, and a neurohormone.

- Hormonal actions simultaneously affect several bodily functions
- Hormones regulate responses generated by internal (genes) and external (environmental) signals to promote reproduction and to maintain homeostasis
- The repertory and efficiency of integrative hormonal responses, which are optimally available during adulthood, diminish with advancing age and, thus, compromise strategies for adaptation and survival

The evaluation of endocrine function in humans often relies on relatively noninvasive measurements of blood, urine, and saliva, under basal conditions (resting or steady state) and under stress. Such an assessment often leads to incomplete and erroneous conclusions, since an adequate endocrine evaluation must assess several levels of endocrine action as well as assess the relationship between endocrine and other bodily systems (primarily the nervous and immune systems), hormone–receptor interactions at the target cell, and molecular events inside the cell, as listed in Table 1. Although none of these aging-related changes alone may be sufficient to irrevocably damage physiologic competence, a number of minor changes may desynchronize the appropriate signal at the target cell/molecule and alter hormonal actions. Factors involved in the design of the experimental protocol (e.g., sample size, health, and sex of subjects) to assess endocrine function may also influence the evaluation of changes that occur with old age.

An ideal "global" approach (as outlined above) to the study of endocrine aging is currently very difficult to achieve in humans. This global approach may be implemented more easily in experimental animals and in cultured tissues or cells. Such in vivo and in vitro models represent an important corollary to human studies. As illustrated in Figure 1, changes with aging may occur at all levels of the endocrine system:

- At the endocrine gland level, weight loss with atrophy, fibrosis, and vascular changes occur in most glands, with or without the concomitant occurrence of glandular tumors (adenomas).
- Under basal conditions, blood plasma hormones (free, biologically active hormones or hormones bound to plasma proteins) in humans and in animals are generally not altered in healthy old age, although some hormones (such as sex hormones) decrease significantly.
- Hormone release depends on nervous and environmental stimuli as well as positive and negative feedback from circulating hormones.
- Some hormones act exclusively on one type of target cells, while other hormones act on many cell types (targets) and by several mechanisms. Thus, the same hormone may have different actions in different tissues.
- With aging, one of the many hormonal actions or one of the many targets may be selectively affected while other actions and targets are preserved.

TABLE 1 Factors that Influence an Evaluation of Endocrine Function

Biologic factors
Physiologic factors
Metabolic state
Body composition
Dietary regimen
Physical exercise
Exposure to stress (environmental and psychosocial)
Relationship to other endocrines and bodily systems
The rate of secretion of secretory cells
Transport of the hormones to target cells
Metabolism of the secreted hormones
Metabolites may be more or less biologically active than the secreted hormones (e.g., conversion of T to the more active DHT (Chapter 11) and conversion of T4 to the more active T3 (Chapter 12))
Number and affinity of hormone receptors
Intracellular postreceptor molecular events
Occurrence of disease and use of medications
Experimental design factors
Sample size
Health status of subjects
Conceptualization of age categories
Comorbidity of subjects
Sex of subjects
Subjects under steady state or under stress
Quality, intensity, timing, and duration of stress
Outcome of study
Parameters measured
Duration of parameters measured (long- vs. short-term experiments)

Abbreviations: DHT, dihydrotestosterone; T, testosterone; T4, thyroxine; T3, 3, 5, 3', -triiodothyronine;.

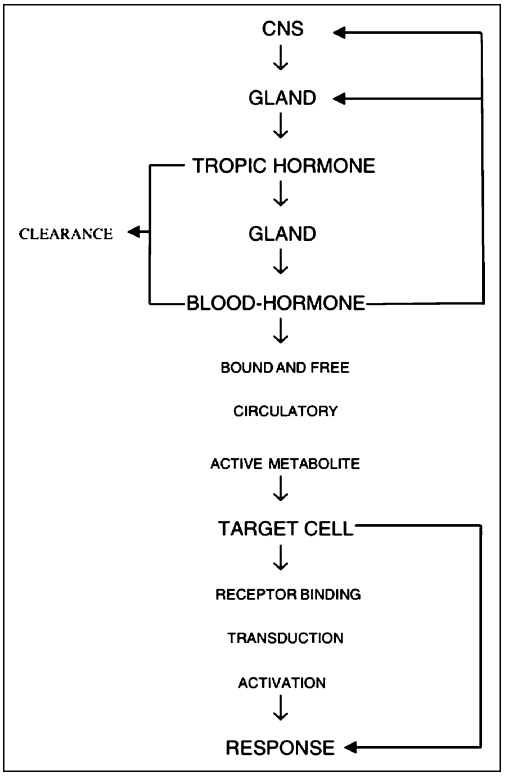

FIGURE 1 Diagrammatic representation of a typical sequence of hormone action and regulation. *Abbreviation*: CNS, central nervous system.

- Secretory and clearance rates often decrease, although it is not clear in these cases whether the primary defect involves hormone secretion or hormone clearance. The pertinent question is to what extent the capacity to maintain stable levels of plasma hormones is preserved. To better understand at which levels defects may be occurring, it is important to study hormonal biosynthetic precursors, their enzymes, and intermediary metabolites.
- Receptors located on target cells mediate specific actions of hormones on particular cells and the number of receptors may increase (upregulation) or decrease (downregulation) depending on the stimulus. Hormone-receptor complexes are usually internalized by endocytosis, bind to the nucleus, and stimulate or repress the transcription of selected RNAs or the activity of specific enzymes. Cellular responses are determined by the genetic programming of the particular cell. With aging, receptor binding and intra-cellular responses vary greatly depending on the hormone and the target cell.

THE ADRENAL CORTEX

The adrenals are paired glands that lie above the kidneys (Fig. 2). They have an inner medulla and an outer cortex (Fig. 3). The medulla is considered a sympathetic ganglion and it secretes the catecholamines [epinephrine (E) and norepinephrine (NE)], which are amines derived from the amino acid tyrosine. The cortex secretes several steroid compounds, characterized chemically by a 17-carbon ring system. The following are derivatives of cholesterol and share the same steroid structure: sterols, bile acids, vitamin D, and hormones from the ovary (e.g., estrogens), the testis (e.g., testosterone), and from the adrenal cortex (corticoids). Corticoids are distinguished into three categories:

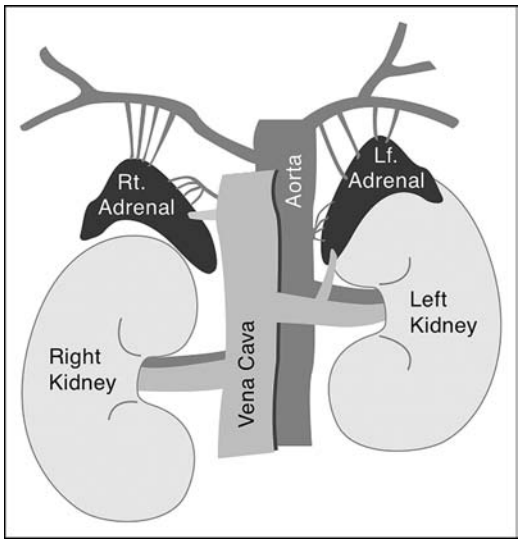

FIGURE 2 Diagram of the kidneys and adrenals.

- *Glucocorticoids*: In this group, cortisol is the principal glucocorticoid secreted in humans, and corticosterone is the principal glucocorticoid secreted in rats (Fig. 3).
- *Sex hormones*: Dehydroepiandrosterone (DHEA) is the principal adrenal androgen in humans. Cortisol and DHEA are secreted by the cells of the zona fasciculata and zona reticularis, and corticosterone is secreted by these and also the zona glomerulosa.
- *Mineralocorticoids*: Secreted by the cells of the zona glomerulosa, aldosterone is the principal hormone of this group.

The HPA axis is the most important system to guarantee adaptation and survival of an organism upon exposure to stress (Table 2). Given the complex interrelationships among the hypothalamus, anterior pituitary, and adrenal cortex, it is necessary, in evaluating the function of each component, to consider the entire axis as one entity (Fig. 4). Secretion of the adrenocorticotropin or adrenocorticotropic hormone (ACTH)

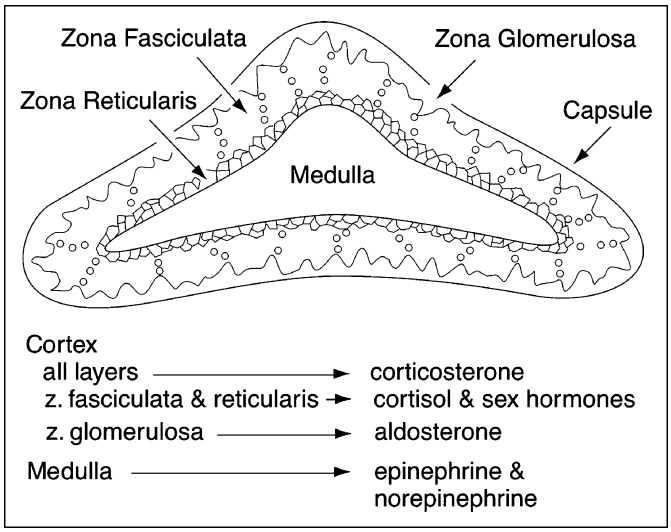

FIGURE 3 Diagram of a section of the adrenal grand illustrating the various zones and hormones.

TABLE 2 Some Characteristics of Stress

Stress induces defense mechanisms for maintenance of homeostasis in response to challenges
Some types of stress known to stimulate the HPA axis
Physical stress
Hypoglycemia
Trauma
Exposure to extreme temperatures
Infections
Heavy exercise
Psychological stress
Acute anxiety
Chronic anxiety
Anticipation of stressful situations
Novel situations
Consequences of exposure to stress
Specific responses (varying with the type of stimulus)
Nonspecific responses (always the same, regardless of the stimulus and mediated through stimulation of neural, endocrine, and immune axes)

Abbreviation: HPA axis, hypothalamo-pituitary-adrenal axis.

from the anterior pituitary is stimulated by the action of the hypothalamic corticotropin-releasing hormone (CRH). In turn, ACTH causes the release of cortisol (with a half-life in plasma of 60 to 90 minutes and a proportion in plasma of approximately 10% free and 90% bound to plasma proteins). The effects of old age on the HPA axis have been studied extensively given its importance in the maintenance of homeostasis.

■ Changes with Aging in Adrenocortical Hormones Under Basal and Stress Conditions

With aging, the adrenal cortex undergoes some structural changes. For instance, its weight is decreased in humans, and in the various animal species that have been examined, nodules (i.e., localized hyperplastic changes, perhaps reactive to a reduced blood supply or consequence of multifocal adenomas) occur frequently. The adrenocortical cells, which are typical secretory cells rich in mitochondria and endoplasmic reticulum with numerous lipid droplets where the steroid hormones are stored, undergo several changes. Of these, the most widespread is the accumulation of lipofuscin granules (Chapters 3 and 6), ultrastructural changes in mitochondria, and the thickening of the connective support tissue (as shown by the thick capsule and the fibrous infiltrations around blood vessels). Major actions of glucocorticoids are described below and in Figure 5. DHEA, the principal adrenocortical sex hormone, has weak androgenic (masculinizing) and anabolic (protein building) actions, and mineralocorticoids, such as aldosterone, regulate primarily water and electrolyte metabolism through their action on the renal tubule (Chapter 18).

Glucocorticoids

Under basal conditions, the following parameters remain essentially unchanged in men and women well into old age (10–12):

- Plasma levels of cortisol and ACTH
- Circadian rhythm of ACTH release
- Cortisol release
- Responses of ACTH and cortisol to administered CRH

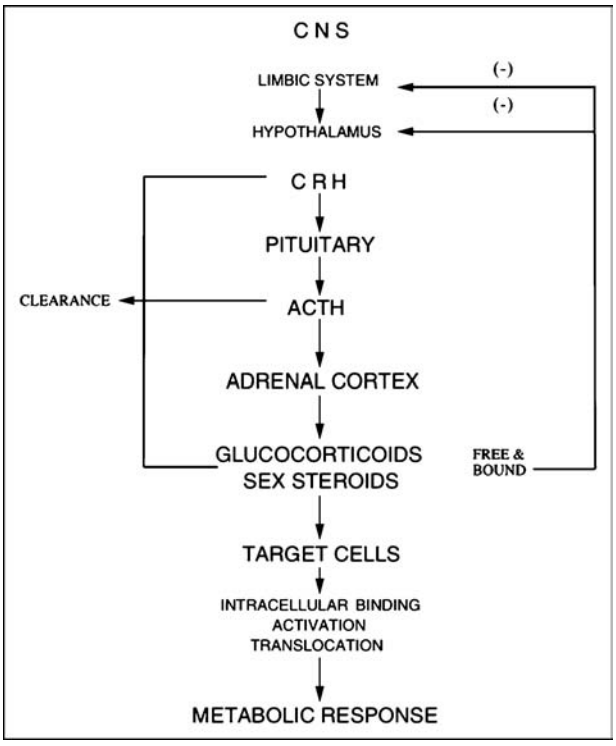

FIGURE 4 Diagrammatic representation of the HPA axis. *Abbreviations*: ACTH, adrenocorticotropic hormone; CNS, central nervous system; CRH, corticotropin-releasing hormone; HPA, hypothalamo-pituitary-adrenocortical axis.

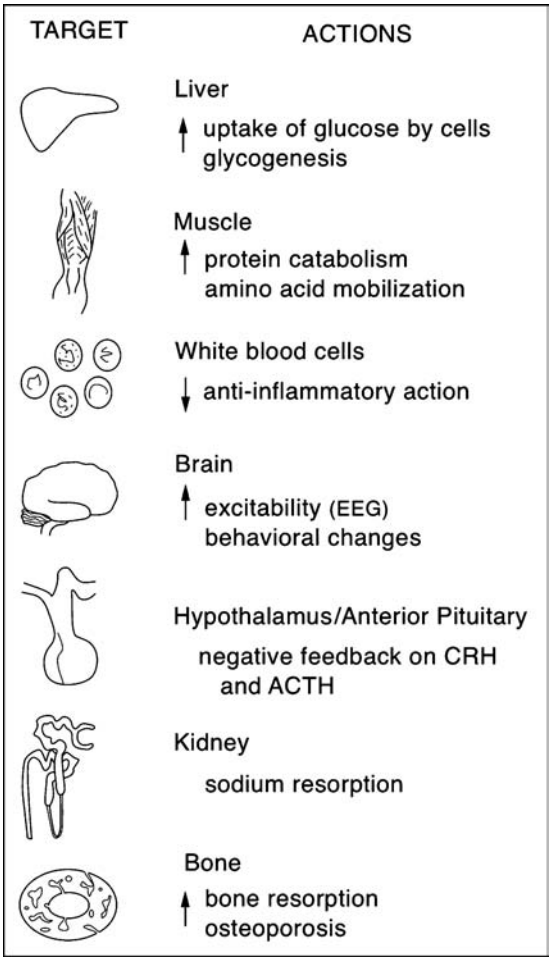

FIGURE 5 Diagram of the major actions of glucocorticoid hormones. *Abbreviations*: ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; EEG, electroencephalogram.

- Number of glucocorticoid receptors in target cells or affinity of these receptors for cortisol

A number of early studies suggested that secretion of cortisol is reduced in old age. However, the reduction of corticoid secretion be compensated for by a decreased clearance (i.e., reduced metabolism and excretion), or increased hormone production may be compensated for by an increased removal (clearance). Such metabolic compensatory mechanisms could remain operative into old age, with the body adapting to decreasing production rates of the hormone by reducing the rate of its removal or vice versa and, thus, maintaining normal circulating levels. However, more recent studies indicate that the production and clearance of cortisol are unchanged if the elderly subjects are in good health (12). Yet other studies have reported that in some species [e.g., rats (13), vervet monkeys (14), tree shrews (15), baboons (16)], glucocorticoid levels are slightly increased with senescence.

Stress stimulates the entire HPA axis, resulting in increased synthesis and secretion of CRH, ACTH, and glucocorticoids. Stress also stimulates the sympathetic nervous system and the adrenal medulla to increase E and NE secretion. In some animal species, under conditions of stress (physical or psychological) or after injection of exogenous glucocorticoids, the levels of glucocorticoids are more highly elevated in the older animals. This is the case for injections of corticosterone, which not only cause corticosterone levels to increase above those of controls of the same age, but also cause the higher levels to persist for longer periods in older rats (of some strains) (Fig. 6). These persistently higher corticosterone levels after stress or after administration of exogenous corticosterone have been interpreted as a loss of resiliency of the HPA axis. That is, it is thought that the HPA axis fails to set into action the negative feedback necessary for returning the elevated hormone blood concentrations to basal levels (Box 2) (13–17).

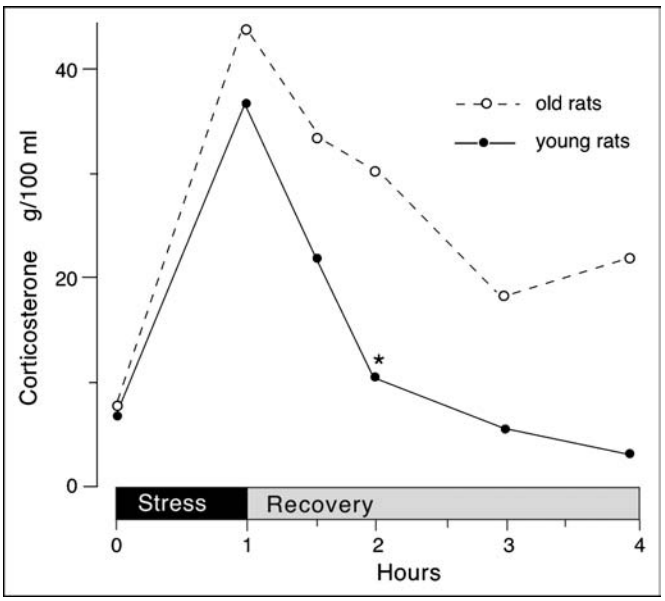

FIGURE 6 Corticosterone levels in young (three to five months) and old (24–28 months) Fisher 344 rats during one hour of immobilization stress followed by four hours of post-stress recovery. Corticosterone levels were higher and persisted higher in the old compared to the young subjects. *Indicates the times when levels are no longer significantly elevated above base line (determined by two-tailed paired *t*-test). In the case of young rats, this was one hour after the recovery period; for aged rats, such recovery did not occur during the monitored time period. *Source*: From Ref. 17.

In the rat, high glucocorticoid levels are toxic to neurons, particularly those of the hippocampus in which there is a high concentration of glucocorticoid receptors (18). Hippocampal cells under basal conditions inhibit CRH release. Therefore, when some of these cells are lost due to the toxic action of high corticosterone levels, CRH inhibition is also lost. Consequently, secretion of ACTH and glucocorticoids is increased and the levels of corticosterone in the blood continue to increase, thereby generating the "glucocorticoid cascade hypothesis of aging" (13). Young rats stressed for several weeks or treated with high glucocorticoid doses show hippocampal cell loss and changes in HPA axis function resembling those in old, stressed animals (13,17–20). In healthy humans, the relationship among the three components of the HPA axis do not appear to change significantly after long exposure to stress or with increasing age (21).

In contrast to levels of glucocorticoids that remain steady under basal conditions and rise under stress conditions, levels of the other adrenocortical steroids appear to decline with aging. This is the case for aldosterone in which values are almost undetectable beyond the age of 65 years (22). For DHEA, values for those aged 60 and older are approximately one-third of those for individuals around age 30 (23,24).

Adrenal Sex Steroids and DHEA Replacement Therapy

DHEA, the principal adrenal androgen, is considered a prototype of the adrenal sex hormones. DHEA follows a characteristic life cycle in which levels are

- very high in the fetus,
- low in childhood,
- rising before puberty,
- high in the adult, and
- progressively declining to low or negligible levels by the age of 70 years.

DHEA secretion is regulated by ACTH. Under conditions of stress, the secretion of cortisol and DHEA is increased, but the ratio of DHEA to cortisol falls as the enzymatic pathways for the biosynthesis of both hormones use the same intermediates, with preferential formation of cortisol (23,24). The reduced plasma levels together with the lower response of DHEA to ACTH administration have led to the suggestion that DHEA may have some antiaging effects, perhaps attributable to an antiglucocorticoid action. For example, severely atherosclerotic individuals have lower DHEA levels compared to normal individuals (25–27). This and other evidence have led to the claim that DHEA replacement may prevent some of the functional decrements and pathology of old age. It may be recalled that the physiologist C.-E. Brown-Séquard, by early 1889, recognized an association between aging and secretory actions attributed to an organ (the testis), and he extolled the antiaging properties of testicular secretions (androgens) (28). Testicular transplants and administration of androgens have been used repeatedly as possible rejuvenating measures to delay or reverse aging, but these attempts have met with little success. Indeed, high levels of androgens in aging men may even aggravate the incidence and severity of prostate hypertrophy and cancer (Chapter 18).

Effects of DHEA replacement therapy have been examined in animals. Long-term DHEA administration in old mice has reduced the incidence of mammary cancer, has increased survival, and has delayed the onset of immune dysfunction (29). DHEA administration also leads, in animals, to decreased

BOX 2 *Feedback Mechanisms Applicable to Hypothalamo-Pituitary-Endocrine Axes and the Portal Pituitary Blood Vessels*

Hypothalamo-pituitary-endocrine axes use feedback signals to regulate their secretory activity around a *set-point value* necessary for homeostasis. The set-point is maintained by negative feedbacks operating in a manner similar to an engineering control system with a set-point, a controlling element, a variable element, an integrator, and a feedback signal.

In almost all physiologic systems, if a discrepancy arises between the set-point and the variable element, an error signal is delivered to the controlling element to produce an adjustment in the direction opposite to the original deviation from the set-point. This type of control system, in which a variable provides a signal for compensatory reduction in the value of the variable, is referred to as a *negative feedback mechanism*. In the case of the hypothalamo-pituitary-adrenal axis, corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and glucocorticoid secretions are inter-regulated by feedbacks operating at each level. *Low blood glucocorticoid levels increase CRH secretion and CRH stimulates ACTH release, which, in turn, stimulates adrenal cortex glucocorticoid secretion. High levels of blood glucocorticoid levels inhibit CRH and ACTH secretion and, consequently, decrease adrenal glucocorticoid secretion. In each case, the needed result is the return of glucocorticoid levels to the original "set-point" level*.

Signals are relayed from one component of the axis to the other and from the periphery to the axis by short- and long-term loops. The short-term-loop feedback signals are carried through the portal blood vessels from the hypothalamus to the pituitary and vice versa (by retrograde flow). In the long-term loop, feedback signals are relayed from the peripheral endocrine gland and the target tissues to the pituitary and the hypothalamus through the general blood circulation.

Portal pituitary vessels represent a direct vascular link between the hypothalamus and the anterior pituitary. On the ventral surface of the hypothalamus, capillary loops from the carotid arteries and the circle of Willis form a vascular plexus that carries blood down the pituitary stalk to the capillaries of the anterior pituitary. This arrangement constitutes *a blood portal system beginning and ending in capillaries without going through the heart and general circulation*. Hypothalamic hypophysiotropic hormones are carried without dilution in the peripheral blood, directly to the anterior pituitary where they stimulate synthesis and release of the pituitary hormones.

food intake and body weight loss. This suggests that, despite its minor anabolic activity, DHEA may act in a manner similar to caloric restriction in extending the life span and in retarding tumorigenesis and immunosenescence (30–32) (Chapter 23).

Mineralocorticoids

Secretion, blood levels, and clearance rates of aldosterone decrease in the elderly (22). This decrease has been attributed to a declining adrenergic receptor activity (33); yet, the persistence of normal plasma electrolyte balance despite lower aldosterone levels demonstrates the efficiency of compensatory mechanisms even in old age (22). Impaired conservation of urinary sodium, which may occur in old age (Chapter 18), has been attributed to defects in the renin-angiotensin-aldosterone axis (34). While renin concentrations remain stable or decline with advancing age, plasma aldosterone levels decline. Reduced aldosterone levels have been attributed not only to the decrease in renin (when renin declines do occur) (35), but also to the reduced activity of biosynthetic enzymes for the hormone (as well as to the reduced number of calcium channels) (35,36).

Adrenal Steroid Receptors

The classical view of the mechanism of action of adrenal steroids is that adrenal steroids exert their cellular and molecular actions by binding to cytoplasmic (cytosolic) and nuclear receptors and that the degree of cellular responsiveness is directly proportional to the number of occupied receptors. The hormone-receptor complexes are then translocated to nuclear receptor sites in the nucleus where they modify gene expression (Fig. 7). The ensuing action occurs with a lag time lasting hours or days. It is now recognized that hormone-receptor responses are mediated, in addition to genomic mechanisms, by nongenomic mechanisms. Nongenomic mechanisms are characterized by rapid-onset actions that are mediated through binding of the hormone to membrane receptors, which, in turn, activate second messengers and various signal transduction cascades (37).

Adrenocortical steroid receptors are members of the steroid hormone/nuclear receptor family comprised of the vitamin D receptor, retinoid receptor, and thyroid hormone receptor, as well as a number of so-called "orphan" receptors (because their ligand and function are not well identified) (38–40). All classical steroid receptors (androgen, AR; estrogen, ER; glucocorticoid, GR; mineralocorticoid, MR; and progesterone, PR) are phosphoproteins that, in the absence of the activating signal, are

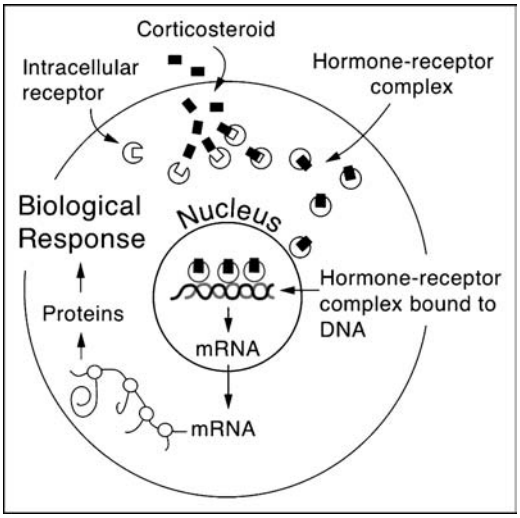

FIGURE 7 Schematic diagram of corticosteroid action in a target cell.

associated with heat shock proteins (HSPs) (41). They all act as transcriptional regulatory proteins and are able to interact with select target genes (40–42).

Numerous mechanisms account for this selectivity, such as interaction with DNA-bound transcription factors, the presence of chaperones, phosphorylation, and subnuclear trafficking pathways that facilitate receptor scanning of the genome (37–43). Several steroid receptors can be activated in the absence of the hormone. This is the case of ERs that bind competitively to antagonist or agonist nonsteroidal molecules (i.e., selective estrogen receptor modulators, Chapter 10), but this does not seem to be the case for glucocorticoids (43) despite data on the binding of the antagonist RU486 (44). The finding that some of the receptors may be activated by signal transduction pathways in the absence of the specific hormone, although not immediately applicable to adrenocortical receptors, may be worth pursuing in future studies considering the current progress in our understanding of the role of coactivators and corepressors in modulating action of estrogen and progesterone receptors (44,45).

All molecular events in the hormone-cellular response pathway subsequent to receptor binding are subject to alteration with age, although the nature and magnitude of these age-related changes are variable depending on the hormone, the target cell, and the animal species. Overall, the concentration of corticosteroid receptors decreases either in early adulthood or during senescence (46). For example, in the rat brain, glucocorticoid receptors are detectable on day 17 of gestation, the receptors increase gradually after birth to adult levels by 15 days of postnatal age, but then are significantly reduced in aged animals (24-months-old) (47). The concentration of cytosolic corticosterone receptors in the primary glucocorticoid-concentrating region of the brain, the hippocampus, decreases with aging, with no change in receptor affinity or capacity for nuclear translocation (47). Some of the receptor physicochemical properties (e.g., activation, transformation) seem to be more susceptible to aging than the number of receptors. Such age-related changes have been reported in glucocorticoid receptors in liver, in skeletal muscle, and in the cerebral hemisphere. Aging changes in corticosteroid receptors that alter the responsiveness of target cells and molecules to hormones may contribute to the decline in the effectiveness of adrenocortical responses to stress.

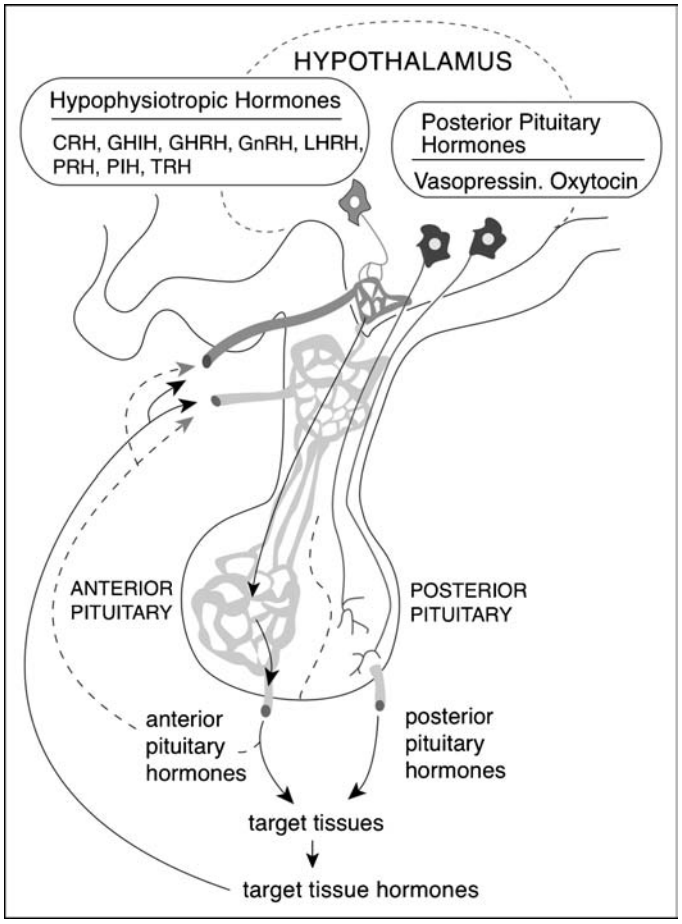

FIGURE 8 Diagram of the relationships among hypothalamus, pituitary, and target tissues. The neuroendocrine cells of the hypothalamus secrete both (1) hypophysiotropic hormones that are carried by a local portal system directly to the anterior pituitary where they stimulate the synthesis and release of anterior pituitary hormones, and (2) hormones that are carried to the posterior pituitary and released from there into the general circulation. Major hypophysiotropic hormones include GnRH, CRH, GHRH, GHIH or somatostatin, PRH, PIH, and TRH. The hypothalamic hormones that are carried to the posterior pituitary include ADH or vasopressin and oxytocin. The arrows indicate the presence of regulatory feedbacks between the circulating levels of the hormones and their release from the hypothalamic neuroendocrine cells. *Abbreviation*: CRH, corticotropin-releasing hormone; GHIH, growth hormone-inhibiting hormone; GHRH, growth hormone-releasing hormone; PIH, prolactin-inhibiting hormone; PRH, prolactin-releasing hormone; TRH, thyrotropin-releasing hormone; ADH, antidiuretic hormone; GnRH, gonadotropins releasing hormones.

■ Regulation of Adrenocortical Secretion

As illustrated in Figure 8, circulating levels of adrenocortical hormones depend on a hierarchy of regulation, from the hypothalamus to the pituitary, to the adrenal gland (Box 2) and, ultimately, to the target tissues, cells, and molecules. With aging and under conditions of stress, a disruption of this complex regulatory system at one or more levels may result in failure of homeostasis and adaptation.

CRH, a polypeptide released from neurons in the median eminence of the hypothalamus, is transported via the portal system to the corticotropes of the anterior pituitary, where CRH stimulates synthesis and release of the ACTH. ACTH, a protein released from the anterior pituitary, stimulates cells of the two inner zones of the adrenal cortex to synthesize and release the glucocorticoids and sex hormones (Fig. 3). Thus, after ablation of the pituitary, these two zones atrophy, and the circulating levels of the corresponding hormones decrease. Conversely, in tumors of the pituitary in which ACTH levels are increased (as may occur in Cushing's disease), the two adrenocortical zones hypertrophy, and the hormonal levels increase (48).

ACTH is secreted in bursts throughout the 24-hour day, with the pulses being most frequent in the early morning and least frequent in the evening. The resulting circadian (diurnal) rhythm in cortisol secretion is largely preserved during aging in humans, but there may be a modest flattening and shift of the diurnal rhythm. Regardless, sustained nighttime cortisol levels (i.e., a reduced nocturnal drop in cortisol levels compared with daytime values) have been correlated with (*i*) reduced renal clearance of the hormone (Chapter 18), (*ii*) reduced muscle mass and generally reduced basal metabolism (Chapter 24), and (*iii*) alterations in sleep patterns and insomnia (Chapter 7).

In addition to ACTH, the adrenal cortex is stimulated to secrete glucocorticoids by the action of the antidiuretic hormone (ADH), one of the two hormones of the posterior pituitary. The major action of ADH is to stimulate retention of water by the kidney in which urine becomes concentrated and its volume

decreases (Chapter 18). Other functions of ADH include elevation of arterial blood pressure (hence the alternative name of vasopressin) and maintenance of blood homeostasis. ADH also has some metabolic actions and causes glycogenolysis in the liver. In relation to the *adrenal cortex, ADH increases ACTH secretion by stimulation of the corticotropes (pituitary cells secreting corticosteroids)*. Lastly, a variety of stimuli increase ADH secretion, such as pain, nausea, stress, some emotions, and some drugs.

■ THE ADRENAL MEDULLA

The adrenal medulla is part of the sympathetic division of the autonomic nervous system, and, as such, it functions in unison with the other sympathetic structures. The main secretions of the adrenal medulla are E, NE, and, to a lesser extent, dopamine (DA) (Fig. 3). These chemicals are derived from the amino acid tyrosine and are chemically classified as catecholamines. NE also acts as a neurotransmitter in the central nervous system (CNS).

The adrenal medulla is interrelated anatomically and functionally with the adrenal cortex by a rich vascular network in which blood flowing from the cortex to the medulla provides high concentrations of glucocorticoids which induce in the medulla some of the enzymes for catecholamine synthesis [e.g., the enzyme phenylethanolamine-*N*-methyltransferase (PNMT)]. NE is formed by hydroxylation and decarboxylation of thyrosine, and E is formed by methylation of NE by the enzyme PNMT. In addition, glucocorticoids have some metabolic interaction with medullary hormones (e.g., mobilization of free fatty acids in emergency situations). Major actions of the catecholamines are summarized in Figure 9, and mechanisms of cellular stimulation are summarized in Figure 10. The catecholamines, like other transmitters, are released at the synaptic cleft where their actions are terminated by three mechanisms: (*i*) they bind to the postsynaptic receptors to stimulate the target cell; (*ii*) they bind to presynaptic receptors for reuptake into the presynaptic cell; and (*iii*) they are metabolized by the enzymes monoamine oxidase and catechol-*O*-methyltransferase (Chapter 6, Fig. 6.11). Thus, the efficiency of neurotransmission depends on both the release and the removal of the chemical transmitter at the synapse.

The autonomic nervous system is comprised of sympathetic and parasympathetic divisions, and its dysfunction is a well recognized, although poorly understood, consequence of old age. One of the anatomical characteristics of the autonomic sympathetic division is its organization into a paravertebral sympathetic ganglion chain that, under emergency conditions of stress, can discharge as a unit, as in "rage and fright," when sympathetically innervated structures are stimulated simultaneously over the entire body (Fig. 11). This emergency response causes heart rate to accelerate, blood pressure to increase, bronchioles and pupils to dilate, and many other changes (Table 3). *The contribution of the adrenal medulla to "the emergency function" of the sympatho-adrenal system involves the perception of stress and functional responses to it.* Although the adrenal medulla is not essential for life under nonstress conditions, as its absence may be relatively well compensated for by activation of other sympathetic neurons, it is indispensable under stress conditions.

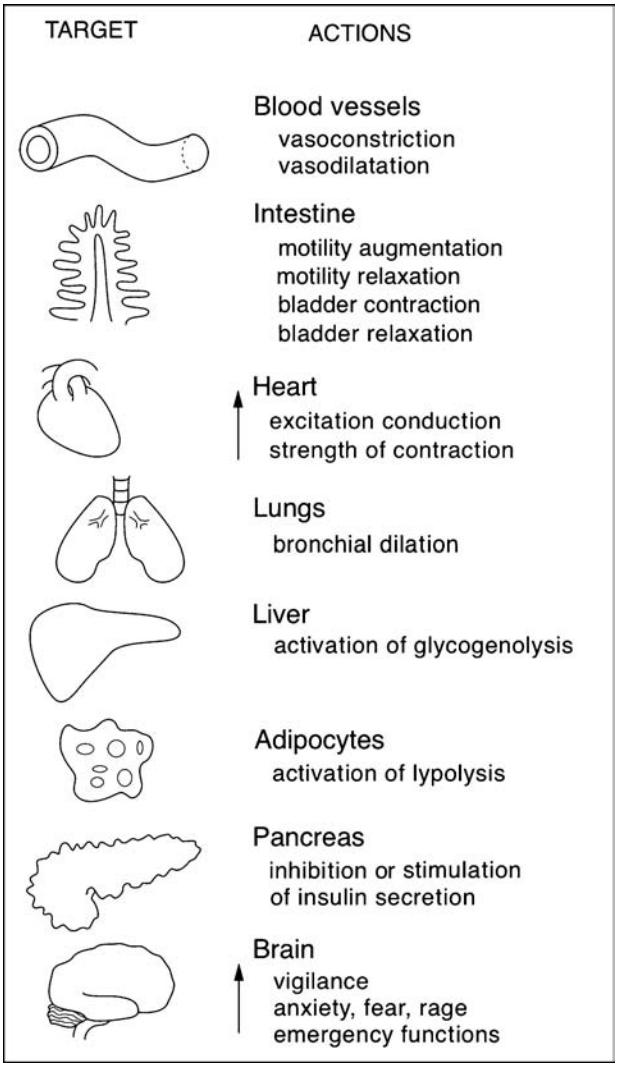

FIGURE 9 Major actions of adrenal catecholamines, E and NE. Both E and NE act on α and β receptors, with NE having a greater affinity for α-adrenergic receptors and E- for β-adrenergic receptors. *Abbreviations*: E, epinephrine; NE, norepinephrine.

■ Variability of Changes with Aging

The structure and function of autonomic neurons appear to be altered with aging. Major structural changes include swelling of axonal neurons with neurofilament aggregates, accumulation of lipofuscin, and decreased catecholamine fluorescence. These structural changes are associated with dysfunction of body temperature, bowel motility, cardiovascular maintenance (also partly regulated by parasympathetic inputs), blood pressure, water and electrolyte distribution, and energy metabolism. Several studies indicate that basal sympathetic activity increases in some elderly individuals, and the increase may be associated with dysregulation of the ability of the sympathetic nervous system to respond to a variety of challenges (49,50). Under basal conditions, in humans, plasma levels and urinary excretion of E and NE are highly variable. With aging, these hormones may do the following:

- Remain unchanged
- Show a reduction in absolute and average circadian amplitude
- Show an increase, with the increase being greater after standing and physical exercise

Elevation of plasma and urinary catecholamines, reported after a variety of stimuli, has been interpreted as a compensatory reaction to the apparently increasing refractoriness with

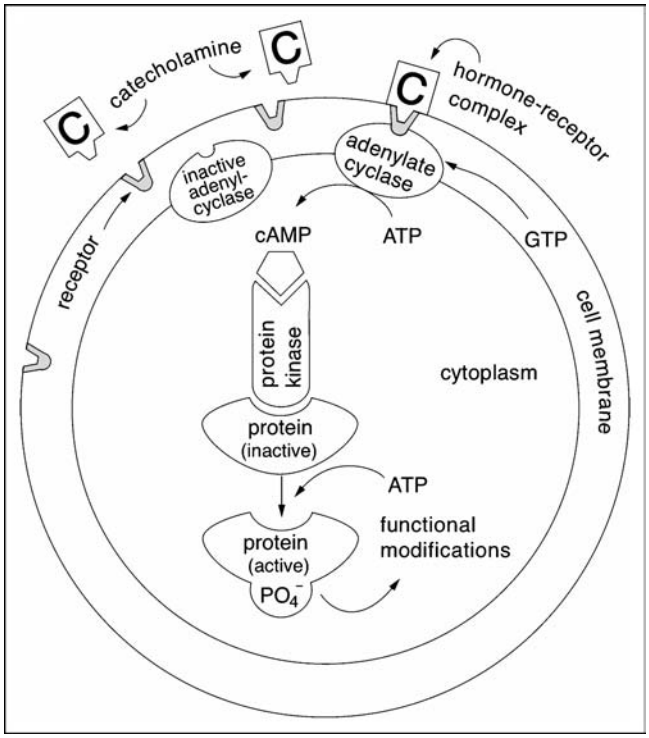

FIGURE 10 Schematic diagram of the action of catecholamines, E and NE, in a target cell. *Abbreviations*: E, epinephrine; NE, norepinephrine; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; GTP, guanosine triphosphate; PO_4.

aging (perhaps due to receptor downregulation) of target tissues to catecholamines (33,49–52). However, the apparent increase in NE plasma levels does not occur with all stimuli, and, additionally, the time it takes to return to baseline levels is prolonged in the elderly. Also, NE and E often show differential responses. For example, in an elderly subject during a mental stress test, plasma NE might be elevated, whereas plasma E might stay stable. Given the wide distribution of NE throughout the sympathetic nervous system and CNS, in contrast to the circumscribed localization of E in the medulla, the differential response of NE and E suggests hyperactivity of the sympathetic system in general, rather than that of the adrenal medulla in particular.

■ Target Differential Responsiveness

In the elderly, high levels of catecholamines may be due to either a higher release from the adrenal medulla or a reduction in peripheral clearance. *One proposed explanation for the overall increase of sympatho-adrenal activity with aging is an increased refractoriness (or decreased sensitivity) of target tissues to catecholamines due to alterations in transport and binding, and, therefore, the need for enhanced NE and E secretion.* A decrease in the number of adrenergic receptors with aging has been reported in some organs and cells (e.g., cerebellum, adipocytes) and a decrease in receptor affinity has been reported in others (e.g., lung), but in many cases, changes are not observed. Although current findings do not support the view that the number of receptors decreases with aging, several concomitant factors (e.g., aging-associated decrease in cell membrane fluidity) may mask true declines.

NE and E both act on α and β receptors, but NE has a greater affinity for α adrenergic receptors, whereas E has a greater affinity for β adrenergic receptors. Both α and β receptors are G protein receptors that span the cell membrane; G proteins are nucleotides, regulatory proteins that bind to GTP (guanosine triphosphate protein) (Fig. 10). Clinical and experimental observations have been conducted primarily of β1 receptors and the other β2 and α adrenergic receptors. An important finding is that the responsiveness of the receptors to adrenergic stimulation depends on the type of tissue in which the receptors are located (47,51,52). Decreased responsiveness may be found in the diminished efficiency of hemodynamic and cardiovascular responses to changes in posture (Chapter 7) and the slower dark adaptation of pupil size (Chapter 8). In contrast, increased responsiveness occurs in those organs and tissues regulating blood pressure. It is worthwhile to recall here that the loss of dopaminergic neurons in the cerebral basal ganglia is a major cause of Parkinson's disease (Chapter 6). Other hormones, such as thyroid hormones, that are known to affect catecholamine metabolism, may also increase the effects of catecholamines on blood pressure (Chapter 12).

■ THE PITUITARY GLAND

The pituitary gland (also called the hypophysis) regulates, through the secretion of its tropic hormones, the activity of several peripheral endocrine organs (adrenal cortex, thyroid, gonads) and other target tissues (e.g., bones, muscles). In humans, the pituitary gland is divided into two lobes, anterior and posterior. A third part, between the two, the intermediate lobe, is structurally and functionally rudimentary in humans. Together with cells dispersed in the anterior lobe, the intermediate lobe secretes melanotropin, which is related to skin and hair pigmentation (Chapter 21) and γ lipotropin, whose function is still little known.

The pituitary has close functional ties with the hypothalamus and, indirectly, other CNS centers, especially the limbic system. *The hypothalamus produces a number of peptides, the hypophysiotropic hormones, that are carried directly to the anterior pituitary by a local portal system (Fig. 8). The hypophysiotropic hormones stimulate or inhibit the synthesis and release of the anterior pituitary hormones.* Hormones of the anterior pituitary and their major functions are listed in Figure 12. The hypothalamic neuroendocrine cells secrete two peptide hormones that are carried by axonal flow to the posterior pituitary where they are stored until they are released into the circulation. Hormones of the posterior pituitary and their major actions are illustrated in Figure 13. *The hypothalamus is also involved in the regulation of the autonomic nervous system and of a number of behaviors (e.g., sex, fear, rage) (53,54).*

■ Structural Changes

The pituitary gland changes little with aging. Although changes have been described, it is still unclear if these changes involve the gland globally or involve localized parts of the gland.

In the anterior lobe, changes with aging are relatively few and include cellular changes typical of aging cells (e.g., accumulation of lipofuscin). Tumor incidence, primarily prolactinomas [tumors secreting prolactin (PRL)], increases with aging in rats and mice, and more so female. In the posterior lobe, studies in old rodents reveal a number of changes, such as decreased size and number of neurosecretory granules, reduced number of hormone receptors, increased autophagic activity, increased perivascular space, decline in cell volume, and

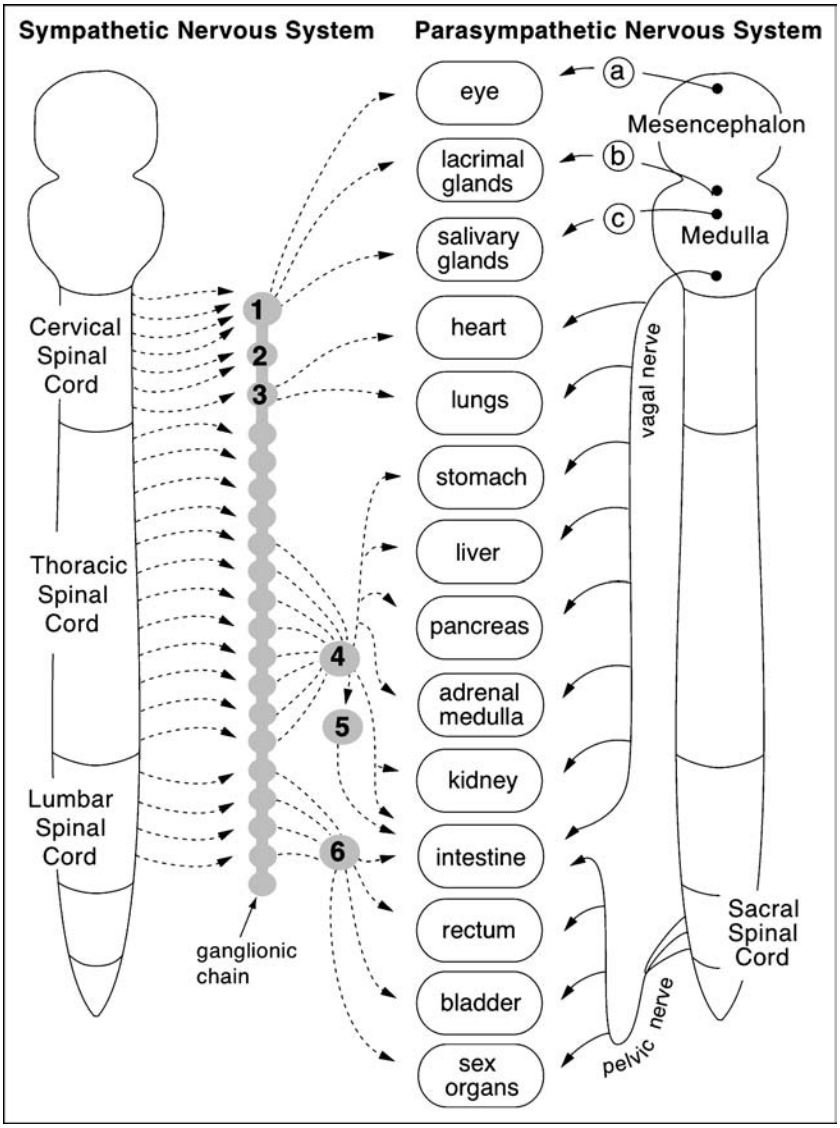

FIGURE 11 Diagram of the efferent pathways of the autonomic nervous system. The two systems, the sympathetic and the parasympathetic, often act antagonistically: for example, sympathetic stimulation usually accelerates the speed of cardiac contraction and increases blood pressure, whereas parasympathetic stimulation decreases both of them. Stimuli from the sympathetic neurons, located in the lateral columns of the spinal cord, are relayed to the paravertebral sympathetic ganglion chain and from the ganglia to the peripheral viscera. In the case of stress, they are activated simultaneously and generate those multiple responses necessary for survival and adaptation. Other sympathetic neurons include the (1) superior, (2) middle, (3) inferior, cervical ganglia (4), celiac ganglion (5), superior mesenteric ganglion (6), inferior mesenteric ganglion. The parasympathetic neurons are located proximal to the organs they innervate and stimulate them individually. They include the cranial ganglia that supply the visceral structures in the head through the (a) III, (b) VII, and (c) IX cranial nerves, and to the organs of the thorax and upper abdomen by the X cranial nerve (vagus). The pelvic nerve originates in the sacral spinal cord and supplies the lower abdomen viscera and the sex organs.

reduction in endoplasmic reticulum activity. In humans and other examined animals (e.g., cattle), such changes are rare.

■ Growth Hormone, Growth Hormone–Releasing Hormone, and Somatostatin

Aging is associated with a decrease in protein synthesis of lean body mass and bone formation as well as with an increase in adiposity (fat). This association suggests the involvement of growth hormone (GH) because of its anabolic (i.e., protein synthesis promoting) and metabolic actions (Box 3). Although some studies have reported a decrease in basal levels of GH, the number of somatotropes (i.e., pituitary cells secreting GH), the pituitary content of GH, the basal plasma levels of the hormone, and its clearance remain essentially unchanged into old age.

Obesity lowers circulating GH levels in young individuals, and the presence of obesity in some elderly may contribute to the decreased GH levels. Contradictory changes in GH levels have been reported in the elderly after exposure to stimuli known to cause GH release. In rats, GH elevation after stimulation by a variety of means is less marked in old animals, and, consistent with those findings, in humans after stress, surgical trauma, exercise, and arginine stimulation, the expected increase of GH secretion is often considerably blunted or even entirely lacking (55).

TABLE 3 "Fright, Flight, or Fight" Responses to Stress

Increased blood pressure
Increased heart rate
Increased force of heart contraction
Increased heart conduction velocity
Shift of blood flow distribution away from the skin and splanchnic regions and more to the heart, skeletal muscle, and the brain
Contraction of spleen capsule (increased hematocrit i.e., increased proportion of red blood cells to whole blood volume)
Increased depth and rate of respiration
Mobilization of liver glycogen to glucose (glycogenolysis)
Mobilization of free fatty acids from adipose tissue (lipolysis)
Mydriasis (widening of pupil)
Accommodation for far vision (relaxation of ciliary muscle)
Widening of palpebral fissure (eyelids wide open)
Piloerection (erection of hair)
Inhibition of gastrointestinal motility and secretion, and contraction of sphincters (ringlike muscles closing an orifice)
Sweating (cold sweats as skin blood vessels are constricted)

GH secretion in humans undergoes a nocturnal peak during the first four hours of sleep, coinciding with stages III and IV of slow-wave sleep. These stages are the most affected in aging (Chapter 7). Studies in older persons have shown a decrease in sleep-related GH secretion and an occasional decrease in the nocturnal peak. The latter finding has been attributed to low levels of growth hormone–releasing hormone (GHRH) and high levels of somatostatin or growth hormone–inhibiting hormone (GHIH) (56). However, the exact nature of the relationship between GH levels and sleep quality over age remains controversial (and so too does the exact nature of the relationship between GH levels and body weight over age).

The effects of GH on growth and protein metabolism depend not only on GH levels, but also on the interaction between GH and somatomedins, which are polypeptide growth factors secreted by the liver and other tissues in response to stimulation by GH. The majority of growth factors act by paracrine communication, that is, they reach their neighboring cells directly without being carried by the blood to distant targets. The principal and, in adult humans, probably the only circulating somatomedins is insulin-like growth factor-1 (IGF-1). Insulin-like growth factor-2 (IGF-2), is present and active primarily during the embryonic/fetal periods. As noted in Chapter 3, suppression of IGF-1 receptors (IGF-1Rs) and, hence, suppression of the actions of this hormone, lengthens the life span in worms, flies, and mice, and has several concomitant actions such as increasing resistance to stress (Chapter 3). In humans, IGF-1 levels decrease with old age, but the functional consequences of their decrease still remain controversial.

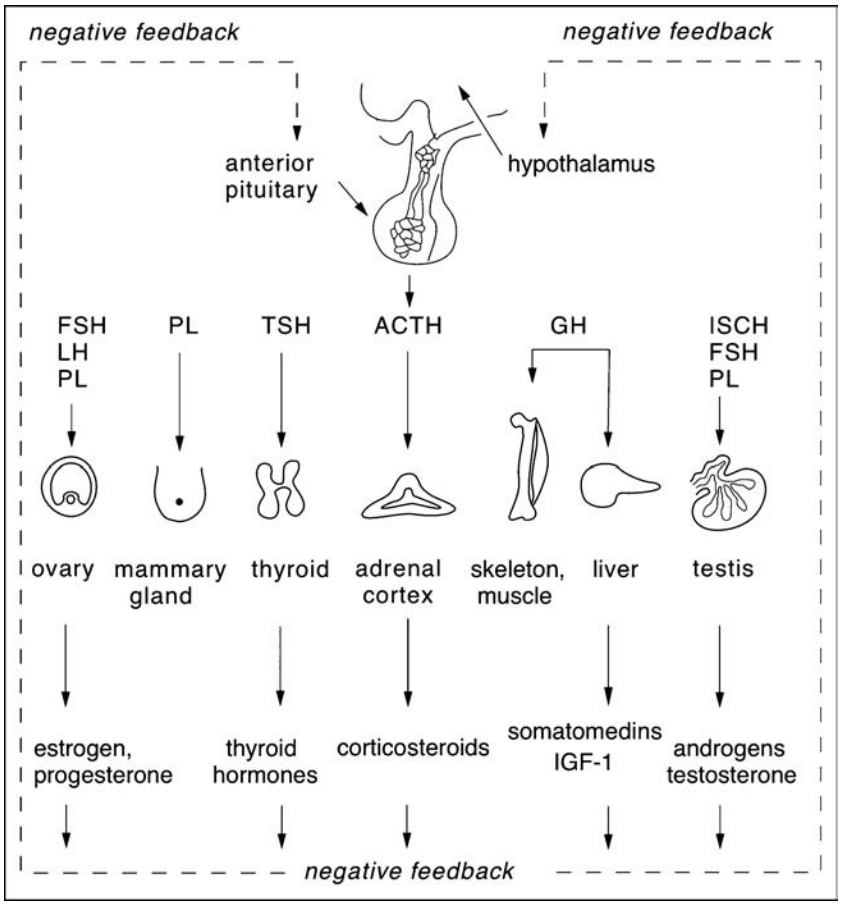

FIGURE 12 Diagrammatic representation of the major hormones of the anterior pituitary and the endocrine glands and tissues on which these hormones act. Note that the regulation of hormone levels depends on negative feedback (Box 2) except for the positive feedback of estrogens on LH secretion from the pituitary (Chapter 10). *Abbreviations*: FSH, follicle-stimulating hormone; LH, luteinizing hormone (women); ICSH, interstitial cells–stimulating hormone (men); PL, prolactin; TSH, thyroid-stimulating hormone; ACTH, adrenocorticotropic hormone; GH, growth hormone; IGF-1, insulin-like growth factor-1.

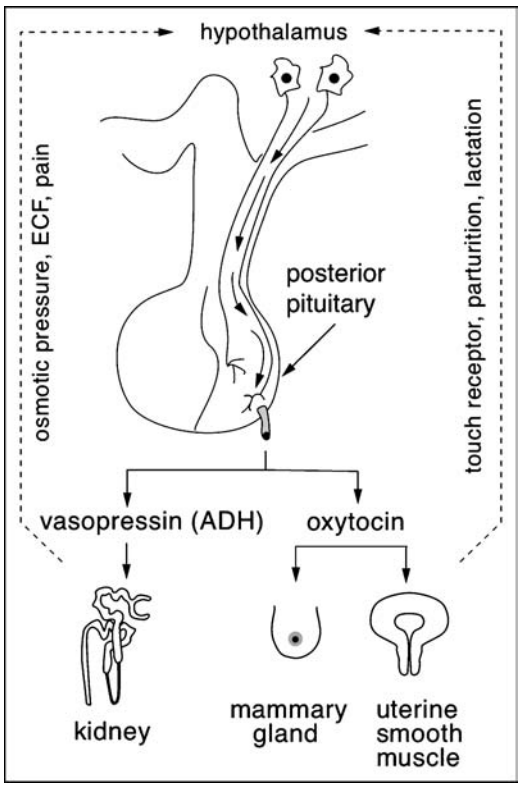

FIGURE 13 Diagrammatic representation of the major hormones of the posterior pituitary and the endocrine glands and tissues on which these hormones act. ADH and oxytocin are synthesized and secreted by the hypothalamic, supraoptic, and paraventricular nuclei; they are transported by axonal flow to the posterior pituitary, from where they are released directly in the general blood circulation. *Abbreviations*: ADH, antidiuretic hormone; ECF, extracellular fluid.

GH Replacement Therapy

Some elderly individuals have low blood levels of GH as well as low IGF-1 levels (57). In these elderly, relatively long-term administration (six months) of biosynthetic human GH increases muscle and bone mass and decreases adipose tissue. These beneficial effects are small (10–14% change) and last only as long as the hormone is administered. However, the hormone may decelerate the decline in muscle and bone with aging and consequently help to prevent the falls and bone fractures that are major causes of disability and mortality (Chapter 21) (58,59). These small and temporary observations, together with those of the potentially beneficial effects of other hormone replacement therapies (Chapters 10–13), have led to speculation about whether reduced GH and IGF-1 levels found in old age contribute to physiologic decline and increased pathology and whether GH and/or IGF-1 administration may delay aging and prolong the life span. Despite the enthusiasm about possible GH therapies, GH administration in a variety of conditions in humans (including cardiac failure, healing of burns and other wounds, and Alzheimer's disease) has been proven disappointing (60).

A number of experiments in animals testing for the possible beneficial effects of GH/IGF-1 on longevity are controversial. For example, *the levels of GH/IGF-1 in young calorie-restricted animals are reduced compared to young non–calorie restricted (i.e., fed ad libitum) animals.* In old fed ad libitum animals, there is also a reduction in GH/IGF-1 levels when compared to the young control animals. In old animals caloric restriction postpones the decline of the levels of GH/IGF-1. The persistence of relatively high levels of GH/IGF-1 in calorie-restricted old animals has been interpreted to mean that these high levels of GH/IGF-1 may mediate the beneficial effect of caloric restriction in old age and thereby help explain the animal's longevity. This latter interpretation would, then, justify the use of GH or IGF-1 administration in the elderly to delay the onset of functional impairment and

BOX 3 *The Structure and Actions of the Growth Hormone*

The growth hormone (GH) is a protein encoded by five genes on chromosome 17. GH has a high degree of species specificity, and it is bound, in plasma, to two proteins. The GH receptor is part of the cytokine receptor superfamily. Major actions of GH include:

Before adulthood

- Growth stimulation
- Increased protein anabolism
- Stimulation of insulin-like growth factor-1 (IGF-1) by tissues

In adulthood

- Stimulation of IGF-1 by tissues
- Increased lean body mass and metabolic rate
- Decreased body fat with increased plasma free fatty acids, thereby providing a ready source of energy for the tissues during hypoglycemia, fasting, and stressful stimuli
- Decreased blood cholesterol
- Increased hepatic glucose output (diabetogenic effect)
- Anti-insulin effect in muscle
- Stimulation of pancreatic B-cells, thereby making them more sensitive to insulinogenic stimuli, with resulting diabetes due to B-cell exhaustion
- Growth [GH was originally thought to produce growth by direct action on tissues (e.g., bones, muscles), but it is thought today that GH acts both directly and indirectly through the stimulation of a somatomedin, the IGF-1 (in the adult)]

GH secretion is controlled via the hypothalamus which secretes into the portal blood both growth hormone–releasing hormone, which stimulates GH secretion from the anterior pituitary, and growth hormone–inhibiting hormone, or somatostatin, which inhibits GH secretion. GH secretion is under feedback control as are the other anterior pituitary hormones. Additional factors can stimulate (e.g., hypoglycemia, fasting, stress) or inhibit (e.g., glucose, free fatty acids) GH secretion.

to prolong life. However, this view is not supported by studies in GH receptor knockout dwarf mice in which the phenotype induced by GH/IGF-1 deficiency is that of a much healthier animal with a longer survival rate.

In humans, it is well known that GH excess has detrimental effects and has been associated with acromegaly, diabetes mellitus, arthritis, and hypertension. In the interventions conducted so far in the elderly, administration of GH to individuals with low or normal GH/IGF-1 levels has resulted in increased morbidity (e.g., joint swelling and pain, cardiac arrhythmia, insulin resistance). These unwanted side effects and the possibility of increased mortality, together with the lack of solid evidence of beneficial effects, diminish the usefulness of GH treatment (60). Generally speaking, bone and muscle mass can be improved with good nutrition and physical exercise at all ages, and, therefore, these measures should be encouraged in preference to other, less efficacious and more risky interventions such as GH treatment (Chapter 24).

Somatostatin

Although the major function of GH is to promote whole-body growth (e.g., visceral organs, bones, tissues) during childhood and adolescence, GH continues to be secreted throughout life. Like GH, somatostatin also continues to be secreted throughout life and it regulates GH secretion. *In addition to inhibiting GH release, somatostatin also inhibits thyrotropin or thyroid-stimulating hormone (TSH) release. Somatostatin acts locally (by paracrine communication) and its levels in plasma are negligible. Somatostatin is secreted from the hypothalamus and other tissues (e.g., pancreas, intestine) and has multiple biologic actions (in addition to the inhibition of GH secretion). For example, somatostatin secreted in the pancreas and intestine inhibits the secretion of pancreatic and intestinal hormones*. Basal plasma levels (originating primarily from the pancreas and intestinal wall neurons) are higher in the elderly compared to young adults, but daytime variations and responses to administered meals are lower in amplitude in the elderly (61–63).

In patients with Alzheimer's and Parkinson's diseases, brain somatostatin levels are decreased in regions that also have cholinergic deficits (62) and in the cerebrospinal fluid (Chapters 6 and 7) (63). Somatostatin secretion is also influenced by neurotransmitter release and is stimulated by increased NE and DA discharge from brain catecholaminergic neurons. It is not clear whether the decline in NE and DA that occurs during aging (Chapter 6) accounts for the decrease in GH by way of a reduction in GHRH release or an increase in somatostatin release. Somatostatin-secreting or -containing tumors have equal incidence in men and women, with a peak incidence in the fifth decade of life. Half of these affected subjects also have other endocrine diseases (64).

Insulin-Like Growth Factor-1

Although not a pituitary hormone, IGF-1 is mentioned here because of its close functional relationship to GH. IGF-1 is produced locally in the brain and acts through widely distributed receptors (65). In addition to regulating of somatic growth and metabolism (along with GH), IGF-1 plays a role in postnatal growth and development and may have several other actions:

- It helps in neuroprotection and regeneration in the adult CNS (by improving metabolism, dendritic growth, learning, and memory).
- It is involved in the regulation of longevity (Chapter 3).
- Studies in old mice (regardless of their diet) show a significant loss in the total number of cells in the supraoptic and paraventricular nuclei of the hypothalamus. However, when only the IGF-1 sensitive cells (i.e., cells that are binding to the IGF-1R) are considered, the IGF-1 cells are selectively protected in the older, calorie-restricted mice as compared to the older, fully fed mice (66,67).

Maintaining IGF signaling may provide the persistent paracrine growth factor activity necessary for delaying neuronal and neuroglial degeneration associated with aging. Alternatively, the maintenance of IGF-1 signaling in calorie-restricted mice may simply be an adaptive response to diminished energy availability. Caloric restriction may be energy conserving since it selectively promotes the loss of cells not critical to the survival of the whole organism, while, at the same time, preserving IGF cells, thereby reducing overall energy expenditure and perhaps prolonging the life span (Chapter 23).

■ Gonadotropins and Thyrotropin

Gonadotropins (Gn) undergo significant changes with aging in males and, particularly, in females. These changes are discussed in Chapters 10 and 11 in relationship to their role in aging of the respective target peripheral endocrine glands. Likewise, changes in thyrotropin-releasing hormone (THRH) (67) and thyroid-stimulating hormone (TSH) are discussed in Chapter 12.

■ Prolactin

PRL stimulates lactation and has anabolic, diabetogenic, and lipolytic actions (68). In humans (in males), plasma PRL levels increase with aging, perhaps because of the reduction in hypothalamic DA (the hypothalamic inhibitor of hypothalamic PRL release) with aging and the high incidence of pituitary PRL-secreting tumors. Low PRL levels in old women may be attributable to low estrogen levels after menopause (68). Further, dampening of day and night PRL levels, combined with declining rhythmicities of GH, adrenal, thyroid, pituitary, and pineal secretions, and the cessation of ovarian cyclicity, contribute to the progressive failure of chronobiologic regulations with aging (Chapter 13).

■ Vasopressin (ADH) and Oxytocin

ADH and oxytocin are small peptides secreted by magnocellular neurons of the supraoptic and paraventricular nuclei of the hypothalamus and transported within the axons to the posterior lobe of the pituitary, where the peptides are stored before being released into the circulation (Fig. 13). Within each hypothalamic nucleus, some neurons produce oxytocin and others ADH. The peptides are synthesized as part of larger precursor molecules (neurophysins) and are secreted in response to neuronal stimulation. In response to stress, ADH secretion stimulates the release of ACTH from the anterior pituitary, and both stimulate the adrenal cortex.

In aging rats (except perhaps in the very old), vasopressin- and oxytocin-secreting neurons are largely spared the morphologic changes that occur in neurons in most other hypothalamic nuclei (69,70). Some of the changes that occur have been related to functional decrements in the secretory activity of neurons in the hypothalamic, paraventricular, and supraoptic nuclei. Degenerating neurons often alternate with hypersecreting neurons, which compensate for cell loss and impaired function; this diverse aging pattern may account for the disparate observations of low, high, or unchanged hormone levels (69,70).

The principal action of vasopressin is to promote the retention of water by the renal distal tubules and collecting ducts (Chapter 18). The principal actions of oxytocin in stimulating contraction of smooth muscle of the uterus during delivery and the mammary gland during lactation are thought to be relevant primarily to the reproductive years. *However, oxytocin and vasopressin have significant CNS actions: they are reported to ameliorate long-term memory and attention impairments, neural connectivity, and social behaviors (71).* Intraventricular administration of oxytocin reduces levels of anxiety behavior and HPA responses to stress in female rats (72,73). If expressions of anxiety and stress do increase with aging for certain individuals, these individuals may benefit from oxytocin administration (69–73).

■ STRESS AND ADAPTATION

Successful survival of each organism depends on an environment favorable to the optimal expression of the organism's function. This expression is crucial for single-celled organisms and even more so for multicellular, complex organisms. For humans, the environment is comprised of both external conditions (e.g., atmospheric temperature, food availability, social interrelations) and internal conditions (e.g., metabolism, coordination of regulatory signaling among bodily systems, integration of multiple cellular and molecular functions). Thus, maintenance of a constantly stable internal environment in response to internal or external challenges (stress) is key to an individual's survival and reproduction (Table 3). Closely related to these concepts *is the idea of homeostasis, which refers to the body's "ideal steady state," or a "constant" internal environment (Box 4).* Since in order to survive and reproduce, an organism must vary the parameters of its internal environment to suit environmental demands, homeostasis can be achieved only through dynamic adjustments that reflect repeated fluctuations of various physiologic systems. *The term allostasis refers to these fluctuations and adjustments necessary for maintaining homeostasis* (Table 4) (79).

The cumulative effects of stress may affect health and longevity of the individual organism. The goal of "stability through change" or "homeostasis through allostasis" is attained by paying a long-term price in terms of decreased function and increased pathology. *Allostatic load captures the idea that over the life course, the body's response to stress carries a cumulative physiological toll that affects multiple biological systems.* Given the multitude and variety of environmental changes/challenges, allostasis must continuously be active in order to maintain homeostasis. *With aging, the allostatic efficiency may decline or deviate from what is optimal, thereby endangering homeostasis and generating abnormal (pathologic) responses and diseases.* Ultimately, then, the build-up of allostatic load might contribute to the increasing morbidity and mortality of old age (Tables 4 and 5).

Unlike allostatic load, which focuses on the negative consequences of some types of stress, *the concept of "hormesis" refers to the health-enhancing aspects of other types of stress. That is, a stress of low severity and/or short duration can promote survival and longevity by boosting the efficacy of physiologic adaptation and the reparation of health injuries, eventually inflicted by severe and long-lasting stress.* With the decoding of the human genome, physiologic genomics (i.e., gene expression that articulates the return to an optimal state after stress) is expected to provide the important integrative link between one or several genes and their function(s) in regulating the responses of the living organism to the environment in which the organism lives (Chapter 3). It is important to note that the idea that some amount of stress may be good for an organism has yet to be fully appreciated (or operationalized) in the allostatic-load literature.

The impact of the hormonal changes with aging on the ability of the individual to adapt and survive has generated a number of theories based on the hypothesis that specific endocrine signals, together with neural and immune signals (probably genetically linked), may direct aging and death. Neuroendocrine and, more recently, neuro-immuno-endocrine theories propose that aging is not due to intrinsic deteriorative processes in all cells or molecules, but rather to a programmed regulation by "pacemaker cells," perhaps situated in the brain (particularly, the hypothalamus) and acting through neural, immune, and endocrine signals (1,28,83–86). These signals might orchestrate the passage from one stage of the life span to the other, thereby timing the entire life cycle, including development, growth, maturation, and aging.

■ The Role of the HPA Axis in Response to Stress

A cornerstone of stress physiology is that widely different types of stress (e.g., physical, social, emotional) induce a series of responses that are mediated through adrenal (both adrenocortical and adrenomedullary) stimulation (83–86). Responses to stress involve the participation of some of the hormones of the adrenal cortex together with those of the adrenal medulla (as discussed in earlier sections). Most animal species so far studied, including humans, show increased glucocorticoid levels in response to stress and administered CRH or ACTH. *With removal of the adrenal gland in experimental animals or with a deficiency of the adrenal cortex in some diseases in humans (i.e., Addison's disease), defense mechanisms against stress fail to take place, and this failure leads to death.*

The glucocorticoid levels in old animals in response to stress may be lower or higher than in young animals, but, in many cases, differences with aging are small or absent. In humans, studies indicate that increased plasma cortisol in response to ACTH is preserved in old individuals. In contrast, the response of DHEA to ACTH appears to be significantly reduced with aging. Similarly, stimulation of aldosterone secretion leading to increased reabsorption of sodium from the urine and to sodium conservation in the body is less efficient in older subjects compared to younger ones (Chapter 18).

In vitro experiments show that corticosterone secretion from isolated adrenal cortical cells of old rats (24 months of age) is less responsive than that of young rats (three months of age) to a synthetic subunit of ACTH and to cyclic adenosine monophosphate (cAMP). Such experiments suggest that the intracellular changes with aging involve impaired steroidogenesis or receptor function, perhaps secondary to telomerase expression (87,88) or increased free radical production and membrane alterations.

Other tests indicate that the feedback mechanisms for ACTH control as well as the stimulatory action of ACTH on adrenocortical secretion are maintained in older men and women. Given the extreme heterogeneity of aging processes in adrenocortical as in many other functions, it appears that some old individuals remain quite capable of allostatic adaptation.

It is notable that in the case of exposure to stress, the activation of the HPA axis has precedence over all other neuroendocrine, hypothalamic, and pituitary functions that are not directly related to stress. Since survival represents the first objective of the stress-endangered individual, the hypothalamus and the pituitary respond by establishing a priority for

BOX 4 *Historical Notes: The "Milieu Interieur," Homeostasis, Allostasis, and Hormesis*

The French physiologist *Claude Bernard* (1813–1878) published, a book on the similarity of requirements for life in animals and plants (74). In this book, he formulated what was to be a landmark in the history of modern physiology, the concept of the "milieu interieur" (internal environment); *the preservation of the constancy of the internal environment is essential to the stability of the living organism, notwithstanding any external change.* He further stated that all vital body functions, varied as they are, act in concert to preserve constant the conditions of life in the internal organization.

Another physiologist, *Walter B. Cannon* (1871–1945), published *The Wisdom of the Body* (75), in which he analyzed the mechanisms of the internal regulation of body activity and coined the word "*homeostasis*" to indicate a relatively stable state (steady state) of equilibrium among the various functions of an organism in its response to changes in the environment. He underlined the importance of the autonomic nervous system, specifically, the sympathetic nervous system in the "fright, flight, or fight" reaction of an individual facing a threat.

In 1948, *Hans Selye* (1907–1982), published the first of several books on stress (76,77), in which he described the important role of the hypothalamo-pituitary-adrenal axis in mediating the responses of the organism to different types of stress in order to preserve the constancy and the stability of body functions. From the efficiency of these two sets of responses would depend the success or failure of the organism to adapt. In the absence of the adrenal gland (e.g., by surgical removal, or pathologic insufficiency), neither of these responses would occur, and the animal would not adapt and die.

Responses to stress could be grouped into three sequential phases as part of a general adaptation syndrome characterized by (*i*) an initial phase in which defense mechanisms are acutely challenged (alarm reaction), (*ii*) a period of enhanced adaptive capacity (stage of resistance) and, (*iii*) the loss of the capacity to adapt (stage of exhaustion). Repeated exposures to stress may either lead to successful adaptation with more efficient ability to withstand subsequent stress or induce pathology, the so-called "diseases of adaptation" (e.g., cardiovascular diseases, immunosenescence) and shorten life.

Among the contemporary investigators, *Roger Guillemin*, a former student of Hans Selye, was the first to identify the hypothalamic source and function of the hypophysiotropic hormones for which he received the Nobel Prize in 1977 with Andrew V. Schally, who described the chemical structure of these hormones, and with Rosalyn S. Yalow, who developed the radioimmunoassay technique for their identification. In 1992, *Robert M. Sapolsky* extended the neuroregulation of stress to include the brain limbic centers (e.g., hippocampus, amygdala) involved in behavior, emotions, and memory (13,78). Glucocorticoids administered in high doses would be toxic, particularly to hippocampal neurons rich in glucocorticoid receptors and cause their death. Hippocampal cells, under no-stress conditions, inhibit corticotropin-releasing hormone (CRH) release from the hypothalamus; therefore, loss of hippocampal neurons would result in increased CRH secretion and consequently of adrenocorticotropic hormone (ACTH) (from the anterior pituitary) and of glucocorticoids (from the adrenal cortex). His "glucocorticoid cascade hypothesis of aging" implicated that the resulting high glucocorticoid levels would be responsible for or contribute to some of the increased pathology of aging.

In 2002, *Bruce S. McEwen*, a neuroendocrinologist, in collaboration with the epidemiologist *Teresa E. Seeman* demonstrated that the cumulative effects of stress may affect negatively the health and longevity of individual organisms (79,80). They stated that the goal of "stability through change" is attained by paying a price: decreased function and increased pathology, the so-called "allostatic load."

Alongside the classical concept of homeostasis and the more recent one of allostasis other studies focus on a more favorable outcome. Thus in 1991, the radiobiologist *Thomas L. Luckey*, comparing the effects of small doses of radiation to those of large doses on animal longevity, demonstrated that small doses prolonged longevity while large doses shortened it. Accordingly, he suggested to add the name of "hormesis" be added to the vocabulary of the effects induced by stress (81). This theme was further developed by several investigators among whom the cell biologist *Gordon J. Lithgow* demonstrated in 2001 that manipulation of insulin/insulin-like growth factor-1 receptor in worms could significantly increase resistance to stress and prolong life (82). The role of hormesis in extending longevity and in increasing resistance to stress seems to be applicable to other animal species (Chapter 3) and continues to be actively studied by many other investigators to maximize the positive effects of stress.

1. an increased secretion of CRH which, in turn,
2. insures a higher secretion of ACTH from the pituitary, and
3. a higher secretion of glucocorticoids from the adrenals.

Simultaneously, sympathetic stimulation, initiated in the hypothalamus, directs the adrenomedullary cells to release more catecholamines. These priorities for stimulation of the HPA axis result concomitantly with an inhibition of the secretion of gonadotropin-releasing hormone, GnRH, and of sexual function in males and females. As shown in Table 6 and Figure 14, under conditions of stress, GHRH secretion is inhibited and body growth impaired.

TABLE 4 Stress, Homeostasis, Allostasis, and Allostatic Load

Stress: threats to physiologic equilibrium (i.e., homeostasis) in the form of internal or external challenges
Homeostasis: an "ideal steady-state" in which a constant internal environment is maintained in order to permit optimal functioning
Allostasis: the process by which an organism actively varies the parameters of its internal milieu to match them appropriately to environmental demands
Allostatic load: the long-term physiological cost to the body stemming from attempts at adaptation (i.e., allostasis). Allostatic load supposedly builds up in a cumulative fashion throughout the life course and affects multiple body systems (e.g., the metabolic, the immune, and the cardiovascular systems)

■ Physiologic Responses to Stress

On the one hand, exposure to a stress elicits physiologic responses that are directed specifically to that stress. On the other, these responses are simultaneously accompanied by a group of responses (e.g., the fright, fight, or flight responses, Table 2) that are always the same, irrespective of the type of stress (Table 3), and depend on stimulation or inhibition by hormonal signals. A consequence of HPA stimulation and increased levels of CRH, ACTH, vasopressin, and cortisol is a concomitant decrease in the secretion of the other hormones originating from the anterior pituitary, particularly a reduction in GH and Gn. Thus, while the stress response produces short-term benefits, it also results in a delay in growth and an inhibition of sexual and reproductive function (Fig. 14). While many of these allostatic responses may be regulated by the HPA axis (79,80), other physiologic systems are involved as well and they may be responsible for some of the consequences of stress (Tables 6–8). Thus, homeostatic competence, as expressed through the HPA axis in response to stress, provides a "panoramic view" of overall physiologic performance (83–88).

As discussed earlier in this chapter, under resting (basal) conditions, few changes occur in HPA function in old age. The nervous, neuroendocrine, and immune systems have many interrelated responses and work together to preserve homeostasis (89–95). However, under stress, evidence of decreased physiologic, competence is plentiful. The increased risk of death following stress in the elderly is well acknowledged and needs no documentation here. Older individuals are less resistant than younger ones to excessively cold or warm temperatures because of the progressive deterioration of thermoregulatory mechanisms that occurs with aging (Chapter 12). Equally diminished is the capacity of older persons to adapt to infections, hypoxia, traumatic injury, excessive exercise, and physical work, all representing types of stress that require complex physiologic adjustments. Emotional stress in the old is also capable of triggering or aggravating a series of physical ailments that, superimposed on an already debilitated state, may contribute to disease and death (Fig. 15).

TABLE 5 Pathophysiologic Responses During and After Stress

During stress
Energy storage ceases because of
↑ Sympathetic activity
(i.e., increased vigilance/arousal)
↓ Parasympathetic activity
↓ Insulin secretion
Use of stored energy is facilitated because of
↑ Glucocorticoid secretion
↑ Epinephrine/norepinephrine secretion
↑ Glucagon secretion
After stress
If adaptation is inadequate, poor health may result (e.g., the body cannot completely restore the loss of stored energy used during the stress response)
Examples of consequences of inadequate adaptation
Muscle wasting
Diabetes (type 2)
Ulcers, colitis, diarrhea
Inhibition of growth (in childhood)
Osteoporosis (in old age)
↓ GnRH, ↓ testosterone

Abbreviation: GnRH, gonadotropin-releasing hormone.

TABLE 6 Functions Stimulated or Inhibited by Physical/Psychological Stress

Functions stimulated by stress	Functions inhibited by stress
All functions immediately necessary for defense and survival are increased	All functions not immediately necessary for defense and survival are decreased
Cardiovascular	Whole-body growth
↑ Cardiac rate	Appetite (anorexia)
Blood pressure	Reproductive function and sex drive
Blood coagulation	Circulation in tissues not involved in stress response
Redistribution of blood from peripheral (skin) and internal systems (gastrointestinal) to heart, skeletal muscles, brain	Response to pain
Respiratory	Immune function
↑ Respiratory ventilation	↓ Thymus size
Metabolic	↓ Thymic hormones and cytokines
↑ Glycogen mobilization	
↑ Glycemia	
↑ Lipolysis	
Hormonal	
↑ CRH, ACTH, and glucocorticoids	
↑ Vasopressin and NGF[a]	
↑ Catecholamines (E and NE)	

[a] Taken as an example of a growth-promoting, paracrine factor (Chapter 6).
Abbreviations: ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; E, epinephrine; NE, norepinephrine; NGF, nerve growth factor.

■ Allostasis or Hormesis? Janus the Two-Faced God

As mentioned in an earlier chapter (Chapter 3), an important indicator of health is the number of illnesses that simultaneously affect the same individual. To capture this dimension of health, comorbidity indices are being utilized to assist in evaluating the so-called "morbidity load" (96). The parameters of one such index are presented in Table 7. A deviation in these parameters from normal values, which may result from repeated exposures to stress, may constitute greater *allostatic load* and put an individual at greater risk for a number of health problems, including cognitive and physical declines (Table 8) (79).

Regarding whether the current measures of allostatic load accurately capture stress experienced over the life course, the evidence is mixed (Box 4). For example, one study investigating this question analyzed a nationally representative data set from

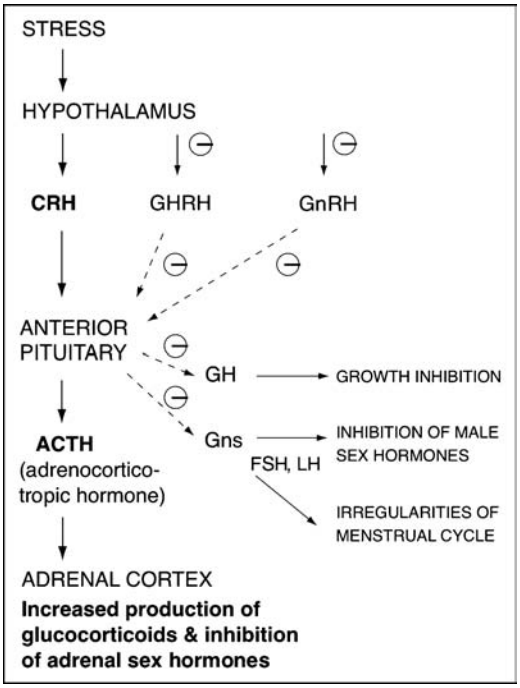

FIGURE 14 During stress, the priorities of the secretions of the hypothalamo-pituitary-peripheral endocrine axes are shifted in favor of the HPA axis. During stress, whereas the HPA axis is stimulated, the secretion of the other hormones is drastically reduced. This shift may explain the decrease in growth and insufficiency of gonadal function during stress. *Abbreviations*: HPA, hypothalamo-pituitary-adrenocortical; FSH, follicle-stimulating hormone; LH, luteinizing hormone (women); GH, growth hormone; CRH, corticotropin-releasing hormone; GHRH, growth hormone–releasing hormone; GnRH, gonadotropin-releasing hormone; Gns, gonadotropins.

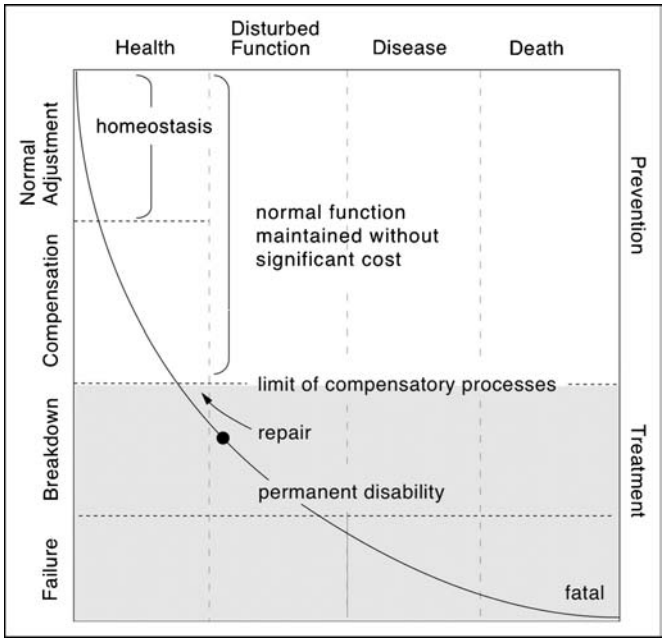

FIGURE 15 Progressive stages of homeostasis from adjustment (health) to failure (death). In the healthy adult, homeostatic processes ensure adequate adjustments in response to stress, and, even for a period beyond this stage, compensatory processes are capable of maintaining overall function without serious disability. When stress is exerted beyond the compensatory capacities of the organism, disability ensues in rapidly increasing increments to severe illness, permanent disability, and death. When this model is viewed in terms of homeostatic responses to stress imposed on the aged, and to aging itself, a period when the body can be regarded as being at the point of "limit of compensatory processes," it is evident that even minor stresses are not tolerable and the individual moves rapidly into stages of breakdown and failure.

Taiwan that included individuals between the ages of 54 to 91. Contrary to expectation, a number of indicators of a stressful life history (e.g., low education, widowhood, living alone, subjective reports of familial stress) were not linked to an allostatic load measure that focused on the neuroendocrine biomarkers (21). *One interpretation of these results is that given a life history of challenge, at older age, the body still retains its ability to maintain homeostasis and respond to new types of stress (97).*

According to the concept of *hormesis*, small (moderate) doses of stress have stimulatory actions that provide benefits for the organism (Box 4) (81). As mentioned in Chapter 3, manipulation of metabolic or hormonal signaling in various animal species increases longevity as well as resistance to stress. Thus, stress may have two different types of influences, positive and negative. This dual action is reminiscent of Janus, the Roman god whose statue was placed outside the gate of large and small Roman cities. One face, smiling and benevolent, looked toward the town, presumably wishing prosperity and health. The other face, frowning and malevolent, looked toward the surrounding countryside, presumably intimidating any would-be attackers. In other words, in humans, a moderate stress of short duration may be protective, whereas a severe stress of long duration may be harmful (Fig. 16).

TABLE 7 Some Parameters Used to Operationalize Allostatic Load

1, 2. Systolic and diastolic blood pressure (indices of cardiovascular activity)
3. Waist-hip ratio (index of long-term metabolic/lipid deposition)
4, 5. Serum HDL and total cholesterol levels (indices of atherosclerotic risk)
6. Blood plasma levels of total glycosylated hemoglobin (index of glucose metabolism)
7. Serum DHEA sulfate levels (index of HPA inhibitor/antagonist)
8. 12-hr urinary cortisol excretion (index of 12 hr integrated HPA activity)
9, 10. 12-hr urinary norepinephrine and epinephrine excretion levels (index of 12-hr integrated sympathetic activity)

Abbreviations: DHEA, dehydroepiandrosterone; HDL, high-density lipoprotein; HPA, hypothalamo-pituitary-adrenal.
Source: Adapted from Ref. 79.

TABLE 8 An Operationalization of Allostatic Load

Elevated physiologic indices (indicating individuals at risk)	
Systolic blood pressure:	≥ 140 mmHg
Diastolic blood pressure:	≥ 80 mmHg
Waist-hip ratio:	≥ 0.94
Total cholesterol/HDL ratio:	≥ 5.0
Total glycosylated hemoglobin level:	≥ 6.5%
Urinary cortisol level:	≥ 25.7 mg/g creatinine
Urinary epinephrine level:	≥ 5 mg/g creatinine
Urinary norepinephrine level:	≥ 48 mg/g creatinine
Lowered physiologic indices (indicating individuals at risk)	
HDL cholesterol level:	≤ 1.45 mmol/L
DHEA level:	≤ 2.5 μmol/L

Abbreviations: DHEA, dehydroepiandrosterone; HDL, high-density lipoprotein.
Source: From Ref. 79.

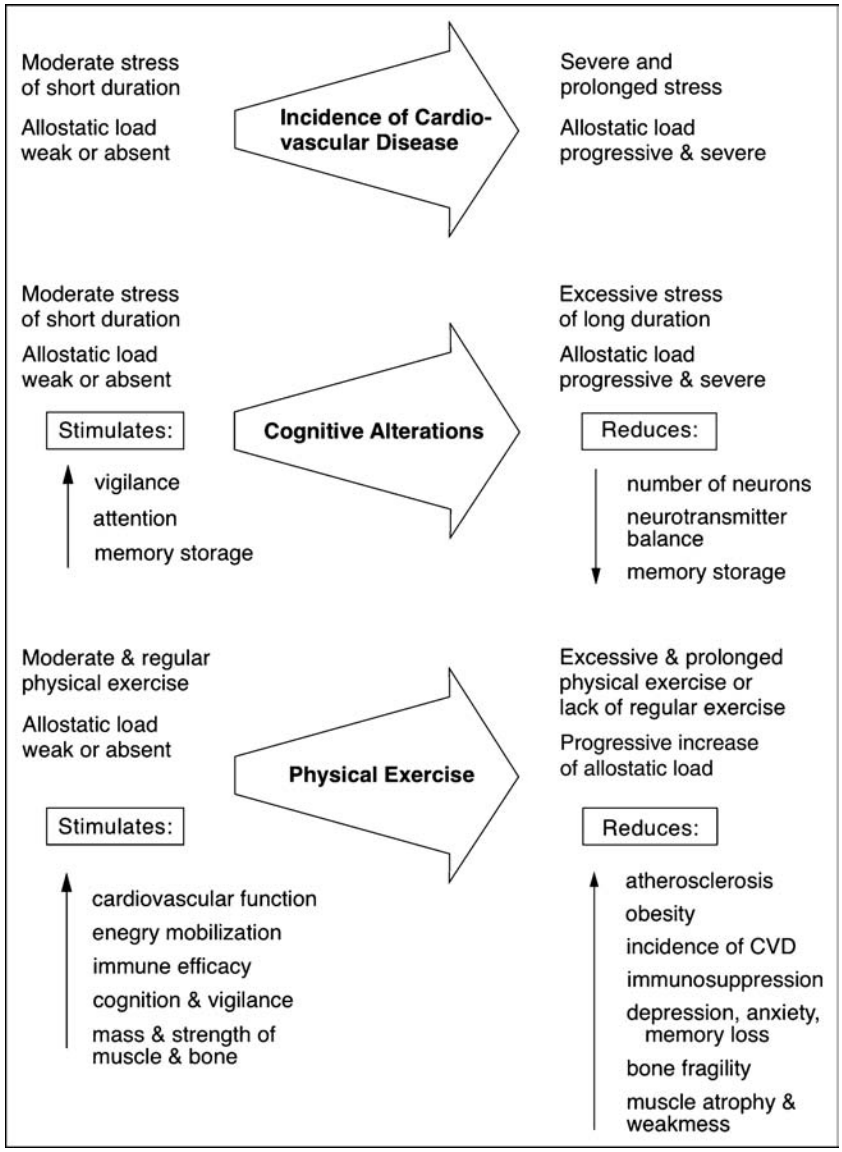

FIGURE 16 Dual action of stress on the incidence of CVD, cognitive alterations, and physical exercise. With moderate stress of short duration, the allostatic load is weak, absent, or may induce beneficial "hormetic" effects (the *left side* of the figure). When stress is severe and prolonged, the allostatic load is progressive and severe, and induces untoward effects, leading to an increase in pathology (the *right side* of the figure). *Abbreviation*: CVD, cardiovascular disease.

Some of the beneficial effects of stress are listed in Table 9. These effects are mediated through several mechanisms such as the (*i*) reduction of free radical production and accumulation (Chapter 5) and the (*ii*) increased production of HSPs. These HSPs act as chaperones by assisting other proteins in the cells to fold in a way that helps in the maintenance of the cells. In addition, HSPs play an important role in immune reactions by modulating antigens and the activation of lymphocytes, cytokines, and natural killer cells (98–104).

■ Strategies for Coping with Stress

Although a number of stress-reducing interventions are often outside of an individual's control (105), *stress management* can still lead to salutary states of adaptation by several approaches: *physical* (e.g., optimal physical exercise, responsible diet, Chapter 24), *pharmacological* (e.g., administration of neurotransmitter agonists, neurotransmitter antagonists, calcium blockers), and *psychological* (e.g., improvement of social networks, finding outlets for frustration, manipulation of feelings). Given the wide heterogeneity among individuals, a wise approach to stress management is a customized set of

TABLE 9 Beneficial Effects of Hormesis[a]

↑	DNA repair
	Chaperones
	Immune-system competence
	Neurologic acuity
	Neuromuscular activity
	Better memory
	Resistance/adaptation to stress
↓	Oxidative stress

[a] Hormesis follows exposure to mild levels and short durations of stress.

hygienic habits that takes into account specific knowledge of both the specific genome and the environment.

In closing, beyond specific techniques for stress management, it is important to remember that the plasticity of our functional responses persists well into old age, and this should give us optimism in our abilities to cope with challenges. It is also important to remember that adapting to our environment is a fundamental and inescapable activity, so, as Selye would say, we ought to try to "learn to enjoy our stress."

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10

Female Reproductive Aging and Menopause

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INTRODUCTION

The process of female reproductive aging in mammalian organisms is unique, very different from aging of nonreproductive tissues and the organism as a whole. Normal aging generally involves accumulation processes: the accumulation of random damage to macromolecular components, DNA, proteins, and lipids and the accumulation of cells within tissues with increasing age. This damage occurs at different rates in different tissues and leads to increased susceptibility to disease and increasing organ dysfunction. In contrast, the female reproductive aging process begins with a full complement of germ cell (oocyte)-containing ovarian follicles around the time of birth. Female reproductive aging is characterized by a steady, inexorable decline in those follicle numbers with increasing age, independent of the fertility or reproductive cycle (e.g., estrus and menstrual) history of the female, until follicles are depleted or nearly depleted.

The depletion of ovarian follicles signals the end of reproductive cycles and reproductive capability and the termination of the female reproductive aging process. Thus, in mammalian organisms, the age at which female reproductive capability terminates appears to be set more by genetic processes, related to the number of ovarian follicles present at birth, and the rate of loss (atresia) of those follicles as the organism ages rather than health-related conditions and could very well explain the discrepancy between increased life span in women over the twentieth century associated with the increasing quality of health care and stable age at menopause.

Environmental processes that are toxic to the ovary, e.g., smoking or chemotherapeutic treatments, further accelerate the rate of ovarian follicle decline and thereby hasten the end of female reproduction. This is not to say that individual organs within the hypothalamic-pituitary-ovarian (HPO) female reproductive axis do not undergo normal aging processes—clearly, they do. In rodents, which along with primates are the most intensively studied species regarding reproduction and the reproductive aging process, age changes in the ovarian steroid-pituitary feedback sensitivity play a role in the female reproductive aging process.

There are two main areas of interest for women in the reproductive aging, or menopausal, process. The first is the loss of fertility. Generally, loss of fertility begins in the mid-to-late 30s, around the same time as the menopausal process starts. Fertility declines at a much faster rate than the rate of transition to menopause and reaches very low levels years before menopause occurs. Thus while women in their early-to-mid-40s may still have regular menstrual cycles, their natural fertility is very low. Many women who wish to have children but postpone childbearing while progressing in their career are very concerned when confronted with this biologic barrier.

The other main area of interest, and the area toward which this chapter is primarily focused, is the increased risk for health problems and conditions that accompanies the transition into postmenopause. It is generally accepted that premenopausal women who are experiencing regular menstrual cycles are protected from the more serious health problems that lead directly or indirectly to increased mortality—cardiovascular disease (CVD) and osteoporosis. For CVD, premenopausal women are at significantly lower risk than men of the same age. As women transition into postmenopause, the gender risk gap narrows considerably until well into postmenopause, where the risk for women and men of the same age is about the same. Risk of fracture due to osteoporosis accelerates in women around the time of menopause and continues to decline well into postmenopause. Menopausal symptoms, such as hot flashes and vaginal dryness, increase across the menopausal transition, although hot flash incidence generally declines in postmenopausal women. With the increasing fraction of older people in the U.S. population, and the realization that women will spend over one-third of their lifetime in this "unprotected" postmenopausal state, this increased risk for serious disease will have a major impact on health-care delivery as society progresses through the twenty-first century.

Knowledge through research has increased substantially over the past several years due to reports from the several large longitudinal studies of the health of women at midlife. Also, because of research results from large randomized, placebo-controlled trials of estrogen therapy over the past few years, we have a much better understanding of the risks and benefits of hormone therapy and concepts of how risks and benefits may differ for younger versus older postmenopausal women.

The focus of this chapter is on the natural history of the menopausal transition in women, with brief comparisons to the reproductive aging process in females of other mammalian species and animal models of menopause (see section entitled Overview of the Female Reproductive Aging in Humans and Other Mammalian Species). Current proposals for staging the menopausal transition, crucial in comparing research results across studies and in informing women and their physicians when menopause might occur for them individually, are reviewed, and a summary of prior and current ongoing longitudinal studies of the menopausal transition is provided (see section entitled Overview of the Female Reproductive Aging in Humans and Other Mammalian Species).

Structural and physiological changes in premenopausal women are summarized in Box 1. Hormonal changes across the HPO axis that occur as the menopausal transition progresses are explored in detail, as are structural and functional changes of the aging ovary and age changes in the hypothalamus and pituitary that impact the menopausal process (see section entitled Hypothalamic–Pituitary–Ovarian Axis During the Menopausal Transition in the Human Female). Current

BOX 1 *Structural/Physiologic Characteristics of the Female Reproductive System*

The major components of the female reproductive tract are the two ovaries, the two oviducts (or Fallopian tubes), the uterus, the cervix, and the vagina. The ovaries, located in the pelvic cavity, are small (in humans, walnut-sized) oval structures containing the germinal cells, the ova, and the endocrine cells, which secrete the two major hormones, estrogen(s) and progesterone. Estrogens are secreted from granulosa and thecal cells lining the follicles and by cells of the corpus luteum; these latter cells are formed at the site of the ruptured follicle at ovulation and produce both estrogens and progesterone. Thecal cells also secrete weak androgens. The ovaries lie on either side of the uterus and are covered by the fimbriae, the fringed ends of the oviducts, the tubes that lead to the uterus and in which fertilization occurs. The uterus serves as a gestation sac for the developing embryo and fetus and the vagina as the receptive organ during intercourse and the birth canal at parturition. The ovary is the primary sex organ, or gonad, and the other structures are secondary sex organs, which also include the breast, or mammary, gland, and the external genitalia (vulva). Dependent on the ovarian hormones are the secondary sex characteristics, which include hair distribution, voice pitch, adiposes tissue distribution, stature, muscle development, and so forth.

The major hormones regulating sexual and reproductive function are operative at four different levels that are all affected by aging:

- In the brain and, particularly, the hypothalamus, the gonadotropin-releasing hormone (GnRH) is a polypeptide secreted into the portal blood vessels, which carry it to the anterior pituitary where it stimulates the synthesis and release of the two gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The midbrain and the limbic system (specifically outputs from the amygdala) sustain ovulation and an increase in LH secretion;
- In the anterior pituitary, the two glycoproteins, FSH and LH, are synthesized and released in response to stimulation by the GnRH, and are also stimulated or inhibited by the positive and negative feedback from the plasma ovarian hormones;
- In the ovary, the major hormones are steroids, estrogens (in humans, the most potent estrogen is 17-β estradiol, followed, in decreasing order of potency, by estrone and estriol), progesterone, and androgens (e.g., testosterone, in very small amounts). Androgens, especially weak androgens, i.e., dehydroepiandrosterone are synthesized by the ovary; this is especially true in the postmenopausal ovary.
- Inhibin is the polypeptide hormone that inhibits FSH secretion. During pregnancy, the ovary also secretes another polypeptide, relaxin.
- In the periphery, ovarian steroid hormones are bound to plasma proteins, metabolized in the liver, and excreted in the urine. At the target cells, estrogens and progesterone follow the same mechanism of action as the other steroid hormones (from the adrenal cortex) and bind to the nuclear receptors to stimulate the RNA and protein synthesis responsible for the many actions of these hormones.

In the female, but not in the male, the reproductive function shows cyclic changes viewed as periodic preparations for fertilization and pregnancy and is associated with underlying cyclic endocrine and behavioral changes. The reproductive cycle of varying length in lower animals is called the estrous cycle; in primates, the reproductive cycle is called the menstrual cycle with ovulation at midcycle.

knowledge of the results from the Women's Health Initiative (WHI) and similar randomized clinical trials is summarized, as well as proposals for the next steps in hormone therapy research (see section entitled The Role of Reproductive Hormones in Menopausal Health Problems and Conditions). The natural history approach is extended to include the development of menopausal symptoms across the menopausal transition and changes in the cardiovascular, metabolic, and skeletal systems as well as changes in brain function leading to cognitive decline and dementia, emphasizing the potential role for several reproductive hormones in the HPO axis, not just estrogen (see section entitled The Role of Reproductive Hormones in Menopausal Health Problems and Conditions). Finally, current recommendations from the U.S. Government and professional societies for estrogen therapy are provided, along with a brief review of current progress in the use of selective estrogen receptor modulators (SERMs), the "hormone" therapy of the future (see section entitled Current Options for Hormone and Hormone-Related Therapy in Menopause).

■ OVERVIEW OF FEMALE REPRODUCTIVE AGING IN HUMANS AND OTHER MAMMALIAN SPECIES

■ Postreproductive Life Span in Mammalian Species

The term "postreproductive life span," as used here, refers to the period of time or fraction of total life span between the end of fertility (or termination of reproductive cycles) and the end of life. The issue of interest in this section of this chapter is that a relatively long postreproductive life span denotes a relatively long period of time during which the adult female mammalian organism continues to live out its life without the capability of reproduction. In women, a relatively long postreproductive life span translates into a long period of life in a condition of increased risk for health problems associated with menopause, such as CVD and osteoporosis. The increased risk is due to the following two factors:

1. Lack of protection from these diseases and conditions in the menopausal women, afforded to the regularly cycling young adult premenopausal women, probably due to hormones associated with menstrual cycling
2. The process of aging

The purpose of this section of the chapter is to take a broad view of female reproductive aging across mammalian species to see if a relatively long postreproductive life span as seen in women is common or relatively unique in mammalian species.

Termination of female fertility in mammals in protected or unprotected environments has been observed in relatively few species and studied in depth in even fewer species. A recent literature survey of studies of 42 mammalian species from eight orders concluded that a postreproductive life span is widespread among mammals (Box 2) (1). Only four of the reported species have a reproductive life span essentially equal to their actual life span—thus lacking a postreproductive life span. Most species observed demonstrate some postreproductive life span, albeit many of these species have a relatively short (10–20% of total life span) postreproductive life span. In only a few diverse species has the termination of fertility and reproductive cycles been documented to occur well before the life span of the organism, resulting in a relatively long postreproductive life span of approximately 25% to 40% of the total life span. There is no apparent direct evolutionary connection among these several diverse species. For example, within the order Primates, human females experience a postreproductive period of approximately 35% to 40% of their average life span; postreproductive life spans in virtually every nonhuman primate studied are short relative to the human (5), with the longest postreproductive life span only about 20% of the maximum life span in captive animals. Within the order Cetacea, the short-finned pilot whale and possibly the killer whale have postreproductive life spans in the wild in the order of magnitude of 40% to 50% of their maximum life spans, in contrast with other whale species (Antarctic minke whale, sci whale, and fin whale) where no postreproductive life span has been observed (1). Finally in the order Rodentia, well-studied strains of female mice (C57Bl/6) and rats (Long-Evans) in the laboratory environment appear to have fractional postreproductive life spans in the order of 40% to 50% of their total life span, with an average 10-to-13 month postreproductive life span (11,12) for an average life span of 26 to 27 months (13).

There is a moderate amount of literature on the topic of evolutionary aspects of menopause, asking questions such as how and why did an extended postreproductive life span evolve in women, and why was reproductive capability not extended as average life span was extended by improved medical care from about 50 years early in the twentieth century to over 80 years today? The interested reader is referred to recent review articles that addresses the various evolutionary hypotheses proposed (14,15).

■ Staging of the Menopausal Transition

The menopausal transition in middle-aged women is a complex process involving substantial changes in reproductive hormone levels and menstrual cycle characteristics compared with premenopausal women. The general hallmarks of the early menopausal transition, which women generally experience in their late 30s or early 40s, are increasing follicular phase follicle-stimulating hormone (FSH) and decreasing inhibin B while cycles remain regular early in the menopausal transition. The late menopausal transition is characterized by the onset of irregular cycles: FSH continues to increase and inhibin B to decline, estrogen levels may start to decline, and luteinizing hormone (LH) levels start to increase. These irregular cycles may be normal cycles that are substantially shorter or longer than a regular cycle, or they may be anovulatory cycles, or women may experience periods of amenorrhea. While most women go through all of these stages, and not necessarily in the simple sequential order of regular to irregular cycles to menopause, some women experience no cycle irregularities and simply transition from regular cycles directly to their final menstrual period (FMP). The median age at natural menopause, defined as 12 consecutive months without a menstrual period, from self-report data from over 14,000 women ranging from 40 to 55 years of age from the Study of Women's Health Across the Nation (SWAN) is 51.4, with only two conditions responsible for lowering the median age of menopause by over a year—current smoking of more than 10 cigarettes/day or a history of heart disease (16).

It is important both for a woman and for her physician, and for valid comparison among research studies into the menopausal process, to know if a woman is in the early or late transition, and if late, whether her FMP might occur in the next year or next three years. To assist in this determination, terms such as premenopause, perimenopause, climacteric, and menopause were defined so that women undergoing the menopausal transition could be categorized in this regard. The most recent World Health Organization Scientific Group on Research in the Menopause definitions of these terms (published in 1996) were overlapping (17) and thus of little apparent benefit. Problems associated with ambiguous definitions of menopausal status or cultural issues involved in a woman considering herself menopausal have been described (18).

Menopause Staging

Currently, various investigators are attempting to define staging categories of menopause based primarily on menstrual bleeding patterns and secondarily on serum hormonal measures. One of the more visible efforts has come from the Stages of Reproductive Aging Workshop (STRAW) (19). Seven stages were defined, ranging from stage –5 (early reproductive) through stage +2 (late postmenopause), with stage 0 indicating the FMP (Fig. 1). Workshop participants recognized that, although most healthy women will follow this pattern progressing from one stage to the next, many "flip-flop" between stages. In rare cases, women will progress from regular cycles directly to menopause (20,21).

To examine the validity of these proposed menstrual cyclicity markers of transition to menopause from STRAW and from similar menstrual flow- or cycle-based menopause staging systems that preceded the STRAW effort (22,23), a subset of TREMIN Trust data (24) were analyzed retrospectively for 193 women with menstrual cycle data from menarche through natural menopause who did not use hormones (including hormonal contraceptives) (25). Increased cycle variability and amenorrhea were predictive of the FMP only in women over age 40. The 60-day or higher intermenstrual interval was deemed the most desirable marker because of both its proximity to the FMP and its ease of determination, thus validating the STRAW marker for the late transition (25).

Is the underlying assumption of the STRAW criteria correct in terms of women progressing through the menopausal transition in a discrete, linear, and orderly fashion? The TREMIN Research Program on Women's Health (this study is described later in this section) reported a lack of uniformity as women progress through the menopausal transition, with a wide variety of perimenopausal stage patterns ranging in

BOX 2 *Animal Models of Menopause*

Most research on the process of female reproductive aging and its connections to the postmenopausal state has focused on rodents or nonhuman primates (NHP), and, generally young adult animals that have been ovariectomized. There are several problems with this approach. First, because the term menopause relates to cessation of menstrual bleeding, rodents do not experience a true menopause because their four-to-five-day estrus cycles lack a uterine bleeding phase. Second, use of young ovariectomized animals, either rodent or NHP, removes the effect of aging and of any continuing involvement of the aging ovary in interactions within or outside of the hypothalamic-pituitary-ovarian (HPO) axis. Because a detailed examination of animal models of menopause is beyond the scope of this chapter, a brief overview will be provided, with references to primary and review articles that the interested reader will find useful in further exploring this topic.

Advantages of the rodent model are

1. the large numbers of animals available for research,
2. the short life span (two to three years),
3. particularly for the mouse, access to transgenic animals affecting specific features of the reproductive aging process,
4. the four-to-five-day estrus cycles involve similar HPO-axis hormonal interactions to humans and other female primates, and
5. a relatively long postreproductive life span to allow exploration of aging changes as well as hormonal changes associated with pathophysiologic processes in somatic tissues.

Disadvantages of the rodent model are

1. for many rodent strains (2,3), initiation of the female reproductive aging process within the hypothalamus-pituitary rather than the ovary as likely occurs in women and some NHP,
2. a prolonged condition of elevated estradiol designated “persistent estrus” in rodents following the transition from regular to irregular cycles, which may be modifiable by transgenic approaches or chemical treatment to accelerate ovarian follicular loss (described later in this section), and
3. differences in the underlying pathophysiologic processes associated with somatic tissues and with menopause in women (e.g., hormone-associated hot flashes, cardiovascular disease, or bone fracture) (4).

The ovariectomized young adult rodent has been used extensively in exploring the role of replacement estrogen to restore the function of somatic tissues and organs associated with postmenopausal health problems, as have other animal species (dog, rabbit, pig, and sheep) to a lesser extent (4).

Several species of NHP, particularly the rhesus monkey, cynomolgus, and baboon, have been proposed as appropriate models of the human menopause (5–8). These animals experience regular menstrual cycles, albeit seasonal for the rhesus, with very similar length and hormonal dynamics as the human female. They go through a menopausal transition at an advanced age with increased follicle-stimulating hormone (FSH), development of irregular cycles, and then termination of menstruation as occurs in middle-aged women. In contrast to women, their post reproductive life span is considerably shorter, with menopause occurring relatively late in their 40- to-50-year life span, and the number of animals available for study is severely limited. The NHP models, including ovariectomized cynomolgus monkeys, develop similar health problems as occur in postmenopausal women, such as serum lipid changes, bone loss, and loss of cognition, thereby offering an excellent model connecting menopause with these health changes.

Over the past five years or so, there has been an interest in developing ovary-intact rodent and NHP models of female reproductive aging. The advantages are that the HPO axis remains intact so that the impact of aging across the full axis can be explored particularly if using middle-aged animals, and this model most closely parallels the naturally menopausal woman. For the rodent, this generally refers to ways to accelerate the loss of ovarian follicles so that ovarian follicles are lost at a younger age, prior to changes in the hypothalamus-pituitary axis, resulting in a female reproductive aging process more similar to the human female. These rodent models may be transgenic mouse models in which the rate of ovarian follicle loss is increased by genetic alteration, or treatment of intact wild-type rats or mice with a follicle-specific ovarian toxicant to accelerate follicle loss (9).

A recent study in cynomolgus monkeys shows promise in using the same ovarian toxicant effective in rodents to hasten ovarian follicle loss in the monkeys (10). If successful in future studies, this technique may result in menopause at an earlier age with an extended postreproductive life span in cynomolgus monkeys.

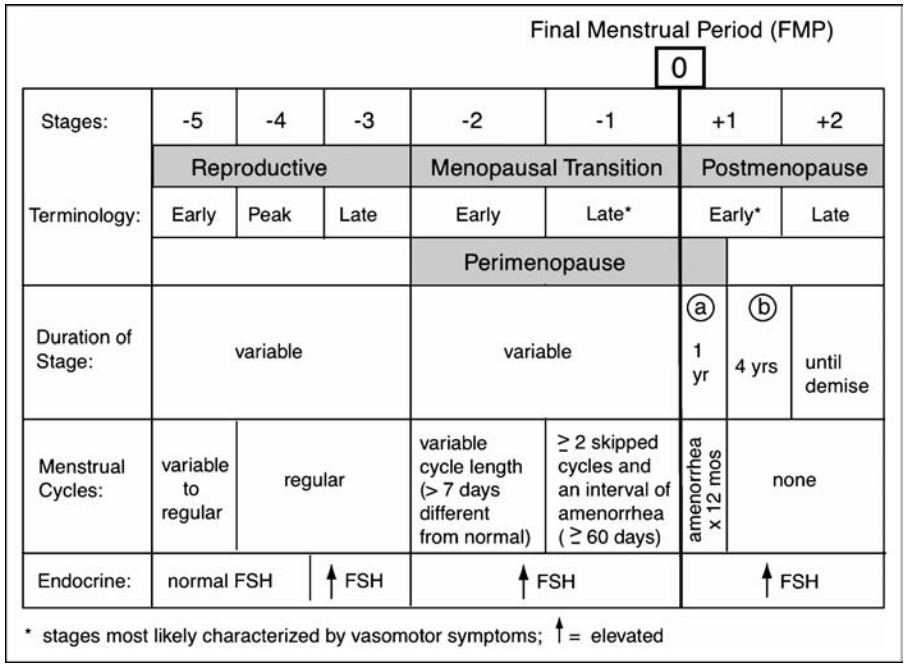

FIGURE 1 Recommended staging system for the menopausal transition from the Stages of Reproductive Aging Workshop. Criteria for staging the menopausal transition from stages –5 to +2 are shown based on menstrual bleeding patterns (regular or indicated characteristics of irregular patterns) and normal or elevated FSH level ["elevated" is defined as an early follicular phase level that exceeds two standard deviations of the mean level for a sample of normal women of peak reproductive age (25–30 years)]. *Abbreviation*: FSH, follicle-stimulating hormone. *Source*: From Ref. 19.

number from 8 to over 20, depending on the analysis (20). The most common pattern was progression from regular cycles to changing cycles to menopause, but some women alternated between regular and changing cycle patterns prior to menopause, while others skipped directly from regular bleeding to menopause. Some women experienced menstrual bleeding after a year or more of amenorrhea.

Another menopausal staging process was recently proposed that is based on measurements of reproductive hormones, particularly estradiol and FSH, in daily urine samples over a 6- to 18-month period from midlife women. The categorization of women according to the stage of menopausal transition is based on both hormonal levels and bleeding patterns (Fig. 2), with a proposed progression of these stages shown in Fig. 3 (26). Full characterization of women according to this staging system is very labor intensive because of the extensive urinary sample collection and hormonal measurements required, particularly for women in stage 3.

Future research into the validity and accuracy of the various proposed staging systems will go a long way in staging

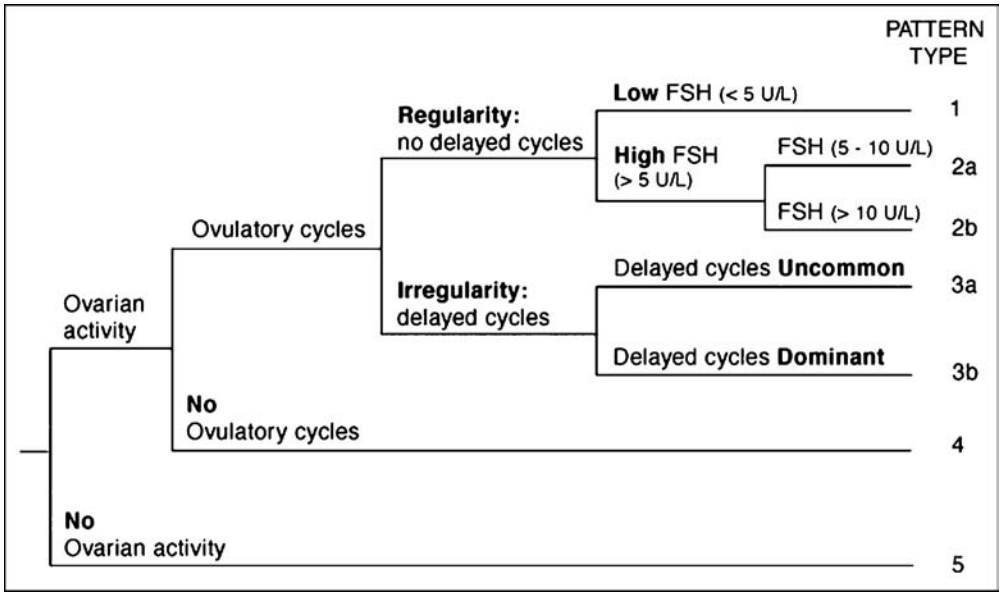

FIGURE 2 Decision tree for staging the menopausal transition. The stage of the menopausal transition for a woman may be approximated based on menstrual bleeding patterns and FSH levels. This scheme is based on urinary hormonal measurements and menstrual bleeding records from a population of 103 women, aged 30 to 58 years. *Abbreviation*: FSH, follicle-stimulating hormone. *Source*: From Ref. 26.

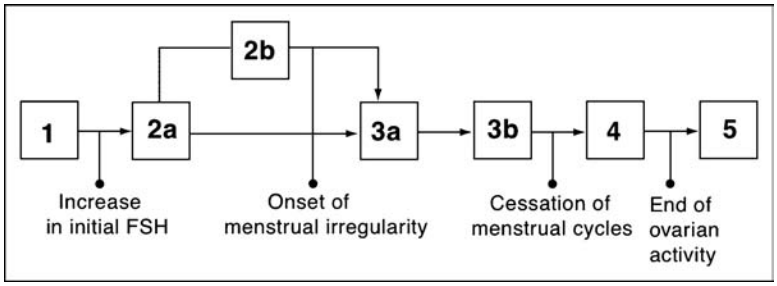

FIGURE 3 Temporal sequence of the endocrine stages 1–5 and episodes (*underneath*) involved in the process of reproductive aging in women. *Abbreviation*: FSH, follicle-stimulating hormone. *Source*: From Ref. 26.

a majority of women traversing the menopausal transition both in predicting the time of their FMP and in being better able to compare findings in women at comparable stages of the menopausal transition.

■ Longitudinal Studies of the Menopausal Transition

Longitudinal studies are crucial to understand the biologic and physiologic mechanisms underlying the transition from pre- to postmenopause, because of the wide variety and unpredictability of patterns of hormonal and menstrual cycle changes that occur in women over this period. There are several ongoing and recently completed longitudinal studies of the menopausal transition that are currently contributing their research findings to the scientific literature. To avoid scattering study descriptions throughout this chapter where findings from these studies are described, a brief description of these studies is provided here (Box 3).

■ Animal Models of Menopause

Despite significant attention of the research community, particularly over the past 10 to 15 years, toward understanding the underlying biological mechanisms of the menopausal process and its connections to health problems and conditions associated with the menopause, many important questions remain. The amount and types of research that can be conducted directly in women are extremely limited for ethical, practical, and financial reasons. Therefore, turning to appropriate animal models will be crucial to shed light on these questions. Animal species that experience a similar menopausal process at a comparable stage of their life history as women present advantages because of the ability to utilize tighter control of genetic constitution and variation in experimental populations and to perform invasive research under controlled experimental conditions. Whether or not a true animal model of the human menopause in all of its ramifications exists will be clear only after extensive comparative studies are performed. Nevertheless, it is possible to answer specific questions about the human menopausal process in animal model systems that exist today or are currently being developed (Box 2).

■ HPO AXIS DURING THE MENOPAUSAL TRANSITION IN THE HUMAN FEMALE

■ Entry into the Late Reproduction and Early Menopausal Transition Stages

Changes in FSH and Menstrual Cycle Length

While still experiencing regular menstrual cycles (Fig. 4), the transition from the peak reproductive stage to late reproductive stage in middle-aged women is marked by a rise in early follicular phase FSH (Fig. 5) (42,43). This transition generally occurs in women aged 39 to 43, with no significant change in LH, estradiol, or progesterone secretion over the cycle. Although a commonly observed feature in studies of the menopausal transition, this rise in early follicular phase FSH is not sufficiently consistent between cycles in the same woman or in different women in this age range to be considered a reliable marker of initiation of the menopausal transition (44,45). The increase in early follicular phase FSH is associated with

- significantly shorter follicular phase length,
- accelerated follicular development (46–48), and
- decreased cycle length (49,50).

This increase in follicular phase FSH resulting in advanced follicular growth in regularly cycling middle-aged women may be due to an earlier rise in FSH relative to younger women in the late luteal phase about four days before menses (50); however, this observation may not be unique to middle-aged women and may also occur in younger adult women (48).

While the association among the observations of the increased follicular phase FSH, earlier follicular phase rise in estradiol, and a shorter follicular phase length had been inferred, a recent study directly demonstrated this association. Using a large database of daily urinary concentrations of FSH, LH, and estrone-3-glucuronide (E1G) from 37 women ranging in age from 30 to 52 years (51), direct evidence was obtained for a gradual decrease in follicular phase length with increasing FSH, with the increased FSH confined primarily to the early follicular phase prior to the initiation of the rise in E1G.

Changes in Serum Inhibin Levels Associated with Increasing FSH

To explore the biologic factors underlying the age-related increase in early follicular phase FSH, investigators focused on inhibin A and B, peptide hormones that are secreted by ovarian follicles and that regulate FSH synthesis and secretion from the pituitary (52). In young, normally cycling women, inhibin B rises and falls in the early follicular phase of the menstrual cycle; inhibin A is low in the early follicular phase, rises at ovulation, and is maximal during midluteal phase (53). Thus, inhibin B is considered to arise from small antral follicles (53) while inhibin A probably originates in the dominant ovulatory follicle (54). The observation that inhibin B, but not inhibin A, levels decline with age early in the menopausal transition (Fig. 6) (43,55) suggests that inhibin B is a key regulator of FSH levels, at least in regularly cycling women early in the menopause transition. This decline in inhibin B probably reflects a diminished follicular pool in older cycling women, either prior to (55) or concomitant with the increase in early follicular phase FSH levels (47,56).

BOX 3 *Longitudinal Studies of the Menopausal Transition*

TREMIN Research Program on Women's Health: The TREMIN Program (27), started in 1934 at the University of Minnesota, is the longest and largest longitudinal study of age changes in menstrual cyclicity. A cohort of 2350 women (cohort I) was recruited to record days of the start and stop of menstrual bleeding. A second group of 1367 women (cohort II) also from the same university was recruited over the period 1961 to 1963. A group of 1000 Alaskan women and girls was added in 1965. In 1984, the program was moved to the University of Utah. In 1990, 505 premenopausal women were recruited into a longitudinal study of the menopausal transition. In 1998, the program moved to Pennsylvania State University.

The Massachusetts Women's Health Study: This was a community-based, longitudinal study of 2569 middle-aged women, age range of 45 to 55 years, from 1982 through 1987 (28,29). The study consisted primarily of six 30-min telephone interviews conducted nine months apart. Each telephone interview involved questions on sociodemographic characteristics, menstrual status, health, and health-care utilization. Three supplemental reviews were completed twice for each woman, 27 months apart, to evaluate social support networks, lifestyle, or help-seeking behavior.

Seattle Midlife Women's Health Study (SMWHS): The SMWHS recruited 508 premenopausal or early perimenopausal women, ages 35 to 55, between 1990 and 1992. Most women were Caucasian (79.4%), with the remainder African American (8.3%), Asian American (8.6%), and Native American (3.3%). After an initial in-person interview, 376 agreed to provide annual data over a three-year period in the form of a questionnaire, daily menstrual calendar, and a health diary. From late 1996 to early 1997, a subset of the original cohort (243 women) began a second phase by providing first-morning voided urine on day 6 of their menstrual period 8 to 12 times per year, and three-day diaries around the collection period from late 1996 to 2001, and quarterly from 2001 through 2006 (30,31).

The Melbourne Women's Midlife Health Project (MWMHP): This community-based cross-sectional and a longitudinal study (32) of middle-aged women focused on the menopausal transition (33,34). The cross-sectional study began in 1991 with 2001 Australian-born women living in Melbourne aged 45 to 55 years. A one-time telephone interview obtained information on menstrual and health status, sexual functioning, use of health services, health-related behaviors, and sociodemographics. A subgroup of 438 women (with menses in the prior three months but no surgical menopause nor hormone replacement therapy) participated in the longitudinal study; they were interviewed annually in their homes on moods, symptoms, sexual functioning, and lifestyle using instruments similar to those used in the Massachusetts Women's Health Study. Blood samples were taken between days 4 and 8 for cycling women, or after three months of amenorrhea (i.e., absence of menses), for fasting glucose and cholesterol, sex steroids, follicle-stimulating hormone (FSH), sex hormone–binding globulin, and inhibin, as well as body composition measures. Those with continuing menstruation completed menstrual diaries between annual interviews.

The Study of Women's Health Across the Nation (SWAN): This is a multiethnic, longitudinal, population-based study of the menopausal transition in women aged 42 to 52 on entry (35). The study addresses the physical, biological, psychological, and social changes during this transitional period (36). A cross-sectional, 15-minute survey of 16,063 women aged 40 to 55 was conducted in person or by telephone and eligible premenopausal women were enrolled into the longitudinal study. At baseline in 1996, 3302 women of five ethnic/racial groups were recruited into the longitudinal study (1550 Caucasians, 935 African Americans, 281 Japanese, 250 Chinese, and 286 Hispanic). Criteria for entry were at least one menstrual period in the previous three months and not currently using estrogens or other medication known to affect ovarian function. There are seven study sites around the United States. Physical measures and fasting morning blood are taken annually at these sites and questionnaires are completed. Data is collected in five major areas: bone mineral density and body composition, cardiovascular measures and risk factors, ovarian hormonal markers, psychosocial factors (such as quality of life, depression, stress, social support, and life events), and epidemiologic issues (e.g., education, income, occupation, marital status, medical history, and diet). Women are given menstrual calendars to complete monthly over the coming year. By 2009, 11 annual visits will have been completed.

The Daily Hormone Substudy (DHS) of SWAN enrolled 848 participants from all seven study sites. The purpose of this substudy is to collect and analyze cycles of daily urinary hormones annually, providing both cross-sectional and longitudinal data (17). Women were instructed to collect first-void urine specimens starting on the first day of menstrual bleeding and end on the first day of bleeding of the subsequent cycle, or after 50 days, whichever came first. These daily urine specimens were analyzed for luteinizing hormone (LH), FSH, estrone conjugates (E1c), and pregnanediol glucuronide (PDG).

The SWAN biorepository contains blood and urine specimens collected at each participant's annual visit, as well as DHS urine specimens and DNA from about 1500 participating women (37). These specimens are available to qualified researchers.

The Penn Ovarian Aging Study: This is a longitudinal, population-based study of hormonal, clinical, behavioral, and demographic factors associated with the menopausal transition in women living in Philadelphia (38,39) that began in 1996 to 1997. Of the African American or Caucasian

(Continued)

BOX 3 *Longitudinal Studies of the Menopausal Transition (Continued)*

women between the ages of 35 and 47 years invited to participate, with menstrual cycles in the normal range for the previous three months and at least one intact ovary, 218 in each racial group agreed to participate. There were six assessment sessions at approximately eight month intervals over four years and a seventh assessment one year later. Each assessment involved blood collection for hormone (sex steroids, dehydroepiandrosterone sulfate, FSH, and LH) measurements taken at two visits, each visit scheduled within the first six days of the menstrual cycle, one month apart. Each visit also included anthropometric measures and completion of standardized questionnaires to collect information on demographics, menstrual history, menopausal symptoms, general health, medical care, and social support.

The Biodemographic Models of Reproductive Aging (BIMORA) project: The BIMORA project (40) at the University of Washington recruited 156 women, age ranging from 26 to 58 years, from the TREMIN Research Program on Women's Health Study, either from cohort II or from younger women recruited after cohort II (41). These women provided daily urine specimens and menstrual cycle data for one six-month interval (mid-January through mid-June) per year over five years from 1998 through 2002. Urine specimens analyzed for estrone-3-glucuronide (E1G), PDG, LH, and FSH levels provide longitudinal age-related hormonal data along with menstrual cycle data.

No Apparent Role for Estradiol in FSH Increase Early in the Menopausal Transition

While one might anticipate that estradiol secreted from the dominant follicle may play a key role in the negative feedback regulation of FSH levels, this does not appear to be the case. Although some studies do not report changes in estradiol over this early segment of the menopausal transition relative to young adult controls (54,57,58), other studies conclude that estrogen levels fluctuate widely across the menopausal transition and are often increased in comparison with earlier stages of reproductive life. A meta-analysis from nine studies reporting follicular phase estradiol levels in perimenopausal women relative to premenopausal controls showed significantly higher average levels in the perimenopausal women (59). Data from women in the Melbourne Women's Midlife Health Study showed the wide fluctuation of follicular phase estradiol levels, with some showing extraordinarily high levels even in women without a period for over three months (34). Two small-to-midsize studies of regularly cycling perimenopausal women in their 40s, one the longitudinal Biodemographic Models of Reproductive Aging (BIMORA) study (41) described in Box 3, Overview of the Female Reproductive Aging in Humans and Other Mammalian Species, showed increased urinary E1G (Figs. 7 and 8) (41,49). Furthermore, in a study of regularly cycling women aged 20 to 50 years, inhibin B, and not estradiol, was a

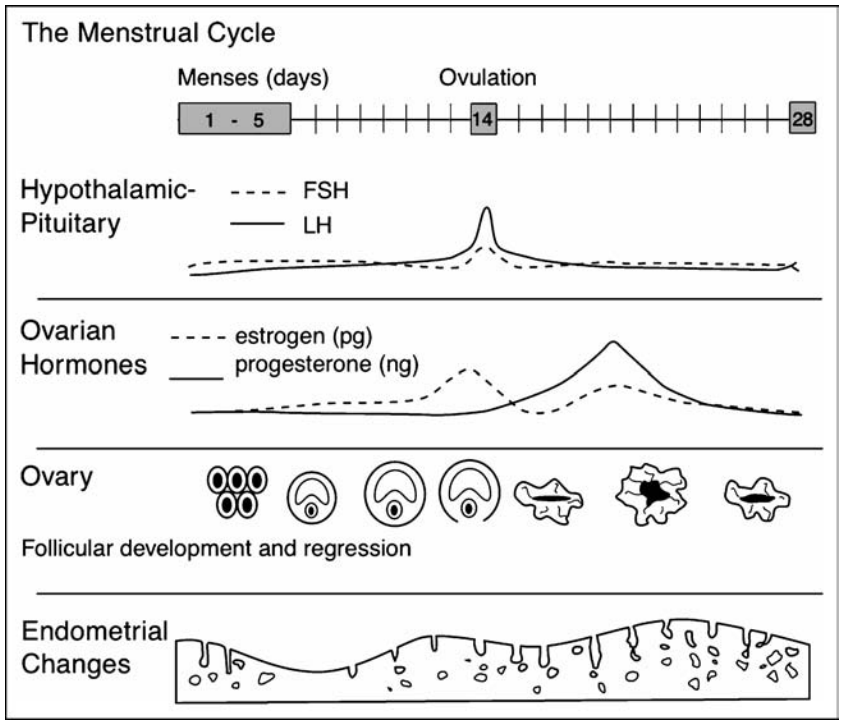

FIGURE 4 Cardinal changes that occur in the normal human menstrual cycle. Follicle growth is accompanied by cessation of menses and a rise in estradiol. Shortly after peak estradiol is attained, a midcycle surge of LH and FSH occurs and initiates the process of oocyte maturation and ovulation. The follicle then forms a corpus luteum and secretes progesterone and estradiol, in an approximate bell-shaped curve, over the next 14 days of the cycle. Gonadotropin levels are at their lowest in the luteal phase of the menstrual cycle. *Abbreviations*: FSH, follicle-stimulating hormone; LH, luteinizing hormone. *Source*: From Ref. 17.

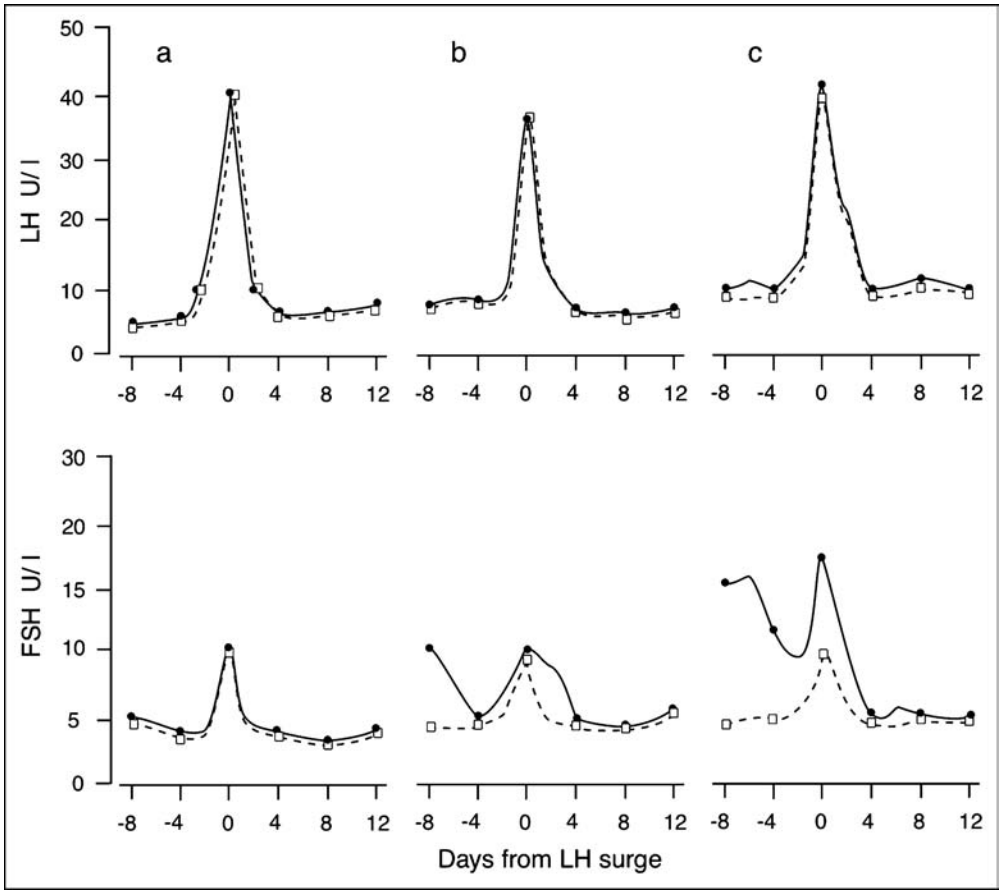

FIGURE 5 Daily serum levels of LH and FSH in control (young) and middle-aged women with regular menstrual cycles. Mean values are shown from 41 women aged 24–35 years (□, the control group, profiles repeated in each section) with (**a**) 19 women aged 36 to 40 years; (**b**) 18 women aged 41 to 45 years, and (**c**) 16 women aged 46 to 50 years (all shown as •). The data points shown in this figure are a small subset of the total data points provided in the original figure. *Abbreviations*: FSH, follicle-stimulating hormone; LH, luteinizing hormone. *Source*: From Ref. 42.

significant predictor of early follicular phase FSH (56). Thus, if estrogen provides important negative feedback control for FSH at this early stage of the menopausal transition, the well-documented rising FSH levels should be associated with declining estradiol levels, but this clearly is not consistent with the data.

Little to No Change in Progesterone, Inhibin A, or LH

Most studies report no change in serum progesterone or urinary pregnanediol glucuronide in women aged 40 to 50 years with regular cycles (42,54,57). Likewise, most studies report no change in inhibin A early in the menopausal transition in regularly cycling middle-aged women with elevated FSH (47,55,60), with one study actually reporting an increase in inhibin A (43) at this early stage of the transition (Fig. 6). In a group of studies that measured LH levels in middle-aged cycling women with elevated FSH, some report small but significant increases in LH (42,49,57) while others report no change (54,58) relative to normal levels in young premenopausal women. One study reports an increase only in older (age 46–50) women in the study (42).

Compensatory Process to Maintain Estrogen and Inhibin A Levels

In view of the declining numbers of primordial and developing ovarian follicles during the menopausal transition (described later in this section), is there a compensatory process to maintain adequate follicular secretory capacity of estradiol and inhibin A in older ovulatory women? To address this question, recombinant FSH was administered to younger (20–25 years) and older (40–50 years) cycling women following pituitary suppression with a gonadotropin-releasing hormone (GnRH) agonist (61). No significant difference was reported in serum estradiol or inhibin A between the two groups despite greater numbers of large follicles in the younger group. Increased aromatase (or estrogen synthetase: the enzyme that synthesizes estrogen from androgen) activity in medium-to-large follicles in older women may account for the sustained estradiol production in older women, despite lower follicle numbers and androstenedione levels in the older women (62). The capacity of the ovary to secrete androgen declines with age in young and middle-aged cycling women, although estradiol levels remain unchanged (63).

■ Entry to the Late Menopausal Transition and Menopause

Development of Irregular/Anovulatory Cycles Late in the Menopausal Transition

According to the STRAW definitions for menopausal staging, women in the early menopausal transition experience elevated FSH with some menstrual irregularity, up to one skipped cycle or interval of amenorrhea of less than 60 days. Cycles in most

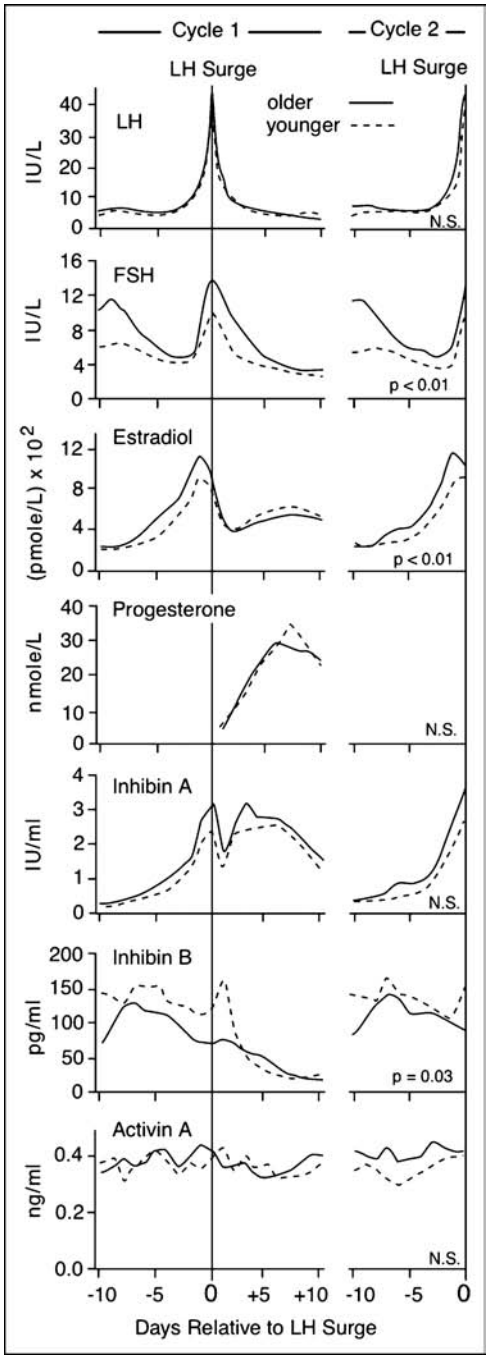

FIGURE 6 Daily serum levels of LH, FSH, estradiol, progesterone, inhibin A, inhibin B, and activin A normalized to the LH surge. Results are shown from two consecutive cycles in regularly menstruating older subjects (40–45 years, $n = 16$) and younger controls (20–25 years, $n = 13$). Mean values are shown. The actual data points are not shown but can be seen in the original publication. *Abbreviations*: FSH, follicle-stimulating hormone; LH, luteinizing hormone. *Source*: From Ref. 43.

women through the late reproductive stage into the onset of the early menopausal transition generally remain within the 21- to 35-day span, tending to alternate slightly above and below a constant mean duration, with deviations as low as 14 days or as high as 56 days seen no frequently than once in 20 cycles (33). Transition to the next stage of menopause, the late menopause transition, occurs with menstrual irregularity in excess of that, i.e., two or more skipped cycles and interval of amenorrhea of 60 days or more in the context of elevated FSH. Usually within three to five years of the FMP, cycle length becomes increasingly longer on average and more variable. Two cycle types were noted in a study of 13 women during this late menopausal transition stage: either normal length ovulatory cycles or presumed anovulatory cycles with a prolonged follicular phase resulting in a failure to detect the luteal phase rise in progesterone during the study's four-week collection period (64). These presumed anovulatory cycles, at least some of which could be due to an extended follicular phase resulting in prolonged cycles (Fig. 9) (65), were not seen in any of the women in the study prior to 27 cycles from the FMP, and the proportion of these cycles increased toward the FMP, reaching 62% in the final 10 cycles. The SWAN study also reported increased anovulatory cycles lacking a luteal phase in their perimenopausal cohort, more likely to be observed in women 49 years old and above with variable cycle length. Of 833 cycles for which daily hormonal data were available from urinary specimens, 159 (19.1%) lacked evidence for luteal activity (66).

Hormonal Changes Associated with Late Menopausal Transition

The declining inhibin B in the early menopausal transition continues to decline, reaching steady state at the lower limit of sensitivity in inhibin B assays at about a year before the FMP (44,60). Reproductive hormones progesterone, inhibin A, and LH, which changed little, if at all, in the early menopausal transition, decline substantially in the late transition. Progesterone (65,67) and inhibin A (44) decline as cycles become irregular and/or anovulatory, and LH levels increase. (42,58,64) Estrogen levels are maintained until about a year or two before the FMP (Fig. 8), then decline (44,58).

The rise in follicular phase FSH seen in the late reproductive phase and early menopausal transition accelerates as women enter and pass through the late menopausal transition (44,68). This may indicate further release of the negative feedback regulation of FSH by estrogen and inhibin A, as estrogen and inhibin A levels decline in the late transition.

■ Change in Ovarian Function and Structure Across the Menopausal Transition

Folliculogenesis and Follicular Development

Menopause occurs when the ovary is essentially deplete of follicles. According to the current dogma regarding ovarian follicular development in mammalian organisms, the ovary around the time of birth contains all of the follicles it will ever have, hundreds of thousands of primordial follicles in the human female, with follicles depleted, not added, from that time forward (69,70). Follicles are continuously and randomly selected from the primordial follicle pool, by unknown processes, to initiate growth throughout the woman's life span prior to menopause, even during childhood, pregnancy, and while using contraceptives (71). Over 99% of these randomly developing follicles die by atresia through the molecular process of apoptosis (72). Most of these follicles die by an early developmental stage because they develop during the life stage or menstrual cycle phases when FSH levels are low and never enter the monthly (for the human female) growing cohort. In women, a single dominant follicle is formed in each ovulatory menstrual cycle (73) from a cohort of antral follicles that reach about 2 to 5 mm in diameter as follicular phase FSH increases (74,75). The other follicles in the monthly recruited cohort die by atresia. Thus, with 400 to 500 menstrual cycles in a woman's reproductive life span, less than 0.1% of the primordial follicles

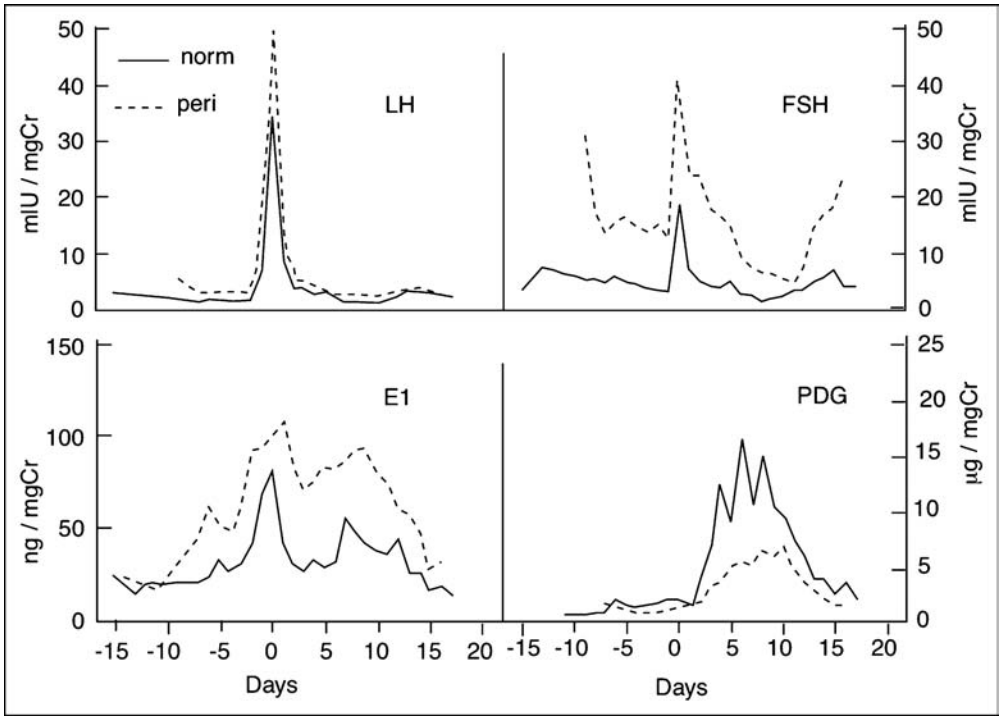

FIGURE 7 Daily urinary gonadotropin and sex steroid excretion patterns in perimenopausal women, age range 43 to 52 years, with regular menstrual cycles (- - -) compared to those in young, normal midreproductive women, age range 19 to 38 years (—). Data are standardized to day 0, the putative day of ovulation, and expressed as mean values. The actual data points are not shown but can be seen in the original publication. *Abbreviations*: E1c, estrone conjugates; FSH, follicle-stimulating hormone; LH, luteinizing hormone; PDG, pregnanediol glucuronide. *Source*: From Ref. 49.

present at birth go on to release ova and form corpora lutea in the luteal phase of the cycle. According to this current dogma, ova released in the late reproductive stage as menopause approaches were present in the woman's ovary for around 50 years (76). An alternative process has recently been proposed for producing oocytes and ovarian follicles in mice (77,78) and humans (79) from germ-line stem cells that continue to seed the ovary in the adult. Further developments will be watched with interest.

Depletion of Ovarian Follicles Leads to Menopause

The total number of follicles in the mammalian ovary declines continuously with increasing age. A log-linear decline was reported with increasing age, with an acceleration in the log-linear rate of follicular loss for women in their mid-to-late 30s with about 25,000 primordial follicles still remaining (Fig. 10) (80,81). At this accelerated rate, follicles are projected to be depleted in women in their early 50s, consistent with the average age of menopause. The age at which the apparent acceleration in follicular loss occurs (mid-to-late 30s) is roughly consistent with the age at which

- early follicular phase FSH levels begin to increase above young adult premenopausal levels and
- fertility begins its abrupt decline.

Changes in Ovarian Size and Structure During the Menopausal Transition and into Postmenopause

With the declining number of growing ovarian follicles in women approaching menopause, total ovarian volume declines as well. In fact, ovarian volume starts to decrease with age in women over the age of 30, with the most rapid decline beginning in the early 40s (82,83). In postmenopausal women, ovarian volume is related to age, years since menopause, and parity (84,85), with a positive association reported for obesity (83). Ovarian volume was smaller in postmenopausal women up to age 59 on estrogen therapy relative to those not taking estrogen, possibly due to the smaller tropic effect of reduced gonadotropin levels, but there was no suppressive effect of estrogen on ovarian volume in women over 60 years of age (82). Predictive equations for ovarian volume as a function of age, pre- or postmenopausal status, and estrogen therapy status provide a way to diagnose ovarian tumors by comparing measured ovarian volume with a calculated age-dependent normal value (86).

Following the virtual depletion of follicles from the postmenopausal ovary, three main cell types remain as the ovary becomes smaller and increasingly fibrotic with increasing age (87,88). These are secondary interstitial (SI) cells, residual cells from the theca interna portion of atretic or ovulated follicles that survive in the ovary; hilar interstitial cells, large steroidogenic cells with structural and functional characteristics similar to Leydig cells of the male testis; and stromal fibroblasts. Both SI and hilar cells can secrete androgens in response to LH stimulation in the premenopausal ovary, but whether that capacity remains in the postmenopausal ovary continues to be explored.

Changes in Capability for Ovarian Hormonogenesis During the Transition to Postmenopause

Gonadotrophin-responsive ovarian follicles in pre- and perimenopause are the primary source of estrogen and the peptide hormones, inhibin A and B. As follicles are depleted during the woman's reproductive life span, the capacity of the ovary for

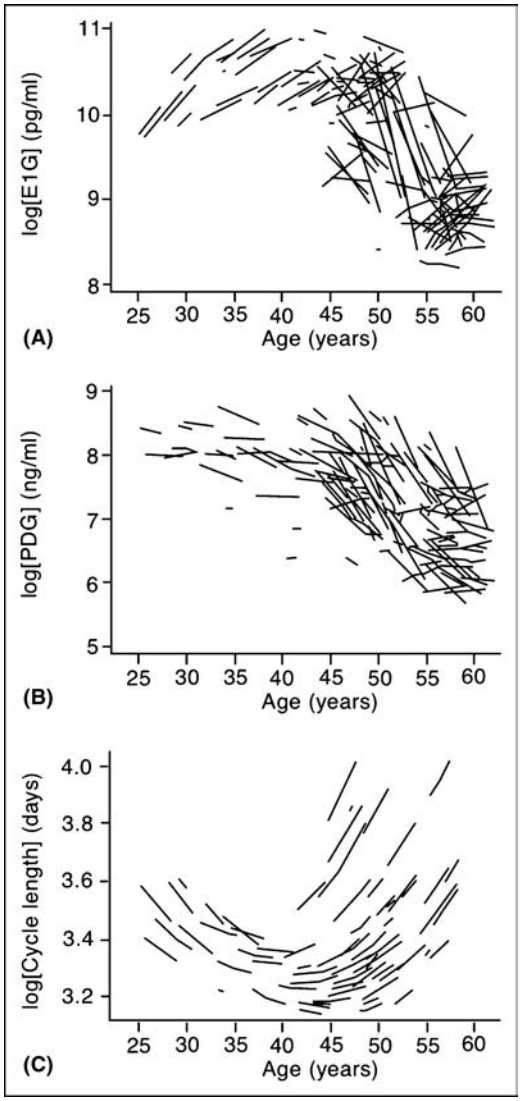

FIGURE 8 Estimated trajectories of steroid hormones and menstrual cycle lengths for individual women. Women collected daily urine specimens and information on menstrual bleeding for a total of six months in each of the five study years. The average log E1G or PDG per week was calculated for each woman, then graphed over the number of years the woman participated in the study and her age range during that period. Log-transform values used in the individual-level quadratic model are presented here; approximate nonlog values are given below. (**A**) Mean E1G per woman-week (n = 145 women), (**B**) mean PDG per woman-week (n = 145 women), and (**C**) menstrual cycle length (n = 89 women) with age. E1G, 8 = 3000 pg/mL (6.4 nmol/L), 9 = 8100 pg/mL (17.3 nmol/L), 10 = 22,000 pg/mL (50 nmol/L), 11 = 60,000 pg/mL (128 nmol/L); PDG, 5 = 150 ng/mL (302 nmol/L), 6 = 400 ng/mL (806 nmol/L), 7 = 1100 ng/mL (2215 nmol/L), 8 = 3000 ng/mL (6041 nmol/L), 9 = 8100 ng/mL (16,311 nmol/L); cycle length, 3.2 = 24.5 days, 3.4 = 30.0 days, 3.6 = 36.6 days, 3.8 = 44.7 days, 4.0 = 54.6 days. *Abbreviations*: E1c, estrone conjugates; PDG, pregnanediol glucuronide; E1G, estrone-3-glucuronide. *Source*: From Ref. 41.

making estrogen and the inhibins declines to undetectable levels, even in the face of increasing levels of pituitary gonadotropins. Any residual follicles in the postmenopausal ovary, as reported occasionally (89), must be refractory to the hypogonadotropic environment. However, with follicles virtually depleted, are there other remaining ovarian cells or structures such as regressed corpora lutea or atretic follicles that retain steroidogenic capability?

The question of steroidogenic capacity of the postmenopausal ovary has clinical relevance because of (*i*) the potential for continued androgen, and possibly estrogen, secretion from the postmenopausal ovary, (*ii*) the potential ability of sex steroids to protect women from menopause-related declines in cognition, bone density, muscle mass and strength, and libido, and (*iii*) the high rate of elective oophorectomies—it is estimated that there are about 600,000 hysterectomies performed each year in the United States alone on women over the age of 40, with about 50% to 60% of these women electing oophorectomies with the expectation of avoiding ovarian cancer (88). If the postmenopausal ovary continues to secrete significant amounts of androgens, then oophorectomized postmenopausal women may be subject to androgen deficiency and, therefore, would need to consider androgen therapy to reduce risk for androgen-related menopausal health problems.

Early studies of steroidogenic capacity and gonadotropin-binding capacity of the postmenopausal ovary concluded that the postmenopausal ovary continues to make androgen (90,91), and in some cases estrogen (90–92), and to contain both gonadotropin receptors and to respond to gonadotropins (91,93). The ovarian tissue/cellular sites with these capabilities were primarily the cortical stroma and hilus. Aromatase was detected by immunocytochemistry in three out of seven postmenopausal ovaries, but no aromatase-positive stain was seen in seven perimenopausal ovaries (94). Two review articles written in the mid- and late 90s (95,96) concluded that the postmenopausal ovary is an androgen-secreting gland responsive to gonadotropins, arguing against dismissing the postmenopausal ovary as a redundant organ unresponsive to the endocrine environment.

A study published in 2001 and almost every study asking this question since then, arrived at the opposite conclusion, i.e., that the postmenopausal ovary does not make significant amounts of sex steroids. This study reported that (*i*) postmenopausal women with complete adrenal insufficiency, thus lacking adrenal steroids, have no circulating androgens, (*ii*) dexamethasone administration to postmenopausal women (ages 50–75 years; n = 10) with intact adrenals dramatically decreased serum androgen levels, (*iii*) intraovarian androgen levels were negligible, (*iv*) immunocytochemistry for aromatase and androgen biosynthetic enzymes showed that these steroidogenic enzymes were either absent or present in very low amounts in postmenopausal ovaries, and (*v*) gonadotropin receptors were completely absent in postmenopausal ovaries (97). The authors conclude that serum androgens in postmenopausal women come from the adrenal, not from the ovary. Subsequent studies have generally confirmed this conclusion. Stroma from the postmenopausal ovary does not express significant levels of P450c17 mRNA or protein, as required for androgen biosynthesis (98), or aromatase mRNA (99) for estrogen biosynthesis, but it does express steroidogenic capacity favoring δ^5 steroid (δ^5-androstenediol) formation over δ^4 steroid (androstenedione or testosterone) formation (100). However, direct measurement of testosterone and free testosterone in over 1400 women aged 18 to 75 years showed that for women aged 55 years and older, those with bilateral oophorectomy (n = 27) had significantly lower levels than intact women (n = 183), thereby suggesting in this study that the ovary is a source of testosterone at least until age 75 (101).

Whether the postmenopausal ovary secretes significant amounts of androgen is an important clinical question that bears on the health and well-being of older women and is worth additional focus in order to reach consensus. There may be interindividual variation in the ability to synthesize and secrete

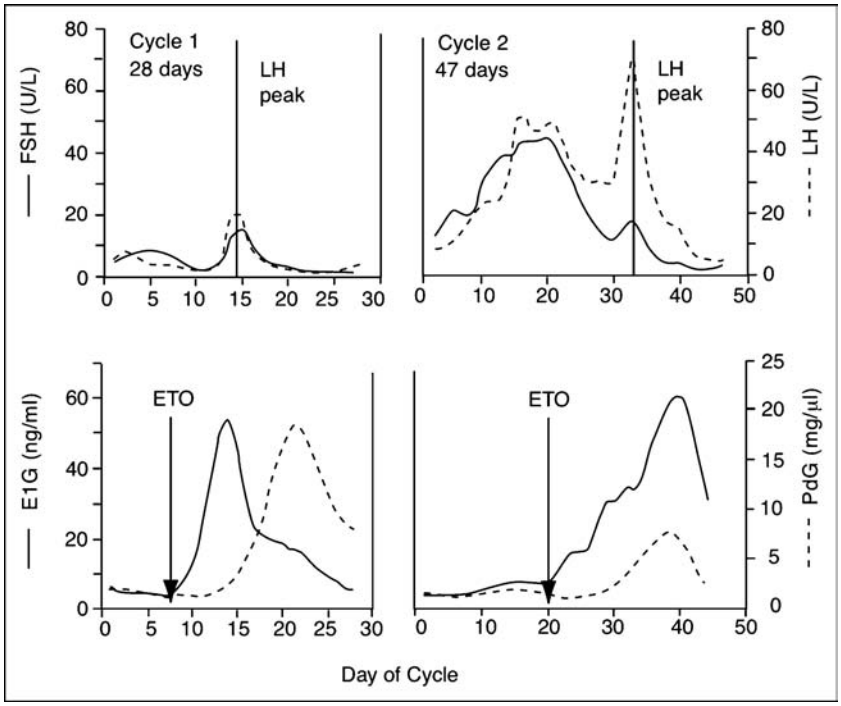

FIGURE 9 Effects of the elongation of the menstrual cycle during the menopausal transition: two menstrual cycles from a woman with menstrual irregularity. Gonadotropin and sex steroid metabolites were measured in daily urine samples. The upper panels show FSH and LH profiles, the lower panels E1G and PDG. Cycle 1 is a 28-day cycle. Cycle 2 is a 47-day cycle. ETO is the "E1G take-off," defined as the time taken from day 1 of the cycle to the start of the first sustained rise in E1G. Comparatively, the 47-day cycle shows delayed ETO, higher FSH before ETO, lower luteal PDG, and higher E1G. *Abbreviations*: FSH, follicle-stimulating hormone; LH, luteinizing hormone; E1c, estrone conjugates; E1G, estrone-3-glucuronide; ETO, E1G take-off; PDG, pregnanediol glucuronide. *Source*: From Ref. 65.

significant amounts of androgen due to ovarian stromal hyperplasia (88), genetic or environmental differences, or even different reproductive histories in which the content of ovarian SI cells or resorbed luteal cells, for example, may vary in women who cycle continuously, are frequently pregnant, or are on oral contraceptives during their reproductive life.

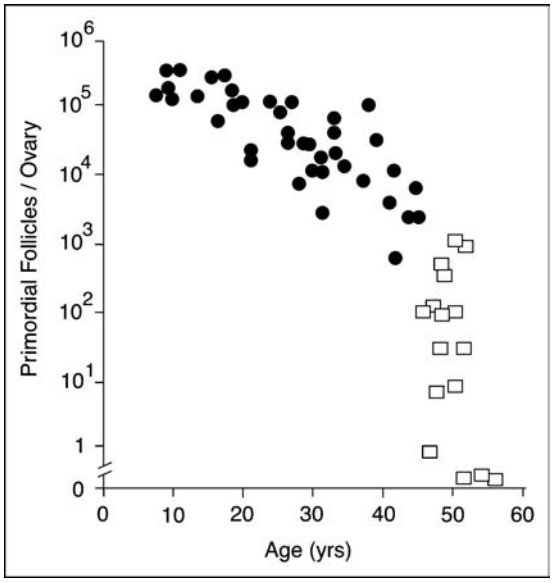

FIGURE 10 Decline in the number of primordial follicles in the ovary with advancing age. Primordial follicles were counted in ovaries obtained postmortem or following surgical oophorectomy from girls and women in the age range of 7 to 55 years. The follicle number decreases in a log-linear fashion up to about 40 years (•); follicle depletion appears to accelerate in the decade preceding menopause (□). *Source*: From Ref. 80.

■ Changes in Hypothalamic and Pituitary Responses Across the Menopausal Transition

The hypothalamus, under control of higher brain centers, releases pulsatile GnRH through the portal vein to the pituitary. Pituitary gonadotropes respond to the pulsatile GnRH signal by secreting gonadotropins, LH and FSH, into the general circulation in a correspondingly pulsatile manner (Box 1). There are two main areas of interest for exploring changes in gonadotropin pulsatility (pulse frequency, width, and amplitude) across the menopausal transition. The first is related to the very well-documented increase in follicular phase FSH that occurs as an early sign that a woman, usually in her late 30s or early 40s, is entering the menopausal transition. Earlier studies have shown that a strong determinant of relative circulating levels of FSH and LH is GnRH pulse frequency, such that a relative decrease in GnRH pulse frequency causes an increase in FSH and decrease in LH (102,103). A slowing of the GnRH pulse frequency preceding, or at least occurring around the same time as, the increased follicular phase FSH would support the argument that changes in brain function may be involved in initiating the menopausal transition.

The second area of interest relates to altered sensitivity to the steroid-gonadotropin feedback relationships that may occur with advancing age or because of changed dynamic hormonal interrelationships across the menopause transition. With reduced feedback sensitivity of estradiol on LH secretion in middle-aged perimenopausal women, for example, the

follicular phase estradiol peak may not trigger the LH surge and subsequent ovulation.

Pulsatile GnRH and Gonadotropin Secretion in Early Perimenopause

Direct investigation of GnRH pulsatility changes in women by current methods is impossible. Access to the brain would be required because serum GnRH levels are undetectable. It is generally accepted, however, that measurement of serum LH pulses, usually over an extended period of time (8–12 hours) in frequently sampled serum (sampled every 10 or 20 minutes) in regularly cycling women, is a valid surrogate for GnRH pulsatility (103). Direct measurement of FSH pulses is more difficult due to the lower amplitude of FSH pulses and the long FSH half-life that challenges peak detection methodology (104,105). There have been four studies in regularly cycling women (ages generally in the late 30s to mid-to-late 40s) with elevated follicular phase serum FSH, thus early in the menopausal transition, of LH pulsatility. All of these studies were relatively small, with numbers of women in their groups ranging from 8 to 16. Studies focused on the early- or mid-follicular phase reported no change in LH pulse frequency relative to a young control group (57,106,107) and one study reported a significant decline in LH pulse frequency (108). The authors in one of the three studies that reported no changed LH pulse frequency (57) in women over 40 years relative to women under 40 years reexamined their data in a subgroup analysis, breaking the under-40-year group into one group with age range of 35 to 39 years ($n = 8$) and the other younger group with age under 34 years ($n = 8$). They then reported a significant increase in LH pulse frequency in the oldest group (over 40 years old) compared with the youngest group (under 34 years old). Thus, there is no consistent evidence for associating the rise in follicular phase FSH in women early in the menopausal transition with a decreased GnRH pulse frequency. Furthermore, utilizing a rhesus monkey model where GnRH pulses were measured directly through a push-pull perfusion method of GnRH neuroterminals in the brain, pulsatile GnRH increased in amplitude but the pulse frequency was unchanged in aged peri- and postmenopausal females compared with young adult females (109).

Change in Hypothalamic-Pituitary Sensitivity to Ovarian Estrogen During Perimenopause

A recent publication from the SWAN study reported evidence for altered hypothalamic–pituitary sensitivity to feedback regulation by estrogen in some of the perimenopausal women who lacked luteal activity, i.e., had no evidence for luteal progesterone secretion in daily urinary hormonal measurements. Of the 840 women recruited into the SWAN Daily Hormone Substudy (DHS) between 1997 and 1999, 680 women demonstrated luteal activity. The 159 women recruited from the DHS who did not produce luteal phase progesterone were the subjects for this study (110). These women were divided into three groups depending on their patterns of estrogen and LH secretion: group 1 ($n = 29$) had normal follicular phase estrogen peak and subsequent LH surge but no luteal progesterone, suggesting a defect at the ovarian level by failure to luteinize the follicle in response to the LH peak; group 2 ($n = 32$) had normal follicular estrogen peak but no LH surge, consistent with reduced hypothalamic–pituitary sensitivity to the estrogen peak; and group 3 ($n = 98$) demonstrated neither an estrogen peak nor an LH surge (Fig. 11). Estrogen levels in group 3 women remained flat for over 50 days at levels seen in the other women during the early follicular phase, indicating capability of the ovary to synthesize and secrete estrogen. However, when the FSH levels rose substantially about days 8 to 15 of the 50-day collection period, there was no increase in the estrogen levels, suggesting lack of ovarian response to FSH.

Thus, less than 5% of the menstrual cycles studied in the SWAN DHS in women who had at least one menstrual cycle in the prior three months showed a change in sensitivity of the hypothalamic–pituitary axis to positive feedback from ovarian estrogen by the failure of the estrogen peak to induce a LH surge. But, in contrast with female reproductive aging in rodents where hypothalamus-pituitary changes precede changes in cyclicity and ovarian responses (111,112), this does not appear to be an early change because luteal phase deficiency in women usually occurs only in the late menopausal transition stage (64,66).

About 15% of the cycles showed a reduced ovarian sensitivity to either LH in the failure to luteinize the dominant follicle or FSH in the failure to make increased amounts of estrogen in response to the increased FSH level during the follicular phase. Thus, defects in sex steroid-gonadotropin feedback relationships across the HPO axis are seen but only relatively late during the menopausal transition.

■ THE ROLE OF REPRODUCTIVE HORMONES IN MENOPAUSAL HEALTH PROBLEMS AND CONDITIONS

■ The Up and Down History of Estrogen Therapy in Menopause

Premenopausal women have a reduced risk for a variety of health problems and conditions such as stroke and CVD, musculoskeletal decline, and Alzheimer's disease, compared with postmenopausal women or men of the same age (113–115). Thus, regular menstrual cycles with normal cyclic variations in reproductive hormones appear to protect premenopausal women from these health problems and conditions (116). From around the mid-twentieth century, the low level of ovarian estrogen in postmenopausal women was thought to be central to the loss of protection and increased development of pathophysiology in nonreproductive tissues. This presumption led directly to the strong encouragement by physicians for estrogen therapy in postmenopausal women bolstered by Dr. Robert Wilson's book "Feminine Forever," suggesting menopause was an estrogen-deficiency disease that if left untreated would rob women of their femininity (117).

Estrogen therapy for postmenopausal women has had a rocky history (118). Premarin™, the most commonly used form of estrogen in the United States and comprised of a number of different forms of conjugated estrogens as well as many other yet unidentified components extracted from pregnant mare urine, was developed by Ayerst Pharmaceuticals in 1942. During the 1950s, Ayerst promoted Premarin use for menopausal symptoms as a "replacement" for estrogen "lost" at menopause, thus the use of estrogen replacement therapy (ERT). About 12% of all postmenopausal women used Premarin in the 1960s. In the mid-to-late 1970s, use of ERT declined following reports of increased risk for endometrial cancer in women. Estrogen use rebounded in the 1980s following reports that addition of a cyclic or continuous progestin, called hormone replacement therapy (HRT), in women with a uterus reduced the risk of endometrial cancer. Also, around this time, reports for decreased risk for heart disease and bone fracture

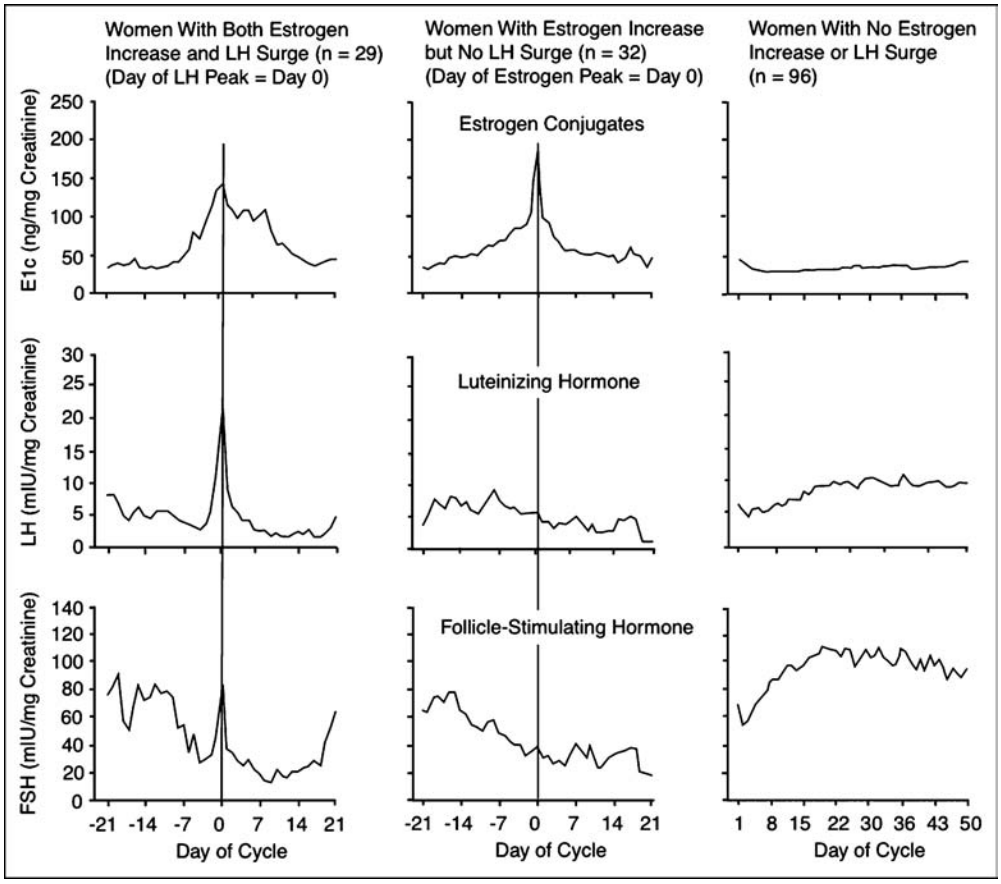

FIGURE 11 Daily urinary hormone levels in older reproductive-age women with and without estrogen increases but lacking luteal activity. Daily urine samples were collected by each woman for up to 50 days. Levels of E1c, LH, and FSH are presented as mean values (the actual data points are not shown but can be seen in the original publication) for each cycle day, where day 0 is the day of the LH or estrogen peak for women with an estrogen increase, or graphed against day of collection for women with no estrogen increase. Hormonal patterns were grouped into three categories: both estrogen and LH peaks; only estrogen peak; neither estrogen nor LH peak. *Abbreviations*: E1c, estrone conjugates; FSH, follicle-stimulating hormone; LH, luteinizing hormone. *Source*: From Ref. 110.

were appearing. In the mid-1980s, the U.S. Food and Drug Administration (FDA) approved use of estrogen therapy for osteoporosis. Epidemiologic studies throughout the late 1980s and early-to-mid-1990s continued to confirm the cardio- and bone-protection properties of estrogen as well as effective treatment of menopausal symptoms such as hot flashes, while acknowledging increased risk for stroke and probably for breast cancer (119–121).

A protective effect of estrogen in the brain, bone, and cardiovascular system was strongly supported by the basic science literature showing that estrogen had many favorable effects on tissue function (122), with increasing numbers of nonreproductive tissues shown to contain estrogen receptors (ER) and thus a response to estrogen. Attitudes regarding the use of ERT remained relatively positive even after the publication of the results from the Heart and Estrogen/progestin Replacement Study (HERS) trial in 1998, a secondary prevention trial (123). In that trial, women with preexisting heart disease were not protected from further heart disease progression by estrogen, and in fact estrogen appeared to exacerbate the disease progression over five years of followup. From the results of that trial, the clinical community concluded that estrogen was not effective in secondary prevention of heart disease but may still be effective for primary prevention.

Results from the WHI

The report that did strongly negatively affect the opinion of women and physicians, and use, of HRT (Box 4) (125,126) was the publication in 2002 of the early termination of the estrogen plus progestin (E + P; daily 0.625 mg conjugated equine estrogens plus 2.5 mg medroxyprogesterone acetate—"Prempro™") arm of the WHI, a very large randomized, double-blind, placebo-controlled primary prevention clinical trial of over 16,000 postmenopausal women in the age range of 50 to 79 years in the E + P arm (127). The E + P arm of the study was terminated early because of the observed increased risk for coronary heart disease (in sharp contrast to the earlier expectations, mostly from epidemiologic studies, of protection from CVD), stroke, pulmonary embolism, and invasive breast cancer; risk reduction was reported for colorectal cancer and hip fractures. Thus, overall health risks of E + P were judged to exceed benefits at the time of study termination. In subsequent data analyses, WHI investigators reported that E + P treatment relative to placebo did not have a clinically meaningful effect on health-related quality of life (128), increased risk of ischemic stroke in generally healthy postmenopausal women (129), increased the risk for probable dementia in women over 65 years and did not prevent mild cognitive impairment (130), increased incident breast cancer and substantially increased the percentage of women with abnormal mammograms (131), increased

BOX 4 *Decline in Use of Postmenopausal Estrogen Therapy May Be Associated with Decreased Incidence of Breast Cancer*

In late 2006, a presentation at the San Antonio Breast Cancer Symposium received prominent media attention. Investigators reported the abrupt and substantial decline in breast cancer incidence beginning in 2003 (124). Public data from the National Cancer Institute Surveillance Epidemiology and End Results (SEER) database over the period of 1990 through 2003 were analyzed. Over the period 1990 to 1998, breast cancer incidence increased (1.7%/yr) but then began to decrease (1%/yr) from 1998 to 2003. In 2003, a sharp decrease (7%) in incidence was reported in this single year. Other tumor registries confirmed this decline in breast cancer, with no abrupt change seen in other major types of cancer. This decline in incidence in the SEER registry actually accelerated during 2003: a 6% decline occurred in the first half of the year and a 9% decline in the second half of the year. This decline was most noticeable in patients over 50 years with estrogen receptor–positive invasive tumors, where a 12% decline was noted in 2003 alone.

What event or events might account for this rather sudden, substantial, and unexpected decline in breast cancer incidence? As alluded to in the section "The Role of Reproductive Hormones in Menopausal Health Problems and Conditions," the report from the Women's Health Initiative of the early termination of the estrogen plus progestin (E + P) arm of study because of an unacceptably high risk-to-benefit ratio in mid-2002 resulted in many postmenopausal women terminating their hormone therapy (125,126). This single event may account for the substantial decline in breast cancer incidence on the assumption that undetectable incipient breast tumors stopped growing, and possibly regressed, within a very short period of time in women who terminated their hormone therapy. Further studies are required to confirm or refute this possibility.

bone mineral density (BMD) and reduced the risk of fracture (132), may increase the risk of breast cancer while having no effect on endometrial cancer rates (133), and decreased the risk of colorectal cancer though tumors found were diagnosed at a more advanced stage in women on E + P (134). Regarding cardiovascular outcomes, E + P did not protect against peripheral arterial disease risk (135), doubled the risk of venous thrombosis (136), did not confer cardiac protection, and may increase risk of coronary heart disease in relatively healthy postmenopausal women, particularly in the first year of hormone use (137).

Although the estrogen (E; daily 0.625 mg conjugated equine estrogens—"Premarin") alone arm of the WHI study, which enrolled over 10,000 postmenopausal women of the same age range with prior hysterectomy, went longer than the E + P arm, this study was also terminated earlier than planned (138). As in the E + P arm, stroke risk was increased and risk for hip fracture decreased. There was no effect on CVD and a possible reduction in breast cancer risk that required further investigation. There was no difference with the placebo group on burden of incident disease. Thus, it was concluded that there was no overall benefit for estrogen therapy and estrogen should not be recommended for chronic disease prevention in postmenopausal women. Subsequent data analyses concluded that E alone relative to placebo did not affect incidence of dementia or mild cognitive impairment (139) and adversely affected cognition in women over 65 years (140), provided no protection against myocardial infarction or coronary death although a lower coronary heart disease risk was suggested in women aged 50 to 59 years (141), increased risk for venous thrombosis, particularly in the first two years of treatment, although less than E + P treatment (142), and caused no increase in risk of breast cancer, although women in the E group had more frequent mammograms with abnormalities that required followup (143). Current information regarding the WHI and research findings that continue to come from that study is available (144). A publication for the lay public is available on the web that describes these findings from the National Heart, Blood, and Lung Institute (NHLBI) at the National Institutes of Health (NIH) (145).

In light of these findings, recommendations for the clinical use of E or E + P in peri- and postmenopausal women were completely revised. A summary of the current recommendations are provided in section Current Options for Hormone and Hormone-related Therapy in Menopause.

■ The Natural History of Pathophysiologic Changes in Nonreproductive Tissues Across Menopause—Are Other Hormones Besides Estrogen Involved?

While the results from the WHI and other clinical studies focused on the use of estrogen with or without progestin on health problems and conditions in postmenopausal women are being further analyzed and debated, the question remains—what aspect of the premenopausal reproductive condition appears to protect these women from the high morbidity and mortality associated with CVD, for example, compared with postmenopausal women or men of the same age? Is it the form and timing of estrogen exposure, which clearly is different in many aspects in the natural premenopausal cycling condition compared with estrogen therapy? Questions regarding modality of exogenous estrogen administration need to be sorted out by clinical studies. The purpose of this section is to review the natural history of the development of menopause-related health problems and conditions across the menopausal transition, pointing out what is known regarding the association and potential involvement of estrogen, the other reproductive hormones, sex steroids, and ovarian as well as pituitary peptide hormones, in the etiology of these health problems.

Development of Symptoms During the Menopausal Transition

A review article written for the 2005 National Institutes of Health (NIH) State-of-the-Science Conference on Management of Menopause-Related Symptoms (146) summarized data on

the more common symptoms associated with the menopausal transition (147) across community-based longitudinal studies of the menopausal transition. Of the symptoms examined, only vasomotor symptoms, vaginal dryness, and sleep disturbance increased in prevalence during the menopause transition. Hot flashes affected about 10% of women in the late reproductive stage and rose to nearly 40% of women during the late menopause transition and into postmenopause. Sleep disturbances affected about 30% of women in late premenopause, rising to about 40% in postmenopause. Vaginal dryness affected less than 10% of women in late premenopause but rose to about 20% by postmenopause. Also, symptom severity increases during the late menopausal transition and into postmenopause, but due to limited follow-up data, it is not clear how long symptoms persist in postmenopause.

Hot flashes are thought to arise because of small elevations in core body temperature in symptomatic women with a reduced thermoneutral zone (Fig. 12) (148). The connection of hot flashes with estrogen levels is largely circumstantial: the percentage of women complaining of hot flashes rises as estrogen levels decline in the late menopause transition (149), although estrogen levels are similar in symptomatic and asymptomatic women. Furthermore, exogenous estrogen is clearly effective in hot flash treatment (150), probably by increasing the sweating threshold temperature (148), although the underlying mechanism is not clear. However, a recent publication from the SWAN study reported that annual serum FSH, but not free or total estradiol or free or total testosterone or dehydroepiandrosterone sulfate when collectively modeled longitudinally, is associated with hot flashes in midlife women (151).

Also, the SWAN study reports that sleep difficulties in women at midlife are relatively common, varying from about 38% to nearly 50% in women in the late menopausal transition and postmenopause (152). The authors suggest that the stage of the menopause transition, rather than older age per se, is associated with self-reported sleeping difficulty. Ethnic differences were noted, with lowest rates in Japanese women (28%) and highest in Caucasian women (40%). To obtain hormonal correlates, urinary hormones were measured in cycling women the morning after the women reported sleeping difficulties. Most women who reported sleeping difficulties experienced them at the beginning or end of their menstrual cycle. After controlling for covariates, hormonal correlates for sleeping difficulties were FSH in premenopausal women and pregnanediol glucuronide (PDG) in early perimenopausal women (153). Hot flashes and mood were the strongest contributors to sleeping difficulties.

Dyspareunia, or vaginal dryness, in postmenopause is associated with an alkalinization of the vaginal pH to around 6.5 to 7.0. In premenopausal women, vaginal fluid pH is tightly regulated and ranges from 4.5 to 5.5. This acidic environment of the vaginal lumen protects the lower genital tract from infections. The alkaline pH after menopause is also associated with urinary tract infections and vaginal atrophy. The observation that estrogen treatment in postmenopause can partially acidify the vaginal environment suggests that low postmenopausal serum estrogen plays some role in this alkalinization process. A study of cultured normal human vaginal-extocervical cells from pre- and postmenopausal women concluded that cells from postmenopausal women are less responsive to estrogen compared with those from premenopausal women, suggesting factors in the aging process other than declining estrogen affects the ability of these cells to acidify the vaginal lumen (154).

Cardiovascular System

Heart disease is the number one killer of women (Chapters 15, 16, and 20). Therefore, it is important to identify the protective factors that support the widely held assumption of premenopausal cardioprotection relative to age-matched men and postmenopausal women (155,156). For example, nearly half of the postmenopausal women (five to eight years after menopause) in one study had clinically significant carotid intima-media thickness, a strong predictor of CVD risk, compared with about 16% of premenopausal women (157), although a recent meta-analysis reported that postmenopausal status and CVD are not related (158).

Hormonal correlates of increasing cardiovascular risk in midlife women include low sex hormone-binding globulin and high free-androgen index, with low estrogen associated to a

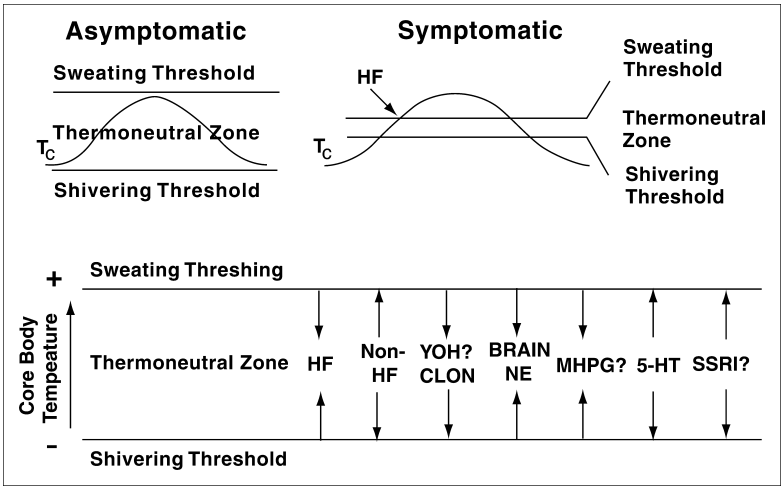

FIGURE 12 Schematic showing how reduced thermoneutral zone in symptomatic women may explain increased HF incidence. Small elevations in core body temperature (T_c) acting within a reduced thermoneutral zone may trigger in symptomatic postmenopausal women. Elevated brain NE in animals reduces this zone. YOH elevates brain NE and should reduce the zone. Conversely, clonidine should widen it. The effects of serotonin (5-HT), MHPG; the primary NE metabolite, and SSRI are uncertain. *Abbreviations*: HF, hot flash; T_c, core body temperature; NE, norepinephrine; YOH, Yohimbine; HT, serotonin; MHPG, 3-methoxy-4-hydroxyphenylglycol; SSRI, serotonin-selective reuptake inhibitor. *Source*: From Ref. 148.

lesser degree (159). Hypertension among pre- and perimenopausal women was associated with ethnicity rather than hormonal correlates, with prevalence two to three times higher among African American and Hispanic women than Chinese, Japanese, and Caucasian women (160). From the SWAN longitudinal study, hemostatic factors were significantly associated with estrogen or FSH levels rather than menopausal stage: lower estrogen was associated with higher levels of fibrinolytic factors plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator; higher FSH was associated with higher PAI-1 and factor VII levels, as well as lower fibrinogen and serum C-reactive protein (161). Also, there was a strong positive association of androgens with many of these same fibrolytic and inflammation markers (162). A recent report from the SWAN DHS directly addresses the effect of normal versus altered cycles in midlife women on metabolic and hemostatic cardiovascular risk factors (155). Altered cycles included anovulatory or extended cycles, or cycles with low follicular estrogen and low luteal progesterone. Failure to ovulate did not significantly affect cardiovascular risk factors; elongated cycles did affect these risk factors, primarily through a common association with body mass index (BMI). Finally, low follicular phase estrogen and progesterone were associated with these metabolic (waist circumference, lipids, and insulin) and hemostatic (PAI-1) risk factors.

Metabolic Changes

Pathophysiologic changes associated with the metabolic syndrome increases risk for CVD. Metabolic syndrome is usually characterized by at least three of the following conditions: central obesity, insulin resistance, dyslipidemia, and hypertension (Chapter 13). Metabolic changes associated specifically with the menopausal process may synergize with aging-related metabolic changes, resulting in metabolic syndrome as women traverse the menopausal transition, thereby explaining at least some of the increased risk for CVD in postmenopausal women.

Most studies find that increased BMI is associated more with aging and low physical activity than stage of menopause in middle-aged women. In a recent study of over 600 53-year-old women in various stages of the menopausal transition, BMI, waist circumference, and waist-hip ratio increased from pre- to perimenopause, then declined in postmenopause (163). The same general pattern in BMI change across menopausal stages was also reported in other studies (164,165). Interestingly, the pattern of BMI, waist circumference, and waist-hip ratio was identical in the same women 10 years earlier at age 43 (i.e., the women in the perimenopausal group at age 53 who had the highest levels of these three measures also had the highest levels 10 years earlier) when all of the women were premenopausal, although the magnitudes of these measures were much smaller (Fig. 13) (163). This result clearly demonstrates substantial age-related increases in all of these measures over this 10-year period and argues against a strong menopause-related component. Although BMI has not been associated with stage of menopause, BMI has been associated with menstrual cycle length and urinary hormone levels in the SWAN study. Women with BMI levels less than 25 have shorter cycles and higher total-cycle LH, FSH, and PDG (66) compared with women with BMI over 25.

Although no changes related to the stage of menopause were seen in blood pressure or high-density lipoprotein (HDL) cholesterol, metabolic syndrome–related risk factors of total cholesterol, low-density lipoprotein (LDL) cholesterol, and hemoglobin A1c did increase steadily over the menopausal transition in the previously cited study of age-matched (53-year-old) women (163). Thus, there is little to no increase in weight or visceral adiposity due to menopause, but there are substantial changes due to aging that could be reversed by increased physical activity (166,167). However, the menopausal transition may be associated with dyslipidemia and insulin resistance (155,156) that in turn could affect incidence of CVD.

Bone Density

Menopause-related bone loss in women actually begins years before menopause when women are perimenopausal (168–170). Although there is a great deal of research showing that estrogen acts to preserve bone (171), serum estrogen levels are generally maintained or even increased during much of the menopausal transition, as described elsewhere in this chapter, arguing against substantial estrogen involvement in the initiation of perimenopausal bone loss. The SWAN longitudinal study and two cross-sectional studies reported that decreasing BMD or serum markers of increasing bone resorption in middle-aged pre- and perimenopausal women were most strongly related to increasing FSH (Fig. 14) or declining inhibin rather than changes in estrogen or androgen levels (170,172,173). More recently, the SWAN DHS, where urinary hormones were measured over an entire menstrual cycle rather than restricting the hormonal measurements only to the early follicular phase (170), reported that both lower integrated urinary estrone conjugates (E1c) and higher integrated urinary FSH were significantly associated with lower BMD (174).

Also, as discussed earlier in this chapter, the earliest known hormonal change signaling the initiation of the menopausal transition is increased follicular phase FSH in response to declining inhibin B from developing ovarian follicles resulting in increased pituitary FSH during the follicular phase of the menstrual cycle. Several groups have provided evidence that both declining inhibin and increasing FSH levels directly and negatively affect bone-cell function. In mouse bone marrow cultures, inhibin suppresses both osteoblastogenesis and osteoclastogenesis, even in the presence of activators of these processes, activin or bone morphogenetic protein (175). Thus, declining inhibin during the menopausal transition may affect the balance between bone formation and bone resorption, resulting in bone loss. A very recent clinical study supports this conclusion. In this cross-sectional study of 188 pre-, peri-, and postmenopausal women, inhibin significantly and inversely correlated with serum markers of increasing bone turnover; FSH was associated with bone resorption only in the perimenopausal women (176). In addition, inhibins were shown to directly affect human osteoblast and osteoclast development in vitro.

Evidence for the ability of FSH to directly affect bone-cell function comes from the observation that transgenic mice lacking FSHβ subunit or FSH receptor knock-out, although severely hypogonadal, showed no evidence of bone loss (177). Further evidence for the dependence of bone density on FSH levels was obtained using heterozygotic FSHβ transgenic mice. These mice are eugonadal, with a 50% reduction in serum FSH levels, but showed strikingly enhanced BMD at several bone sites. Thus, BMD appears to be more responsive to FSH activity rather than serum estrogen levels. In exploring the molecular mechanisms underlying these effects, both osteoclast precursors and mature osteoclasts were shown to contain FSH receptors along with associated signaling pathways.

While there is very strong evidence that estrogen protects from bone loss, there is increasing evidence that the ovarian inhibins and pituitary FSH also modify bone turnover and

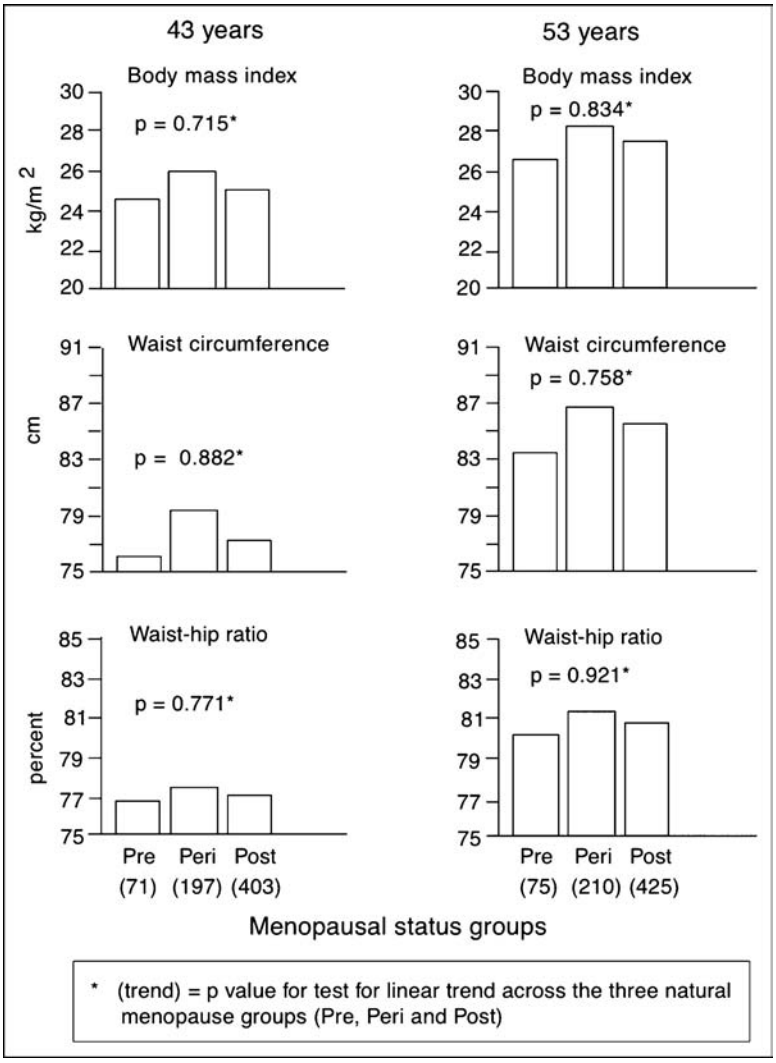

FIGURE 13 Measures of total and central obesity in 53-year-old women during the menopausal transition and 10 years earlier in the same women. Mean values of body mass index, waist circumference, and waist-hip ratio in women 53 years old by menopausal status groups and in the same women at 43 years of age by their menopausal status at age 53. *Source*: From Ref. 163.

affect BMD. The emerging picture is that early in the menopausal transition, both declining inhibin and increasing FSH act to increase bone resorption, possibly with the normal-to-high estrogen levels during this period acting as a brake on rapid bone loss to some extent. However in the late transition to the onset of menopause, declining estrogen levels act in concert with low inhibin and elevated FSH to accelerate bone loss.

Cognitive Function and Alzheimer's Disease

Although several cross-sectional studies of women in the menopausal transition report an increase in forgetfulness over the transition (178,179), more recent longitudinal studies appear unable to reach a consensus (Chapter 7). In contrast to initial expectations, data from the SWAN longitudinal study were consistent with small but significant increases in working memory and perceptual speed across the pre- and perimenopausal stages of the menopausal transition (180). Another longitudinal study of women 40 to 54 years old in Taiwan who were premenopausal when recruited reported no significant cognitive decline, except of verbal fluency (181). A third recent study, this one involving women aged 53 at various stages in the menopausal transition, concluded that menopause adversely

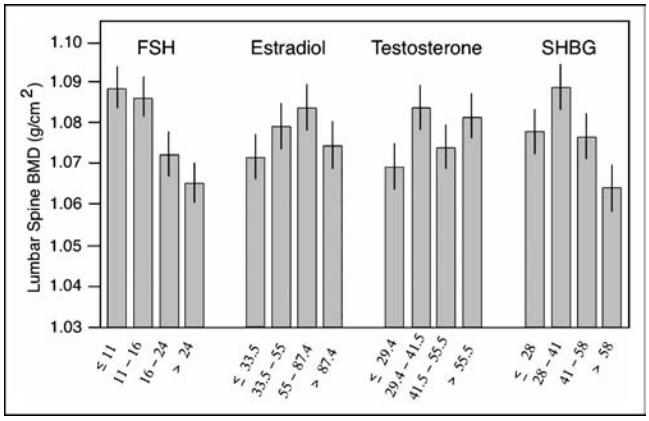

FIGURE 14 The mean lumbar spine BMD values according to quartiles of FSH, estradiol, testosterone, and SHBG in pre- and perimenopausal women, following adjustment for race/ethnicity, clinical site, BMI, and physical activity level. *Abbreviations*: BMD, bone mineral density; SHBG, sex hormone–binding globulin; BMI, body mass index; FSH, follicle-stimulating hormone. *Source*: From Ref. 170.

affects cognitive function (182). Except for search speed and concentration on the National Adult Reading Test, this decline in cognition may be largely explained by premenopausal cognitive function. None of these studies correlates hormonal changes across the menopausal transition with their cognition findings.

There is an interesting series of studies associating increasing gonadotropin levels with increased risk for Alzheimer's disease in postmenopausal women. Female Alzheimer's patients not on estrogen therapy were reported to have significantly higher levels of serum LH and FSH relative to age-matched non–estrogen treated normal controls or in non–estrogen treated women with frontotemporal dementia (183,184). LH was significantly increased in the cytoplasm of pyramidal neurons and neurofibrillary tangles of AD brain compared with age-matched control brain (185). In mice, the GnRH agonist leuprolide, which reduces gonadotropin and sex steroid levels, significantly reduced amyloid-β (Aβ) 1-42 and 1-40. Furthermore, LH treatment of neuroblastoma cells in culture did not affect Aβ precursor protein (Aβpp) expression but did alter Aβpp processing toward the amyloidogenic pathway (186). A very recent paper (187) showed that, in contrast to the adverse cognitive test results in estrogen plus progestin–treated postmenopausal women (140), suppression of LH by leuprolide significantly attenuated cognitive decline and decreased Aβ deposition in an aged transgenic mouse model of Alzheimer's disease compared with placebo-treated animals. Clinical trials for treatment of Alzheimer's disease with GnRH agonists are currently under way (188).

■ CURRENT OPTIONS FOR HORMONE AND HORMONE-RELATED THERAPY IN MENOPAUSE

■ New Recommendations for Estrogen Therapy Reflecting the Outcome of the WHI

The recent series of large randomized, placebo-controlled clinical trials focused on disease prevention in older postmenopausal women (123,127,138) demonstrated

- no benefit of E therapy in contrast to expectations from earlier epidemiological studies, and
- increased risk for CVD on E + P therapy, particularly for postmenopausal women beginning estrogen therapy or in women with preestablished disease.

As described in section The Role of Reproductive Hormones in Menopausal Health Problems and Conditions, despite some benefit of reduced risk for bone fracture and possibly colorectal cancer, the risk versus benefit calculation for the WHI study came down on the side of increased risk. However the effect of E or E + P therapy for ameliorating menopausal symptoms was not tested in these disease prevention studies, largely because the women in the studies were many years past menopause so less likely to be experiencing the typical menopausal symptoms of hot flashes, night sweats, and vaginal dryness.

As a result of the disease prevention results, clinical guidelines for the use of E or E + P therapy in postmenopausal women have recently changed. Long-term use of E or E + P is not recommended for any indication. The FDA recognizes the effectiveness of short-term use of estrogen in treating menopausal symptoms; it recommends that women discuss with their providers if benefits of short-term use may outweigh the risks in their particular cases (145). If use is solely for vaginal symptoms, the FDA recommends topical creams or gels or vaginal rings containing low-dose estrogen to minimize the systemic estrogen exposure. While the FDA recognizes that estrogen use is effective for osteoporosis, alternative effective medications such as bisphosphonates, calcitonin, teriparatid (parathyroid hormone), and SERMs (see later in this section) are available. However, estrogen may be used for osteoporosis if benefits outweigh the risks. If estrogen is used in any of these scenarios, the FDA recommends usage at the lowest effective dose for the shortest possible time. The FDA does not approve the use of estrogen to prevent cognitive decline. Estrogen therapy (E or E + P) should not be used to prevent heart disease or in women with heart disease to reduce risk for other diseases.

The NHLBI recommends lifestyle changes such as not smoking, maintaining a health weight, being physically active, and effectively managing diabetes. Risk for CVD can be managed, if lifestyle changes are not sufficient, with statins to regulate cholesterol levels, and blood pressure medications for hypertension. Increased calcium and vitamin D intake are recommended to maintain bone strength, with the osteoporosis medications listed above if required.

Recommendations from other groups are similar. The American College of Obstetricians and Gynecologists (ACOG) concurs that hormones should not be used for disease prevention but may still be appropriate for treatment of relief of menopausal symptoms (189). ACOG maintains that nothing works better than estrogen for severe hot flashes, at the lowest effective dose for the shortest possible time. They also recommend alternative osteoporosis treatments if this is the only purpose for taking estrogen. Regarding side effects of estrogen therapy, about 10% of women on estrogen experience breast tenderness, fluid retention, and pelvic cramping. Women on E + P, particularly cyclic (with estrogen daily and progestogen on a preset sequence), may have periodic bleeding similar to menstruation.

The North American Menopause Society (NAMS) published recommendations to both clinicians and lay public for estrogen and progestin use in peri- and postmenopausal women for disease prevention as well as for treatment of menopausal symptoms (190). The NAMS recommendations, many of which are similar to recommendations from the FDA, ACOG, and the NIH, are as follows ("estrogen" use here refers to E alone in women without a uterus and E + P in women with a uterus):

> Estrogen should not be used for primary or secondary prevention of CVD, prevention of stroke, and primary prevention of dementia.
>
> Estrogen may be used for systemic treatment of menopausal symptoms, local treatment of moderate-to-severe vulvar and vaginal atrophy, osteoporosis, and long-term use for menopausal symptoms; and osteoporosis is acceptable at the lowest effective dose if the potential risks and benefits are fully understood and medical supervision is provided.
>
> Precautions for using estrogen are as follows: use lowest effective dose and nonoral routes of administration if possible; have a complete health evaluation prior to considering estrogen therapy; only estrogen plus progestin use for over five years probably increases breast cancer risk and may impede diagnostic interpretation of mammograms.

■ Alternative Treatment Approaches

Selective ER Modulators

Prior to the randomized, placebo-controlled clinical trial findings in the late 1990s and early 2000s of lack of protection

of the cardiovascular system, a time when cardiovascular protection was expected based on epidemiologic studies, there was strong support in the clinical community for estrogen therapy in postmenopausal women. Even then, however, downsides to estrogen treatment were recognized, particularly in the uterus with unopposed estrogen and on the breast. Throughout the 1980s and 1990s, the intense research focus on the biological mechanisms underlying the mode of action of estrogen receptors (ER) in specific tissues, primarily nonreproductive tissues, led to the realization that certain nonsteroidal ER ligands can act in a tissue-specific manner, i.e., as an estrogen agonist in some tissues and estrogen antagonist in other tissues. Coupling the clinical concern with the new knowledge regarding the biology of estrogen action suggested the possibility of developing ER ligands that act as an estrogen agonist in tissues where estrogens are protective and as an estrogen antagonist in tissues where estrogens are problematic. Throughout the 1990s, the ideal SERM was considered to be an estrogen agonist in the brain including brain centers involved in thermoregulation, in the vagina and other estrogen-sensitive pelvic organs, and the skeletal and cardiovascular systems, and as estrogen antagonist in the uterus and breast. In this manner, the ideal SERM would ameliorate menopausal symptoms as well as prevent or reduce CVD, cognitive decline, osteoporosis, and urinary incontinence without stimulating uterine and breast cancer in postmenopausal women.

Tamoxifen, a first generation SERM, is used in prevention of breast cancer in high-risk women and in treating advanced breast cancer. It also has beneficial effects on bone and serum lipids (191), and, in breast cancer–focused trials, resulted in fewer fatal myocardial events compared with placebo-treated women (192,193). Raloxifene, a second generation SERM, was developed to be used as a treatment in osteoporosis, but also appears to reduce invasive breast cancer incidence (194). Neither tamoxifen nor raloxifene protects against hot flashes and may even increase their occurrence (195,196). The Multiple Outcomes of Raloxifene Evaluation (MORE) randomized trial concluded use of raloxifene resulted in a 25% reduction in global index of clinical outcomes (earliest occurrence for various measures, including heart disease, stroke, various cancers, hip fracture, or death) compared with placebo (197). However, in the Raloxifene Use for the Heart (RUTH) trial, raloxifene, despite its ability to protect from invasive breast cancer and vertebral fracture, did not significantly affect the risk for coronary heart disease compared with placebo and significantly increased the risk for fatal stroke and venous thromboembolism (198). In a direct comparison with tamoxifen in the Study of Tamoxifen and Raloxifene (STAR) trial, raloxifene was equally effective in reducing the invasive breast cancer risk but had a slightly higher risk for noninvasive breast cancer. Raloxifene also had a lower risk for thromboembolic events and cataracts than tamoxifen (199).

A third generation SERMs, lasofoxifene, has a greater bioavailability than previous generation SERMs and appears efficacious in preclinical and short-term clinical studies in preventing bone loss and lowering cholesterol levels (191). It is currently in phase III development. A major target for future SERM development is to prevent CVD, a feature lacking in current and earlier generation SERMs, the number one killer of older women.

Phytoestrogens and Botanicals for Menopausal Symptoms

Many commercial entities encourage women to use "alternative" and "natural" phytoestrogens and botanicals to relieve menopausal symptoms, thus avoiding the "dangers" of using estrogen therapy (Box 5). Overall, common methodological problems for research studies into all of these phytoestrogens and botanicals stems from lack of standardization of preparation and doses. The Dietary Supplement Health and Education Act of 1994 (DSHEA) (200) treats these materials as "dietary supplements," placing the burden of ensuring the safety and proper labeling of the dietary supplement on the manufacturer before marketing. The manufacturer need not notify or seek FDA approval before marketing. The main role of the FDA is to take action against any unsafe dietary supplement product after it reaches the market. The result is that these compounds may or may not contain what they are purported to contain at the dose levels claimed. Different research studies may not be studying the same compound at the same dose, even if derived from different lots from the same manufacturer. Also, except for soy and isoflavone phytoestrogens where many studies have been done but many more are needed, basic mechanism of action studies are completely lacking for these botanicals.

BOX 5 *Phytoestrogens and Botanicals for Menopausal Treatment*

The recent National Institutes of Health (NIH) State-of-the-Science conference on management of menopause-related symptoms reviewed the current state of knowledge regarding the efficacy of these compounds to relieve hot flashes and other menopausal symptoms (146). Regarding isoflavones and other phytoestrogens, the independent panel, after reviewing the data presented by experts, concluded that several studies of isoflavones extracted from soy suggested some ability to relieve hot flashes but that studies of dietary soy are inconclusive, with the majority of the studies failing to show benefit. Research on botanicals for even the major menopausal symptom, hot flashes, is at a very rudimentary state. Black cohosh, which was thought to have estrogenic properties but recent studies place that conjecture in doubt, is the most studied botanical product. However, there is little evidence of its effectiveness in reducing hot flashes. NIH-supported trials are currently ongoing. Kava effectively reduces anxiety but lacks evidence for reducing hot flashes. Following reports of liver damage, the Food and Drug Administration (FDA) has issued a warning about potential harm from this product. Red clover leaf is thought to contain weak estrogenic compounds, but studies suggest it lacks effectiveness in reducing hot flashes. There is evidence that dong quai root is ineffective for hot flashes, although it is widely used for a variety of symptoms. Bleeding complications may arise from its interaction with warfarin. Ginseng root does not appear to be effective for hot flashes but may be helpful for well-being, mood, and sleep.

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11

The Ensemble Male Hypothalamo-Pituitary-Gonadal Axis

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INTRODUCTION

Healthy aging in men progressively reduces testosterone availability to bone, muscle, brain, fat depots, immune tissue, and sexual organs (1). Free and bioavailable testosterone concentrations fall by 0.8% to 1.5% per year and are reduced by 30% by the seventh compared with third decade of life. Sex-hormone binding globulin (SHBG) concentrations rise twofold in older males. Estradiol bioavailability declines along with that of testosterone, probably contributing to reduced bone density, elevated follicle-stimulating hormone (FSH) concentrations, and decreased prolactin concentrations. Although testis size and firmness diminish, spermatogenesis may be relatively preserved in elderly men.

The causes of hyperandrogenemia in aging include

- less secretion of gonadotropin-releasing hormone (GnRH) by the brain,
- smaller gonadotropin pulses released by the anterior pituitary gland, and
- decreased gonadal steroidogenic responses to luteinizing hormone (LH),
- with concomitant blunting of feedback inhibition of GnRH/LH secretion by testosterone (2–6).

This chapter highlights current insights into the mechanisms that engender impoverished testosterone production. Implications of androgen depletion, listed in Box 1, include muscle wasting (Chapter 24), skeletal demineralization (Chapter 20), visceral adiposity and glucose intolerance (Chapter 13), hyperlipidemia (Chapter 16), diminished libido, decreased potentia (the ability to perform the sexual act), and decreased aerobic capacity (Chapter 17), impaired immune defenses (Chapter 14), reduced activities of daily living (Chapter 3), and (possibly) memory and cognitive loss (Chapter 7). The same features typify aging generally (Box 1).

OVERVIEW OF THE MALE GONADAL AXIS

The reproductive axis in men and women comprise neural structures, the pituitary gland, and gonadal organs, which jointly control fertility, sex-steroid availability, growth in puberty, and anabolism in adulthood (7). The adult male reproductive axis maintains spermatogenesis, sexual behavior, and masculine traits. Spermatogenesis requires an array of testicular factors: systematically delivered FSH and intragonadal testosterone. Inhibin, synthesized by spermatogenesis-supporting Sertoli cells, and sex steroids, secreted by Leydig (interstitial cells), feed back to inhibit FSH secretion. Sexual behavior is influenced by gonadal steroids, psychological factors, visuals cues, and social context. The masculinizing action of testosterone sustains external and internal genital structures (e.g., phallus size, epididymis, seminal vesicles, and prostate gland), central neuronal connectivity, and male life patterns of gene expression in muscle, bone, liver, and fat (Box 2). Estrogen exerts important effects on bone, brain, liver, and the vasculature. Elevated prolactin concentration may repress libido in both genders.

Reproductive Aging

Reproductive aging impacts the general physical and psychosocial health of men and women (1,21–36). In the males, the primary manifestation of gonadal-axis aging is progressive testosterone deficiency. In addition, concentrations of prolactin decrease and of FSH increase reciprocally in older men.

BOX 1 *Correlates of Androgen Deficiency in Aging*

Physical	Chemical
Libido, potential	Bioavailable estradiol
Bone mineral mass	Sex-hormone binding globulin (SHBG)
Muscle strength	Growth hormone (GH), insulin-like growth factor 1 (IGF-1)
Aerobic capacity	Muscle genes
Visceral fat	Insulin action
Memory	Systolic blood pressure
Immune function	Dyslipidemia

BOX 2 *Structural/Physiologic Characteristics of the Male Reproductive System*

The reproductive system in men includes brain, pituitary gland, and genitalia:

- The male gonad produces spermatozoa and sex-steroid hormones.
- Secondary sexual organs are the epididymis and vas deferens, which transport and mature sperm, and seminal vesicles and prostate gland, which produce seminal fluid.
- In analogy with the female system (Chapter 10), the male gonadal axis is supervised by the hypothalamus, which secretes bursts of the decapeptide, gonadotropin-releasing hormone (GnRH).
- Pulses of GnRH are carried through a portal microcirculation to the anterior pituitary gland (Fig. 1) (8).
- GnRH stimulates the synthesis and release of the two gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) (in the male, LH was initially called interstitial cell-stimulating hormone).
- LH and FSH are dimeric glycoproteins secreted by gonadotrope cells, in which a common α subunit combines with a unique β subunit.
- High- and low-frequency GnRH pulses (but not continuous GnRH exposure) preferentially induce LH and FSH β-subunit genes, respectively. In addition, activin, a gonadal and pituitary protein hormone, upregulates the FSH β-subunit gene.

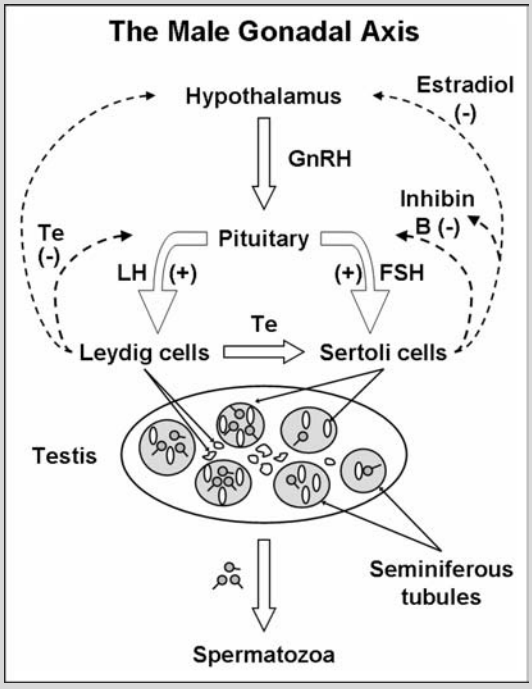


FIGURE 1 Key regulatory interactions within the human male gonadal axis. Basic dynamics include GnRH outflow from mediobasal hypothalamic neurons, LH secretion by pituitary gonadotrope cells, and Te, E_2, and inhibin production by gonadal Leydig and Sertoli cells. Interrupted lines denote negative feedback. Systemic Te concentrations decrease hypothalamic GnRH pulse frequency and amplitude, and via E_2 inhibit GnRH's feedforward on pituitary LH secretion. Te, E_2, and inhibin inhibit FSH secretion. *Abbreviations*: E_2, estradiol; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; Te, testosterone. *Source*: From Ref. 8.

- Synthesis of LH is inhibited by testosterone, estradiol, and FSH by the same sex steroids as well as by inhibin B. Pituitary follistatin lowers FSH secretion by antagonizing stimulation by activin, thus indirectly potentiating repression by estradiol and inhibin. The putative roles of activin, inhibin, and follistatin are inferred from data in other species, since none of these peptides has been infused or blocked directly in the human.

Testosterone and estradiol are transported in plasma bound to the sex-hormone binding globulin, sex-hormone binding globulin (SHBG) (40–50%), and albumin (50–60%). Approximately 1.8% of testosterone and 2.5% of estradiol remain free (unbound) in the circulation. The fraction of a sex steroid that is not bound to SHBG has been called bioavailable, since it remains free and loosely bound to albumin:

- Testosterone bound to albumin dissociates with a half-time of 0.33 seconds, compared with a half-time of 8.4 seconds for that bound to SHBG and a capillary transit time of 6 to 10 seconds.
- Precisely how total, free, albumin-bound and SHBG-bound testosterone moieties differ in driving target-tissue responses in the human remains unknown.

Testosterone and estradiol are metabolized in the liver and excreted as conjugates in the bile and urine:

- In many target tissues such as the brain, skin, and prostate gland, testosterone is converted to 5α-dihydrotestosterone (DHT) by the enzyme 5α-reductase.
- DHT binds to the same receptor as testosterone but several-fold more strongly, thereby amplifying the actions of testosterone.
- Testosterone can also be converted to estradiol via the enzyme aromatase (estrogen synthase), which is expressed in the brain, pituitary gland, kidney, liver, bone, immune cells, and fat. Estrogen derived in this fashion mediates important tissue effects.
- Less potent precursor androgens such as dehydroepiandrosterone (DHEA) and androstenedione are secreted primarily by the zona fasciculata of the adrenal cortex (Chapter 10). Concentrations of DHEA (and its sulfate) decline markedly with age.
- The pathophysiology and clinical consequences of reduced adrenal androgen output in older men remain uncertain (9). This is because testosterone and DHT are principally responsible for the development and the maintenance of male sex organs (seminal vesicles and prostate), secondary sex characteristics (e.g., voice pitch and body-hair distribution), and protein-anabolic and growth-promoting effects of androgens.

Communication within neuroendocrine systems occurs via blood-borne chemical signals, which mediate repeated incremental adjustments toward homeostasis:

- Adjustments require negative feedback and positive feedforward (10–12). Hypothalamic GnRH feeds forward on gonadotrope cells to stimulate release of LH.
- Pituitary LH feeds forward on testicular Leydig cells to drive secretion of testosterone.
- In turn, testosterone feeds back on the hypothalamus and pituitary gland to inhibit secretion of GnRH and LH (Fig. 1).

Stated generally, hypothalamic neuronal outflow regulates the synthesis and secretion of glycoprotein hormones by the

(Continued)

BOX 2 *Structural/Physiologic Characteristics of the Male Reproductive System (Continued)*

anterior pituitary gland; secreted pituitary hormones activate biosynthetic responses in remote target glands; and peripherally produced peptides and steroids feed back on the brain and pituitary gland to repress neurohormone output. A crucial physiological precept is that this set of interactions, rather than any single signal, confers the self-correcting dynamics of a particular axis (13,14).

Secretion of GnRH, LH, and testosterone is intermittent or pulsatile, rather than continuous. This means that single measurements of serum LH and testosterone concentrations vary within 30% to 100% even in the same individual. In young adults, LH and testosterone concentration speak in the morning before 0930 hours, and then decline by 15% and 33%, respectively, later in the day. The normal diurnal rhythm is often blunted in older men, but the reason for this is not known (15).

When viewed as an ensemble, the male gonadal system comprises three main regulatory loci:

1. Mediobasal hypothalamic neurons, which secrete GnRH into hypothalamo-pituitary portal blood
2. Anterior-pituitary gonadotrope cells, which synthesize and release LH and FSH molecules into the general circulation
3. Gonadal interstitial (Leydig) cells, which produce nominally 5 mg testosterone and 50 μg estradiol daily in young men (Fig. 1) (16–18).

The three neuroglandular loci make self-correcting adjustments via feedforward and feedback signal exchange (10,15). Physiological linkages among regulatory loci can be represented formally by time-delayed nonlinear dose-response properties (6,19). According to this integrative concept of regulation, no one component of the gonadal axis acts in isolation(11,12,20). Thus, definitive analyses require evaluating the system as an interconnected unit.

Epidemiological observations, clinical correlations, and androgen supplementation studies indicate that testosterone deprivation predisposes one to physical frailty, sarcopenia, osteopenia, diminished exercise capacity, impaired quality-of-life, glucose intolerance, visceral adiposity, dyslipidemia, reduced cognitive abilities, and sexual dysfunction (22,23,34, 37–41). Although spermatogenesis and fertility appear to be relatively preserved in older men (26,42), populational or longitudinal data are not available to verify this clinical inference. Sperm motility appears to decline in some studies. The fall in prolactin concentrations has no known pathophysiological implications, whereas the rise in FSH secretion putatively reflects diminished feedback by Sertoli-cell inhibin and systemic estradiol. Available data indicate that total, free, and bioavailable estradiol concentrations decrease in aging men and correlate with bone-mineral density changes.

Given that androgen availability declines in most healthy aging men, a practical clinical challenge is to distinguish aging-related from organic disease-mediated testosterone deficiency. Figure 2 schematizes an approach to evaluating and treating possible organic androgen deficiency (1).

■ Testosterone Deficiency in Aging Men

Age-related waning of androgen availability is evident in meta-analysis and prospective investigations (1,24,43–49). The longitudinal New Mexico Aging Process Study estimated that serum total testosterone concentrations decrease by 110 ng/dL per decade after age 61 years (47). The Baltimore Longitudinal Aging study reported an annual decrement in the molar ratio of serum testosterone to SHBG concentrations of 4.9 (pmol/nmol) (46). The Massachusetts Male Aging Study identified a nominal annual decline in testosterone availability of 1% (45). A salient point is that free (non–protein-bound) and bioavailable (non–SHBG-bound) testosterone concentrations diminish more than total testosterone (50,51). In fact, total testosterone concentrations are relatively well maintained in aging men due to a reciprocal rise in SHBG concentrations (Fig. 3) (52). Analytical methods that adjust for higher SHBG and lower albumin levels establish that the daily production rate of testosterone falls by 35% to 50%, while the half-life of free (unbound) testosterone does not change with age (6,16). Thus, reduced secretion of testosterone, rather than elevated SHBG concentrations, lowers free and bioavailable testosterone concentrations in older men. Hypoandrogenemia in elderly men is exacerbated by a variety of factors (1,15,53):

- Acute illness
- Chronic disease
- General anesthesia
- Institutionalization
- Psychosocial stress
- Hospitalization
- Certain medications
- Nutritional deficiency

Common examples include chronic lung disease, type II diabetes mellitus, sepsis, cerebrovascular accident, myocardial infarction, motor vehicle injury, hepatic or renal failure, chest, abdominal, or hip surgery, high doses of spironolactone, antifungal drugs or glucocorticoids, and weight loss or inanition.

■ Decreased LH and Testosterone Secretion in Aging

Clinical investigations indicate that older men compared with young men have low-amplitude LH and testosterone pulses (Fig. 4) (2,16,54,55). An important conceptual point is that lower testosterone concentrations should unleash LH secretion by withdrawing negative feedback (35,49,53). In fact, reduced testosterone availability does not elevate immunoreactive and bioactive LH concentrations maximally in the elderly male (15), thus pointing to diminished GnRH secretion by the brain or reduced GnRH action on the pituitary.

Smaller LH pulses in older men are not due to an impaired pituitary capacity to secrete LH, a larger LH distribution volume, or more rapid LH removal from the blood. These inferences are supported by clinical studies, which infused pulses of biosynthetic GnRH or recombinant LH in young and older volunteers (56–59). In one analysis, LH secretion was

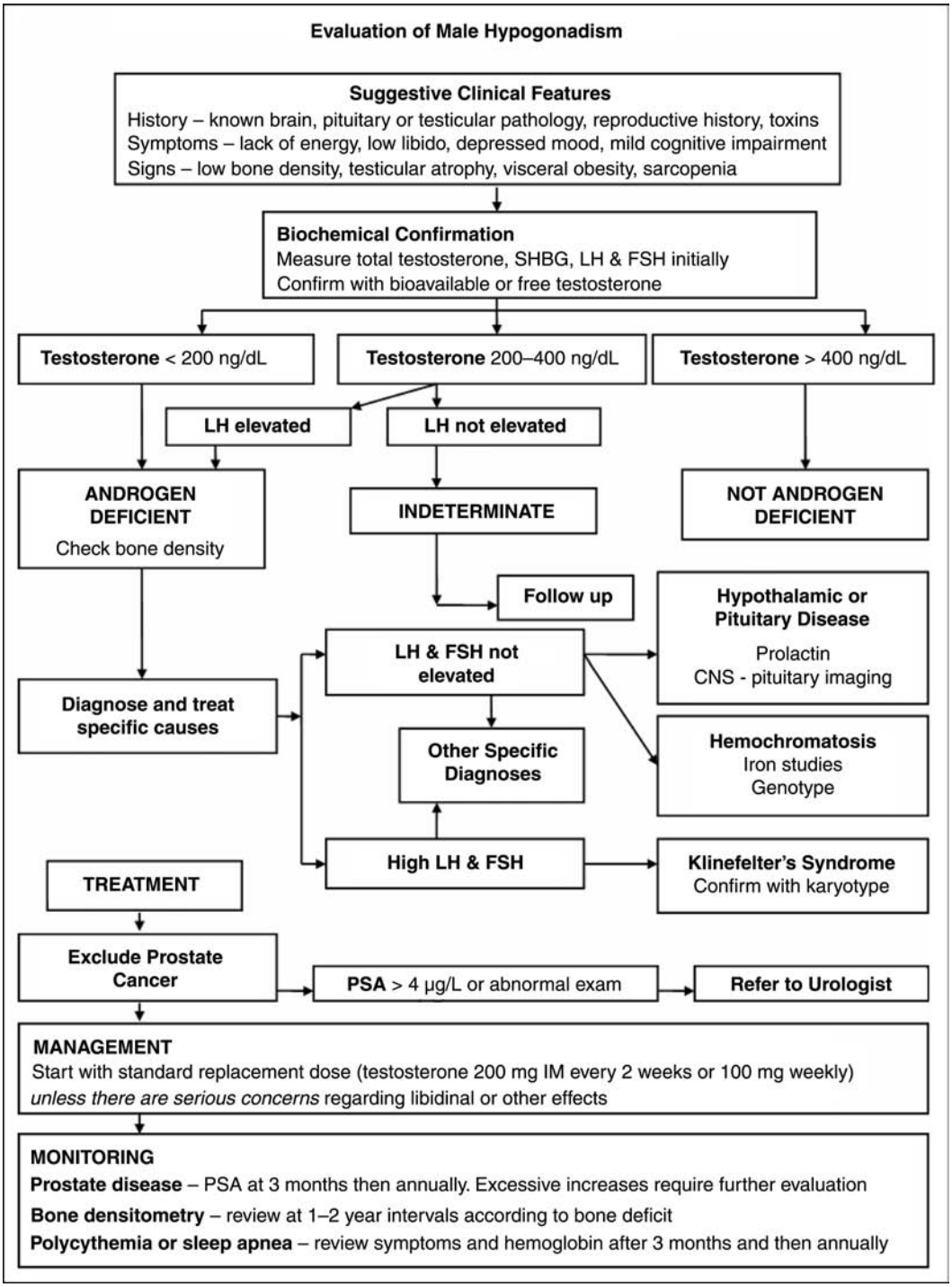


FIGURE 2 Schema of decision-based evaluation of possible hypogonadism in the male. *Abbreviations*: SHBG, sex hormone–binding globulin; LH, luteinizing hormone; FSH, follicle-stimulating hormone; CNS, central nervous system; PSA, prostate specific antigen. *Source*: From Ref. 1.

quantified after injecting a 1000-fold range of GnRH doses in a randomized order on separate days (57). When assessed during experimental testosterone depletion to obviate confounding by negative feedback, age does not alter GnRH efficacy (maximal LH secretion) but enhances gonadotrope sensitivity (slope term) and GnRH potency (leftward shift in dose–response curve).

Therefore, the deduction is that smaller spontaneous LH pulses in aging individuals reflect less hypothalamic GnRH secretion rather than unresponsive pituitary cells. In another study, prolonged intravenous infusion of GnRH pulses every 90 minutes for 14 days elicited comparable pulsatile, entropic, and 24-hour rhythmic LH secretion in older and young men (2). Despite equivalent LH concentration profiles on the last

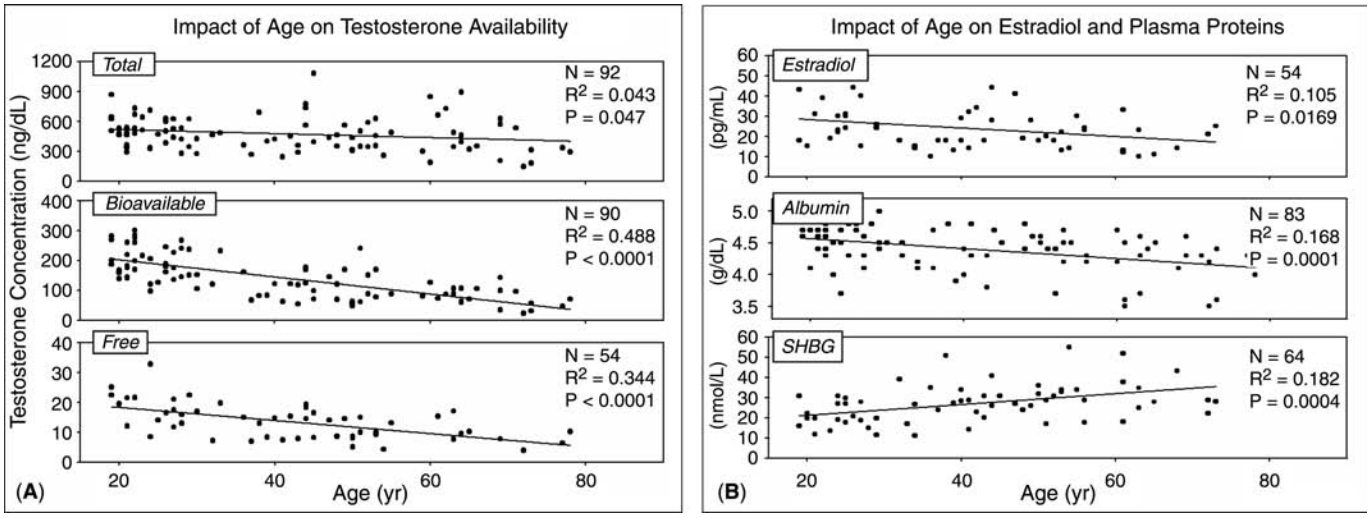


FIGURE 3 Regression of serum concentrations of total (*top*), bioavailable (*middle*), and free (*bottom*) testosterone (**A**) and estradiol, albumin, and SHBG (**B**) concentrations on age in healthy, community-dwelling, ambulatory, unmedicated men from Olmsted County, Minnesota, U.S.A. *Abbreviation*: SHBG, sex hormone–binding globulin.

day of the GnRH "clamp," elderly men exhibited 40% to 50% lower serum concentrations of free and bioavailable testosterone. This outcome signifies that age impairs testicular steroidogenic responses to endogenous LH pulses. Impairment could be due to decreased bioactivity of secreted LH and/or a primary defect in gonadal testosterone synthesis. LH bioactivity measured by in vitro Leydig cell assay is normal in the healthy community-dwelling, unmedicated older male (2,29,53,60–62). However, stress, surgery, trauma, medications, and systemic illness decrease LH biopotency at any age (63). Whether the adverse impact of morbidity on hypogonadotropism is more pronounced in older men than in young men is not known.

To stimulate the testes directly, investigators have injected large doses of human Chorionic Gonadotropin (hCG, a placental hormone resembling LH). hCG stimulation paradigms reveal decreased maximal testosterone output in the elderly male (25,64,65). Recent paradigms of pulsatile intravenous infusions of recombinant human LH to mimic physiological pituitary pulses corroborate reduced gonadal responses (Fig. 5) (58,59). Thus, clinical studies point to the following:

- Attenuated hypothalamic GnRH secretion
- Enhanced pituitary sensitivity to low doses of GnRH
- Impaired Leydig cell responses to LH in aging men

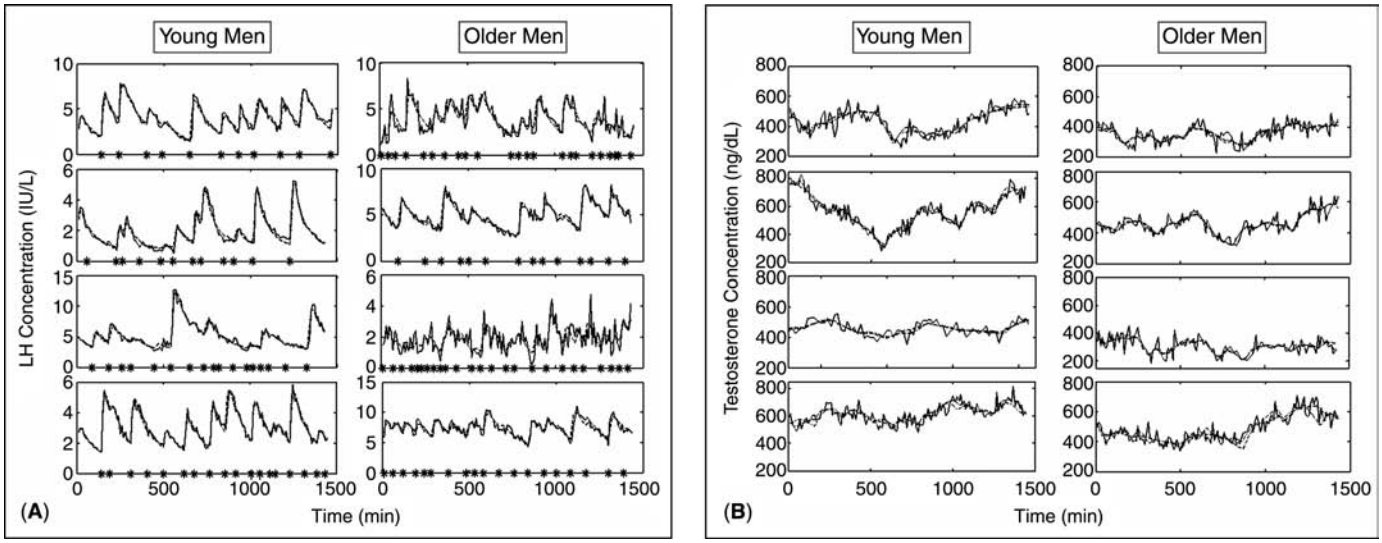


FIGURE 4 Illustrative serum LH (**A**) and total testosterone (**B**) concentration profiles in four healthy young and older men who underwent repetitive (10-minute) blood sampling for 24 hours. Asterisks on the *x*-axis mark computer-identified onsets of individual LH pulses. Continuous and interrupted lines represent measured and mathematically reconstructed hormone profiles, respectively. *Abbreviation:* LH, luteinizing hormone. *Source*: From Ref. 16.

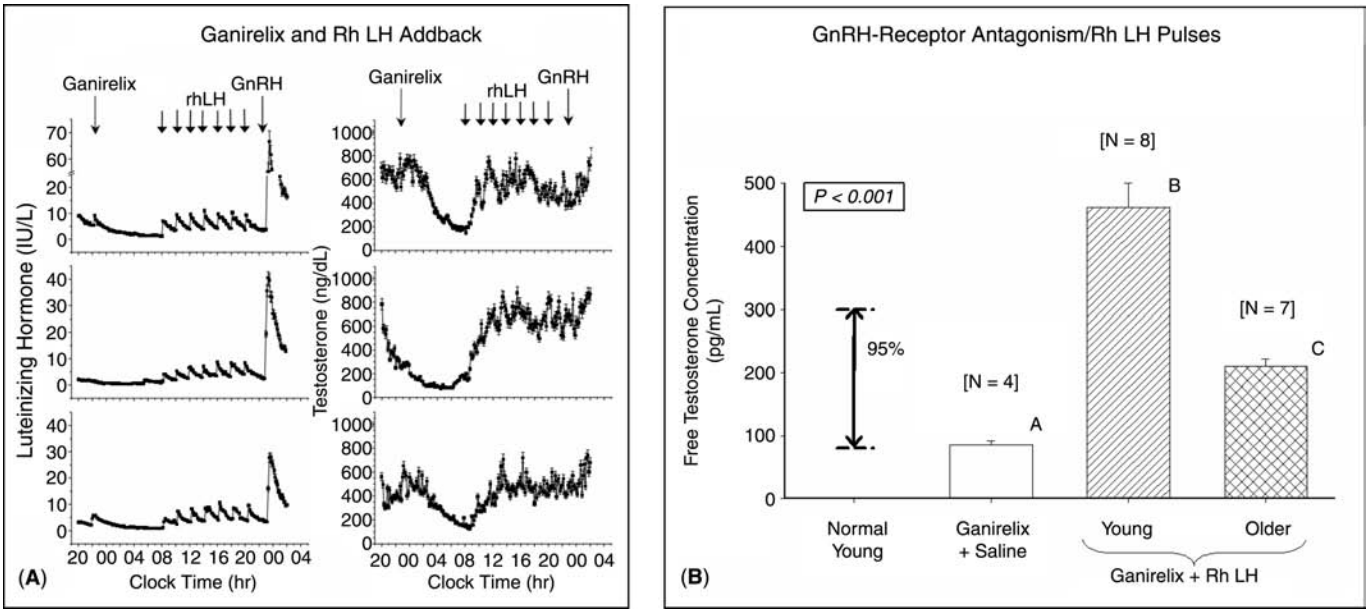


FIGURE 5 (**A**) Paradigm of testis stimulation by a brief train of intravenous pulses of rh LH. To avoid confounding effects of endogenous LH pulses, rh LH infusions were performed in the morning after overnight administration of a GnRH-receptor antagonist (ganirelix), which rapidly suppresses LH secretion. Responses of LH to injected GnRH at 0000 hour verify competitive inhibition by ganirelix. (**B**) Serum-free testosterone concentrations remain lower in older men than in young men after short-term infusion of rh LH pulses. $P < 0.001$ denotes the overall interventional effect. The three alphabetic superscripts identify significant post hoc differences in means. The normal young-adult range is given on the left side. *Abbreviations*: LH, luteinizing hormone; GnRH, gonadotropin-releasing hormone; rh, recombinant. *Source*: From Ref. 58.

■ Feedback Alterations in the Aging Male

Negative feedback onto the GnRH-LH secretory unit by androgens and estrogens is disrupted in the aging male. This conclusion derives from altered LH secretory responses to (*i*) imposition of pharmacological feedback via infused testosterone or estradiol and (*ii*) interruption of endogenous feedback by blocking androgen or estrogen receptors (29,66–71). Noninvasive mathematical analyses corroborate deficient negative feedback (6,71). Blunting of feedback could result from reduced androgen-receptor expression in the brain and/or pituitary gland of older individuals (15).

■ PULSATILE LH RELEASE IN HEALTHY OLDER MEN

The secretion of LH is demonstrably altered in aging individuals. In particular, older men compared with young men secrete LH

- at a reduced pulse amplitude (lesser increment in LH release above basal),
- at a higher pulse frequency (greater number of bursts per 24 hours), and
- with less pattern regularity (less quantifiable orderliness) (2,5,28,28,54,55,72).

Analytical models of the GnRH-LH-testosterone feedback ensemble indicate that the phenotype of the aging male gonadal axis is explicable by tripartite decreases in

- brain GnRH outflow,
- androgenic negative feedback, and
- LH-stimulated testosterone secretion (Fig. 6).

In contrast, no individual mechanism could adequately account for the full set of neuroendocrine features (6,8,11,16,71,73). A fundamental unresolved mechanistic issue

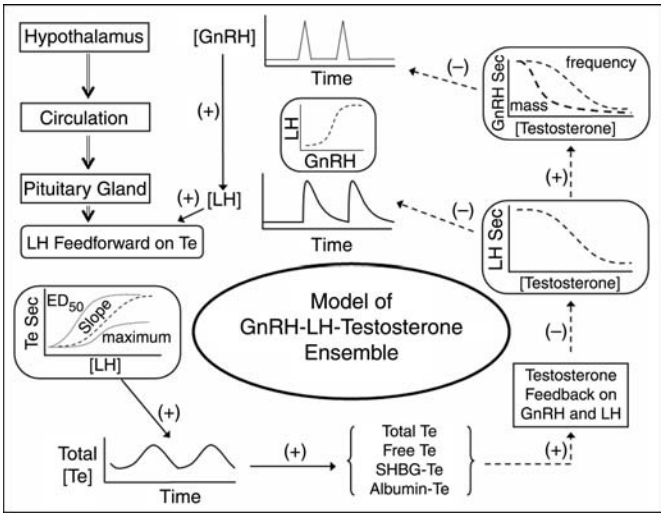


FIGURE 6 Ensemble concept of interconnected male gonadal axis. Dose-response interface functions encapsulate GnRH's drive of LH secretion (LH sec, *top center*), LH's stimulation of Te secretion (*middle left*), and Te's feedback (–) on GnRH burst frequency and amplitude as well as LH pulse amplitude (*top and middle right*). The "[LH] • Te Sec" panel illustrates dose-response properties, which change in selected pathophysiologies. *Abbreviations*: GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; SHBG, sex hormone–binding globulin; Te, testosterone. *Source*: From Ref. 11.

is the relative contribution of each putative deficit to low testosterone output in aging men (6,16,33,47). A corollary question is whether pathway defects arise together or individually and, if individually, in what order. Addressing these queries will require prospective mechanistic assessments in the same healthy individual.

Experimental observations in the old male rodent are also consistent with multilevel pathophysiology of androgen deficiency. For example, the senescent rat exhibits reductions in each of hypothalamic GnRH synapses, in vitro hypothalamic GnRH release, in vivo LH pulse amplitude, and in vitro and in vivo Leydig cell steroidogenesis (10,15).

Pharmacological inhibition of gonadal steroidogenesis can be used to lower androgen secretion reversibly (66,74). In young men, experimentally reduced testosterone concentrations induce high-amplitude, high-frequency, and disorderly patterns of LH release due to feedback withdrawal. Under the same conditions, older men achieve 50% lower interpeak minimum LH concentrations and incremental LH peak areas, signifying impaired GnRH drive of LH secretion (Fig. 7) (67). Such data refute the hypothesis that smaller LH pulses and lower valleys between pulses in older men reflect excessive feedback restraint by circulating testosterone. The same conclusion applies to negative feedback by estradiol.

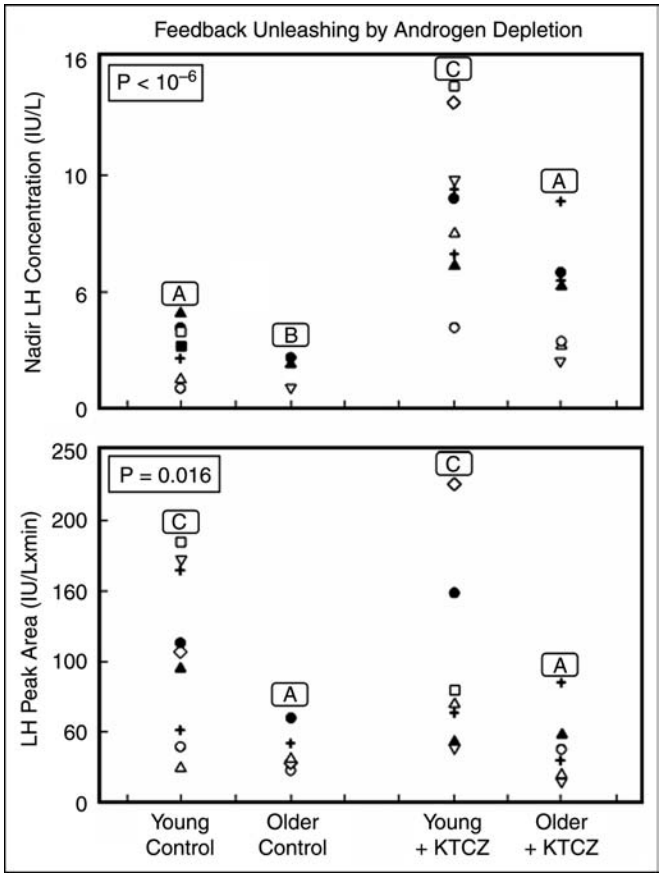


FIGURE 7 Impaired amplification of pulsatile LH release in older men administered the steroidogenic inhibitor KTCZ to decrease testosterone biosynthesis. Computer-assisted analysis was used to quantitate interpeak minimum LH concentrations (*top*) and LH peak areas (*bottom*). Data are individual values ($N = 9$ young and $N = 7$ older men). Alphabetic letters identify significantly different group means. *Abbreviations*: LH, luteinizing hormone; KTCZ, ketoconazole. *Source*: From Ref. 66.

■ EVIDENCE OF PARTIAL GNRH DEFICIENCY

Hypothalamic GnRH deficiency has been postulated to explain small LH pulses in aging men. This hypothesis originates from a variety of indirect considerations. Foremost is the incongruity of normal or minimally elevated mean serum LH concentrations despite reduced testosterone availability in many healthy, ambulatory, community-living, and unmedicated older men (25,29,31,35,49,53,54,61,75,76). Highlights this and other pertinent issues are presented in Box 3.

■ REDUCED ANDROGENIC NEGATIVE FEEDBACK IN THE OLDER MALE

Early clinical studies reported that aging men manifest any one of normal, blunted or accentuated inhibition of LH secretion by pharmacological amounts of testosterone or dihydrotestosterone delivered transdermally, intramuscularly, or by continuous intravenous infusion (32,68,78). An alternative investigative tactic is to avoid pharmacological doses and routes of testosterone exposure by muting feedback using a selective antagonist of the androgen receptor, estrogen receptor, or aromatase enzyme. This strategy discloses that sex-hormone depletion evokes smaller increments in LH pulse size in older than young men (70,83–85). The lesser LH response could reflect decreased outflow of GnRH and/or reduced expression of sex-steroid receptors in the aged brain and pituitary gland (76,86).

Recent technical advances allow one to estimate negative feedback analytically without administering sex-steroid agonists or antagonists (6,19). Noninvasive analyses independently affirm that aging attenuates negative feedback by testosterone in healthy men (6,71,75). Whether attenuation of negative feedback is a primary pathophysiological or a secondary adaptive mechanism in aging is not known. Longitudinal investigations will be needed to address this question and to establish the age dependency of altered negative feedback.

■ IMPAIRED LEYDIG-CELL TESTOSTERONE PRODUCTION IN AGING MEN

The number of Leydig cells and steroidogenic responses to hCG (an LH surrogate) decrease in aged men (35,42,64). Nonetheless, hCG stimulation studies may be criticized on several experimental grounds. First, the half-life of hCG (about 24 hours) is multifold longer than that of LH (about 90 minutes) (87). This means that hCG causes continuous stimulation, quite unlike normal LH pulses (88–90). Second, hCG binds to the LH/hCG receptor nearly irreversibly, thus downregulating gonadal steroidogenesis (91,92). And, third, pharmacological hCG administration evaluates only maximal testis responses (15). Accordingly, decreased responses to injected hCG in aging men would not explain diminished testosterone secretion in the presence of low or normal LH concentrations.

A novel approach to obviate pharmacological confounding by hCG is to stimulate Leydig-cell steroidogenesis using intravenous pulses of recombinant human LH. Infusions are given after rapidly blocking pituitary LH secretion with a selective GnRH-receptor antagonist (58,59). An antagonist is used, because treatment with a long-acting GnRH agonist results in more prolonged withdrawal of trophic LH support to Leydig cells, thereby impairing steroidogenesis (93). In a recent investigation, a GnRH-receptor antagonist was administered

BOX 3 *Disrupted Hypothalamic Regulation of the Gonadotropic Axis in Aging Male*

Major changes with aging include

- small luteinizing hormone (LH) pulses despite increased pituitary LH stores (54,55,77),
- accelerated frequency of LH secretory bursts (12,54,55),
- restitution of high-amplitude LH pulses by intravenous pulses of gonadotropin-releasing hormone (GnRH) (2),
- limited LH secretory response to opiate-receptor blockade (76),
- diminished LH secretory rise following antiestrogen exposure (29),
- impaired LH secretory response to antiandrogen administration (69,70),
- increased feedback restraint of LH secretion by exogenous androgen (32,68,78),
- greater disorderliness of LH secretion patterns (2,3,28),
- disrupted synchrony among LH, follicle-stimulating hormone (FSH), prolactin, testosterone, nocturnal penile tumescence (NPT), and sleep stages (3,4,28),
- normal or low LH bioactivity in the face of reduced testosterone availability (29,61),
- blunted 24-hour rhythmicity of testosterone and LH concentrations (2,27),
- normal or enhanced GnRH-stimulated LH, FSH, and α-subunit secretion (2,57,62,79),
- reduced in vitro GnRH secretion by hypothalamic explants isolated from aged male rats or mice (80), and
- decreased synapses among GnRH-secreting neurons in old rats (81).

Healthy older men manifest a neuroendocrine phenocopy of inappropriately low LH concentrations in the face of reduced testosterone bioavailability. This paradox defines hypothalamic hypogonadotropic hypogonadism when associated with normal or heightened pituitary responses to low doses of GnRH (Fig. 8) (57,79). An analogous condition is evident in

- sheep with surgical hypothalamo–pituitary disconnection,
- men with short-term fasting-induced hypogonadotropic hypogonadism, and
- women with anorexia nervosa, exertional amenorrhea, or hyperprolactinemia (15,31,82).

Limited histopathological studies indicate that the pituitary content of immunoreactive gonadotropins is increased in aging men (77), thus potentially explicating heightened actions of low doses of GnRH. Concomitantly reduced negative feedback by testosterone would be expected to potentiate the effects of GnRH on LH secretion.

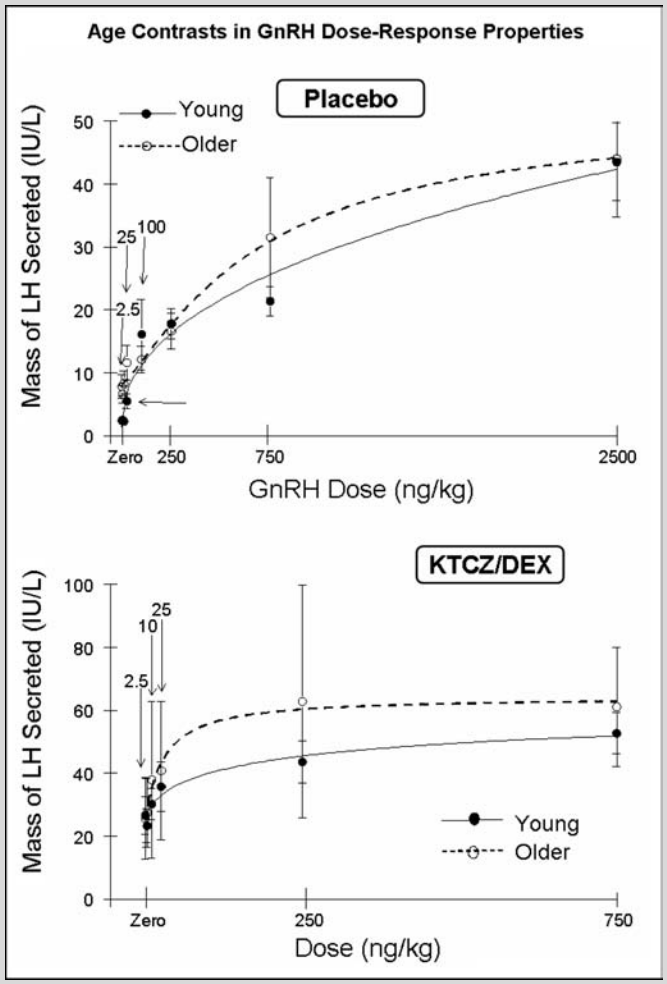


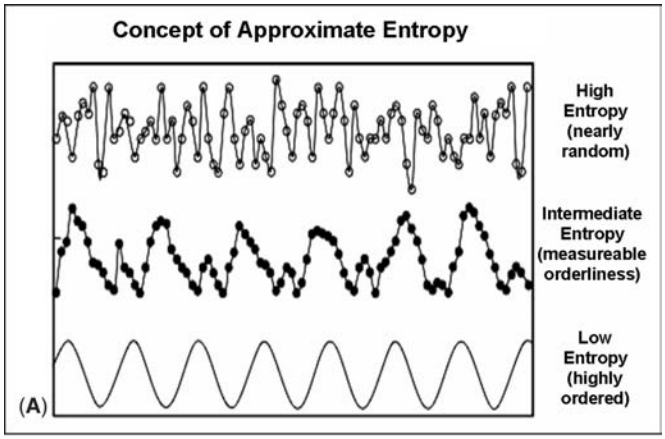
FIGURE 8 Intravenous GnRH dose–LH secretory response curves observed in young and older men. Serum LH concentrations (*y*-axis) were analyzed by immuno-chemiluminescence assay. Data (mean ± SEM) were obtained by sampling blood every 10 minutes after injecting GnRH in a randomly assigned order on separate days in young and older men. Weight-adjusted GnRH doses (*x*-axis) are given on a logarithmic scale. Volunteers were given either placebo (*top panel*) or KTCZ/DEX the night before GnRH injection to maintain or deplete androgenic negative feedback, respectively. *Abbreviations*: LH, luteinizing hormone; GnRH, gonadotropin-releasing hormone; KTCZ, ketoconazole; DEX, dexamethasone; SEM, standard error of the mean. *Source*: From Ref. 57.

once daily for two days to block LH secretion. Concomitantly, a train of 25 pulses of biosynthetic human LH was infused intravenously (as one pulse every two hours for 48 hours). The combined paradigm normalized LH and testosterone concentrations in young men but stimulated testosterone secretion by 35% to 50% less in older men (Fig. 9). In the aged male rat, continuous subcutaneous delivery of LH did not restore subsequent in vitro Leydig cell testosterone production (94). Whether prolonged administration of physiological LH pulses in older men can upregulate gonadal androgen production remains unknown.

■ MULTIPLICITY OF HYPOTHALAMIC REGULATORY ALTERATIONS

New methods of network-level analyses demonstrate that quantifiable orderliness of hormone secretion patterns confers a precise barometer of feedback and feedforward control within an interlinked regulatory ensemble (28,95). A mathematical tool used to quantify pattern regularity or reproducibility is approximate entropy (ApEn) (Fig. 10, left panel). This statistic establishes that aging disrupts the orderliness of LH and testosterone [as well as growth hormone (GH), adrenocorticotropic hormone (ACTH), cortisol, and insulin] secretion patterns (Fig. 10, right panel) (2,5,28,96). Why multiple feedback connections within neuroendocrine axes deteriorate significantly in aging is not yet known.

The complementary statistical tools of cross-correlation and cross-ApEn analyses allow one to quantify pair-wise synchrony of hormone outflow (28,71). Such methods unmask



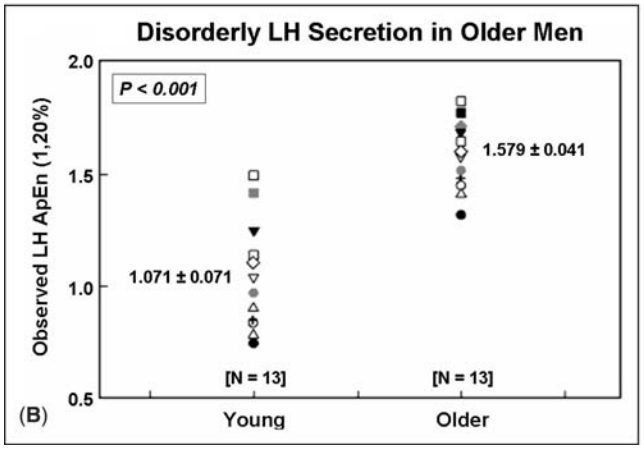


FIGURE 10 (**A**) Concept of ApEn, an ensemble statistic to quantify the orderliness (nonrandomness) of hormone secretory patterns. ApEn is a sensitive and specific barometer of integrative regulation. Cosine functions illustrate irregular (*top*), intermediate (*middle*), and orderly (*bottom*) patterns. (**B**) ApEn of 24 hours LH profiles in 13 young and 13 older men. Higher ApEn values in elderly than young individuals denote more disorderly LH release, signifying impaired feedforward and feedback coordination within the ensemble gonadal axis (28,95,96). ApEn (1, 20%) defines a normalized parameter set of $m = 1$ (window length) and $r = 20\%$ (threshold). Numerical values are the group mean ± SEM. *Abbreviations*: ApEn, approximate entropy; LH, luteinizing hormone; SEM, standard error of the mean.

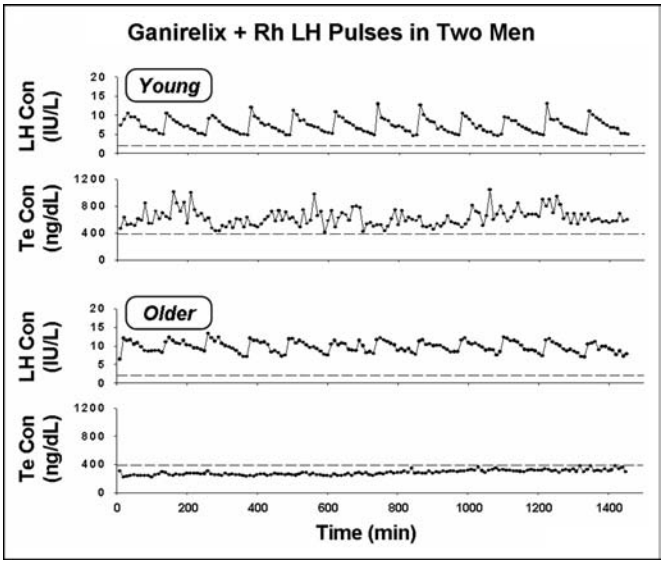


FIGURE 9 Pulses of recombinant human LH given intravenously every two hours for 48 hours normalize LH and testosterone concentrations (*paired profiles*) in young (*top*) but not in older (*middle*) men. Subjects were treated with a selective GnRH-receptor antagonist (ganirelix) to block endogenous LH secretion. Concentrations of LH and testosterone were assayed in serum collected every 10 minutes for 24 hours on the second day of the study. Data are illustrative of responses in one young and one older male. The interrupted horizontal line delineates the lower bound of normal testosterone concentrations in young men. *Abbreviations*: LH, luteinizing hormone; GnRH, gonadotropin-releasing hormone; rh, recombinant; Te, testosterone. *Source*: From Ref. 59.

significant age-related loss of synchronous coupling between LH and testosterone (28,71,75), LH and prolactin (4), LH and FSH (3), LH and nocturnal penile tumescence (NPT) (4), and LH and sleep stage (5) (Fig. 11). The multiplicity of these changes identifies broad deterioration of integrative neuroendocrine control in the aging male (2,3,5,8,28,95–97).

■ ENSEMBLE CONCEPT OF THE INTERLINKED GnRH-LH-TESTOSTERONE AXIS

The dynamic nature of the male gonadal axis can be encapsulated in a simple set of equations embodying deterministic (causally defined) linkages and stochastic (apparently random) variations (6,8,11,12,14,98,99). Deterministic connections include GnRH's drive of LH release, LH's stimulation of testosterone secretion, and testosterone's feedback on GnRH and LH outflow (Fig. 1). Stochastic features, albeit less obvious intuitively, emerge at several levels in neuroendocrine systems (Fig. 12). Variability arises in several ways:

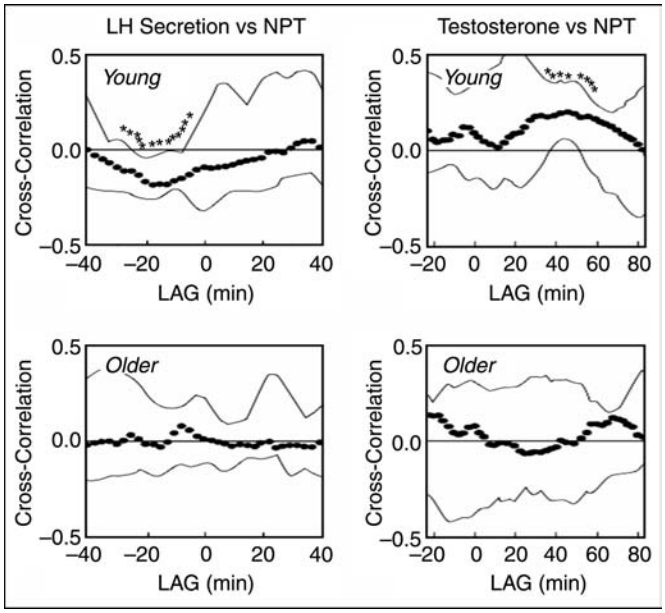


FIGURE 11 Cross-correlation plots of paired (deconvolution-estimated) sample LH secretory rates and NPT oscillations (*left*) and NPT and testosterone concentrations (*right*). Data are the median and range of cross-correlation coefficients (*y*-axis) in young (*top*) and older (*bottom*) men. Lag times (*x*-axis) represent the observed delay (minute) between correlated LH secretion rates, NPT oscillations, and testosterone concentrations. The asterisks in young men designate that NPT declines 7.5 to 32.5 minutes before LH secretion increases (*top*, *left*) and that testosterone concentrations rise 32.5 to 55 minutes after NPT increases (*top*, *right*). Both correlation features are abolished in aged individuals (*bottom*, *left*, and *right*). *Abbreviations*: LH, luteinizing hormone; NPT, nocturnal penile tumescence. *Source*: From Ref. 5.

- Laboratory measurements
- Heterogeneous tissue structure
- Nonuniform dose-response properties
- Random pulse timing
- Vascular-interstitial diffusion and advection (flow)

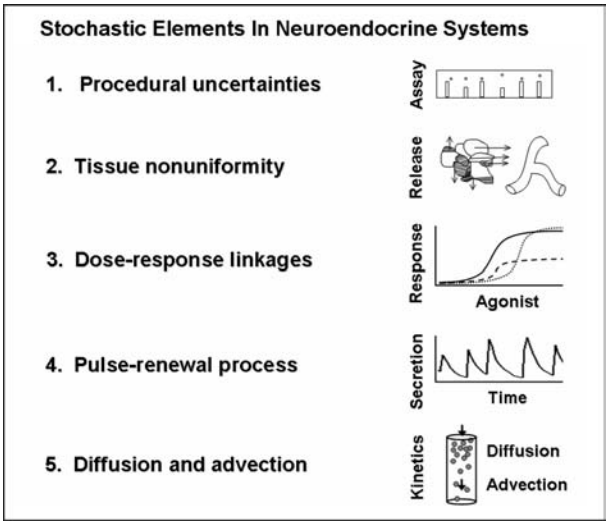


FIGURE 12 Sources of stochastic (apparently random) variability inherent in monitoring in vivo neurohormone secretion. Measurement error and unexplained biological variations together influence measured hormone concentrations.

Allowance for relevant variability in the analytical model is necessary for valid estimates of deterministic features (8,11–13, 19,98,99). Resulting biomathematical constructs of the GnRH-LH-testosterone ensemble predict that aging

- reduces GnRH outflow and thereby LH secretory-burst mass; accelerates GnRH/LH pulse frequency
- blunts the 24-hour rhythm in testosterone and LH secretory-burst massdecreases the efficacy of LH in driving testosterone secretion
- reduces feedback by testosterone concentrations on GnRH-stimulated LH secretion
- does not alter the elimination kinetics of LH or unbound (free) testosterone (Fig. 13) (6,16,19).

Accordingly, multiple interrelated deficits, rather than any single defect, explain hypoandrogenemia in the older male. This conclusion unifies many earlier reports claiming unique impairment of GnRH, LH, or testosterone secretion in agings.

■ Monohormonal Secretory Assessments

The evaluation of hormone secretion and elimination is facilitated by an array of evolving methods. Key strategies developed over the last decade include

- high-frequency and extended blood-sampling paradigms to monitor hormone secretion accurately (10,56,100)
- precise, sensitive, specific, valid, and reliable automated hormone assays (55,56)
- objective peak detection and deconvolution techniques to quantify pulsatility (98,101–103)
- the approximate-entropy (regularity) statistic to appraise the orderliness and thereby integration of hormone release (28,95)
- selective inhibitors of gonadal and adrenal steroidogenesis that cause reversible hypoandrogenemia (66,67,104)
- intravenous infusion of pulses of biosynthetic GnRH or LH by portable pump to impose a "hypothalamic GnRH clamp" or a "pituitary LH clamp" (2,59)
- graded inhibition of endogenous GnRH stimulation of gonadotropes to evaluate homeostatic adaptations in the axis (6,50).

Frequent and Prolonged Blood Sampling

The functional GnRH–LH unit operates via intermittent signaling (18). Because distinct GnRH pulses evoke individual LH pulses, quantitation of LH pulses provides a window to brain GnRH secretion (56). Validating experiments affirm that sampling blood at 5- or 10-minute (but not 20–30 minutes) intervals for 12 to 24 hours will capture the majority of detectable LH secretory episodes in healthy humans (Fig. 14) (10,100). Validated sampling protocols disclose that aging in men elevates the frequency and reduces the incremental amplitude (mass) of LH pulses by 35% to 50% (2,33,35, 54,55). Highly intensive (2.5 minutes) overnight monitoring of the gonadal axis has corroborated this inference (28,54).

Intensive blood-sampling schedules have also unveiled a 30% increase in the frequency of LH pulses in older men (2,54,55,72). More pulses could reflect less inhibition of hypothalamic GnRH pulse-generator activity due to lower concentrations of free and/or bioavailable testosterone (Fig. 15). This hypothesis is based on clinical studies in normal young men, in whom an elevated androgen concentration suppresses, whereas reduced androgen availability stimulates, LH pulse frequency (66,67). Alternatively, aging may be marked by a primary defect in the brain GnRH pulse generator that coexists with impaired Leydig cell steroidogenesis (2,64).

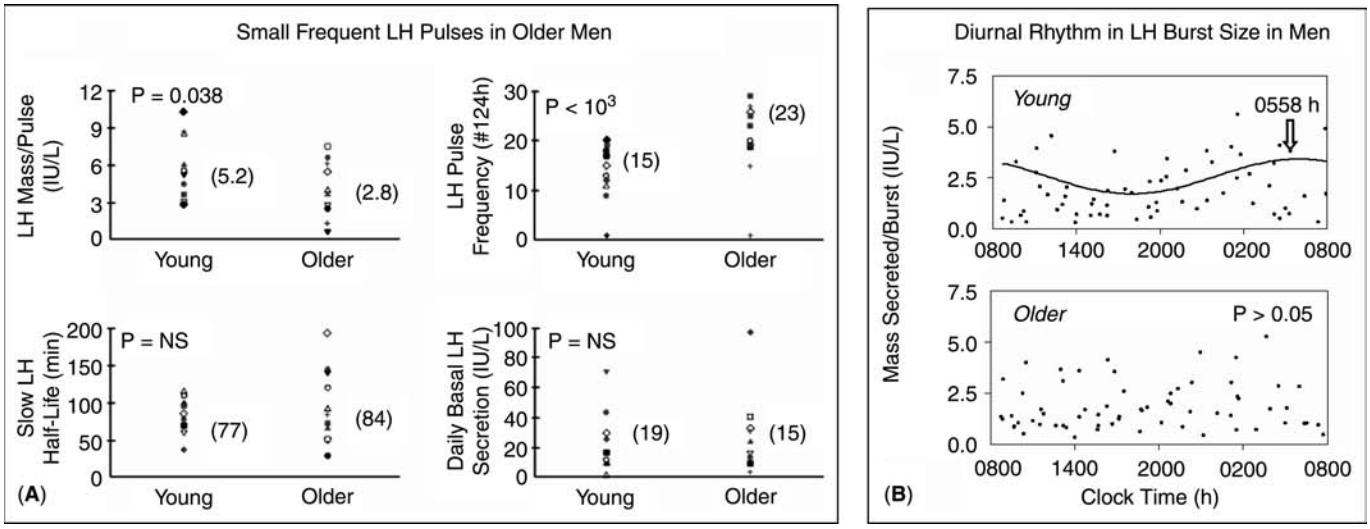


FIGURE 13 (**A**) Decreased size (mass) (*top left*) and increased frequency (number per 24 hours) (*top right*) of LH secretory bursts in older men. The slow (delayed) phase LH half-life (*bottom left*) and the basal LH secretion rate (*bottom right*) do not differ by age (98). Pulsatility was quantified by an objective deconvolution model with stochastic allowance. Numerical values are medians for the cohort of 15 men. (**B**) Loss of 24-hour rhythm in the mass of LH secreted per burst in older men. *Abbreviation*: LH, luteinizing hormone.

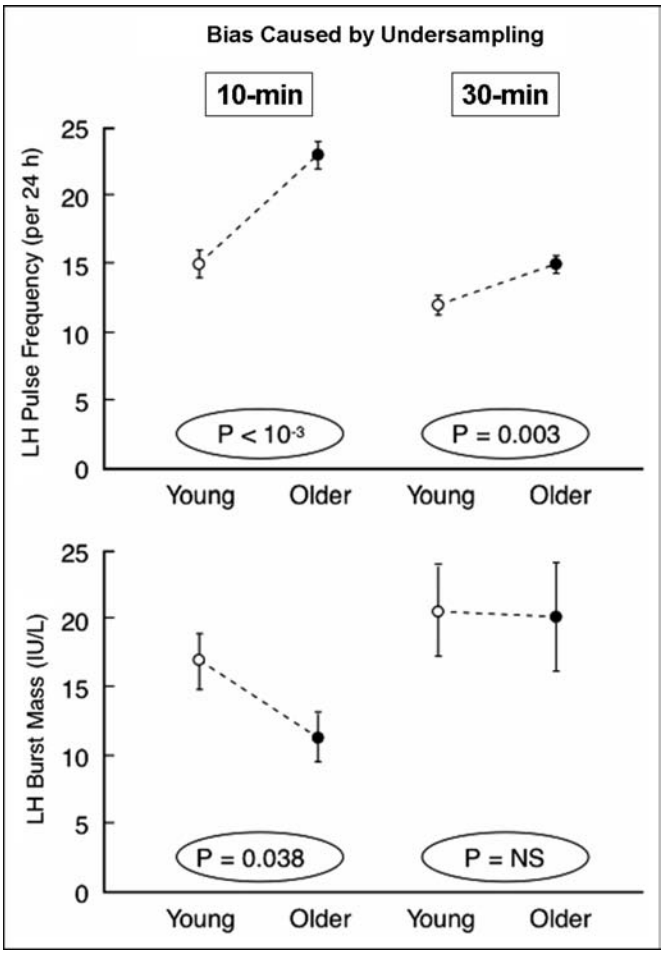


FIGURE 14 Censoring effects of sampling blood every 30 minutes (*right*) rather than every 10 minutes (*left*) on estimates of LH secretory-burst frequency (*top*) and mass (*bottom*) in 13 young and 13 older men. Data are the mean ± SEM. *Abbreviations*: LH, luteinizing hormone; SEM, standard error of the mean.

LH Assays

Quantitation of LH concentrations requires reliable, valid, specific, sensitive, and precise assay technology. Immunoradiometric, immunofluorometric, and chemiluminescence-based assays meet these requirements and correlate well with in vitro LH bioassays (10,29,60,69,87). To ensure high precision, one should use a fully automated (robotics) assay system and model-free data reduction (10,15,95,98).

Enumerating LH Concentration Peaks and Quantifying Underlying LH Secretory Bursts

Discrete peak-detection techniques permit one to identify pulses in serial hormone-concentration measurements, viz.,

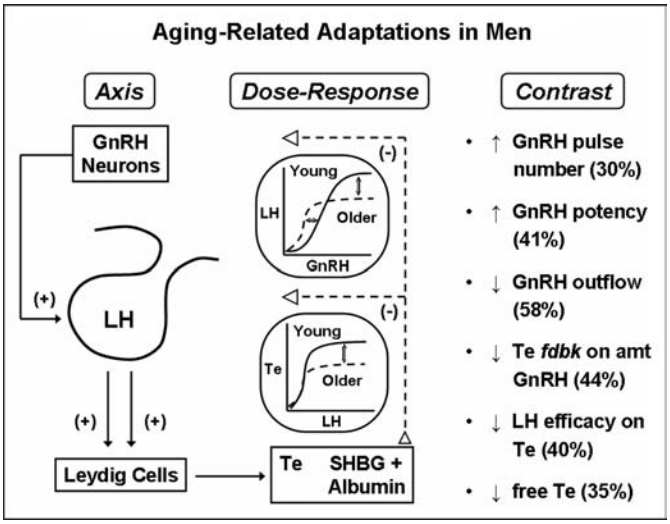


FIGURE 15 Inferred regulatory defects in the aging male GRH-LH-testosterone-SHBG feedback axis. *Abbreviations*: SHBG, sex hormone–binding globulin; LH, luteinizing hormone; GnRH, gonadotropin-releasing hormone; Te, testosterone.

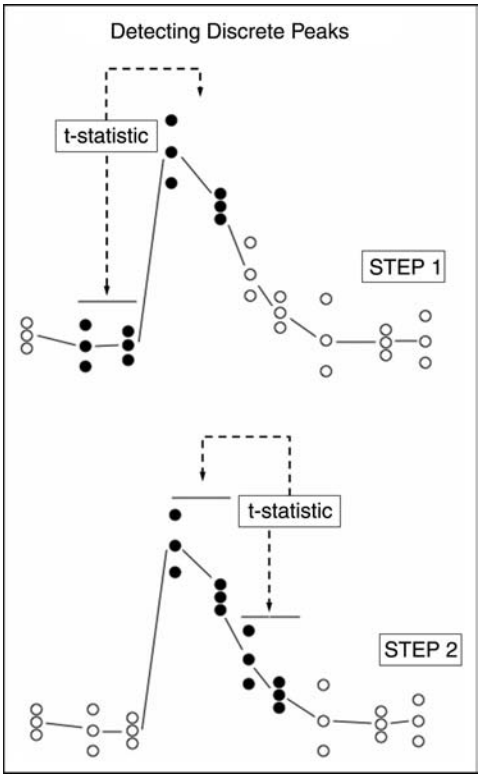


FIGURE 16 Concept of statistically based two-step "cluster" analysis of hormone concentration peaks. This model-free algorithm scans time series using a pooled-variance *t*-test to mark significant upstrokes (Step 1) and downstrokes (Step 2). Upstrokes and downstrokes demarcate a discrete pulse (101).

hormone profiles (10). Such techniques do not quantify underlying secretion, distribution, or elimination per se. One validated method is model-free cluster analysis (Fig. 16) (101), which has been tested by biomathematical simulations, LH infusions in gonadotropin-deficient men, GnRH injections in patients with Kallmann's syndrome (isolated GnRH deficiency), sampling GnRH and LH in hypothalamo–pituitary portal blood in sheep, and recording brain electrophysiological correlates of GnRH release in monkeys (56,105). Sensitivity and specificity of the cluster method are about 85% for 10-minute LH data.

A new generation of methodologies is subsumed under the generic term deconvolution analysis (15,98,102,103). The etymology denotes unraveling or unfolding of interrelated factors such as secretion and elimination rates that jointly determine a hormone profile. From an analytical perspective, four mechanisms contribute simultaneously to measured hormone concentrations, namely,

- the number of pulses and the amount (mass) of hormone secreted within each burst,
- the endogenous hormone half-life (kinetics),
- concurrent basal (nonpulsatile) secretion, and
- prior secretory output that continues to decay during the observation interval (13,98,102,103).

Two complementary classes of deconvolution procedures exist; viz., model-defined (parametric) and waveform-independent (nonparametric) methods. Model-based techniques are designed to estimate the number, size, and shape of secretory bursts and quantify hormone kinetics simultaneously (Fig. 17). Both discrete peak-detection and deconvolution analyses identify smaller and more frequent LH pulses in older men (2,54,55,73,98).

ApEn of Hormone-Release Patterns

In addition to pulsatile features, hormone time series exhibit variable degrees of regularity or orderliness. Regularity denotes the serial reproducibility of subpatterns in the data (Fig. 10). Orderliness is quantified via regularity statistics, such as ApEn (95–97). The ApEn metric is applicable to series containing as few as 30 consecutive measurements. On theoretical grounds, ApEn provides a sensitive barometer of feedback and feedforward alterations within an integrated network (3,28,95,96). For

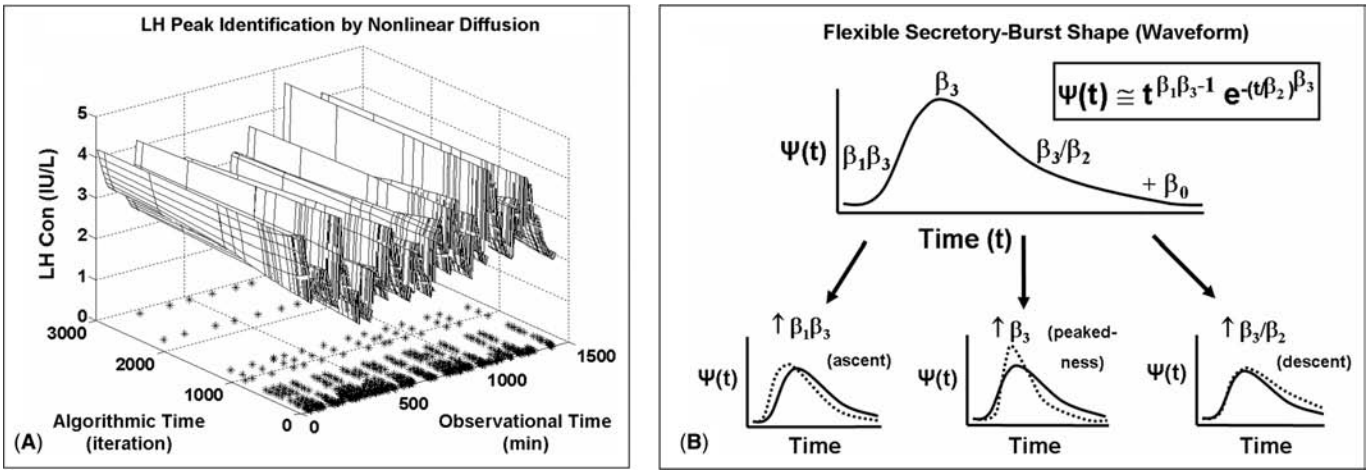


FIGURE 17 Illustrative deconvolution procedure to estimate pulse-onset times objectively by nonlinear diffusion (**A**) and determine the flexible shape of underlying secretory bursts (**B**). The algorithm quantifies hormone half-life and basal secretion simultaneously. *Abbreviation*: LH, luteinizing hormone. *Source*: From Refs. 98, 103.

example, ApEn calculations show that endocrine tumors secrete GH, ACTH, prolactin and aldosterone in a disorderly fashion; GH secretion patterns are less consistent in the female than male rat and human; and LH, testosterone, ACTH, cortisol, GH, and insulin output is more irregular in older than young adults 333 (2,3,28,31,95). Irregularity signifies disruption of multisignal integration, which is required to maintain reproducible patterns in complex systems.

Cross-ApEn provides an analogous two-variable regularity statistic, which quantifies the degree of synchronicity of paired hormones. Cross-ApEn analysis of LH and testosterone profiles in older men discloses marked deterioration of bihormonal synchrony (2,28). To establish the basis for loss of coordinate control requires "localizing" experiments, which test specific feedforward and feedback connections. In this regard, cross-ApEn analyses of paired LH–testosterone, LH–prolactin, LH–FSH, LH–NPT, LH–sleep, and NPT–sleep document decreased synchrony of coupled neurohormone outflow in aging men (Fig. 18) (5).

Exogenous GnRH "Clamp" Paradigms

Infusion of synthetic GnRH can be used to stimulate pituitary LH secretion directly. Illustrative applications include studies of aging, hyperprolactinemia, anorexia nervosa, Kallmann's syndrome (isolated GnRH deficiency), and fasting-induced hypoandrogenemia (2,25,72,79). In one analysis, intravenous infusion of GnRH pulses every 90 minutes for 14 days via a portable pump evoked comparable pulsatile, diurnal, and entropic patterns of LH secretion in five older and five young men (2). However, the GnRH clamp failed to elevate 24-hour mean free testosterone concentrations equivalently in older men (Fig. 19). This outcome points to impaired Leydig cell steroidogenesis and/or reduced biological activity of secreted LH in aging. The latter hypothesis is rendered unlikely by comparable estimates of plasma LH concentrations by in vitro Leydig cell bioassay in healthy young and older men (2,63).

Ketoconazole-Induced Steroidogenic Blockade to Enforce Androgen Withdrawal

High doses of the antifungal agent, ketoconazole, block testicular and adrenal steroidogenesis. Thus, the drug can be used to induce acute reversible hypoandrogenemia in healthy men (66,67,104). Ketoconazole lowers testosterone concentrations by approximately 85% (from 550 to 105 ng/dL), thus withdrawing negative feedback and unleashing more frequent and larger LH pulses (74). Disinhibition of androgenic feedback by this means discloses that older men fail to achieve maximal increments in LH secretory-burst size (Fig. 7) (84). The same outcome is observed after blocking estrogenic negative feedback using the aromatase inhibitor, anastrozole, and antagonizing testosterone negative feedback using the androgen-receptor antagonist, flutamide (70,84,85).

At very high gonadotropin pulse frequencies, short-lived uncombined α subunit peaks are sometimes more vivid then

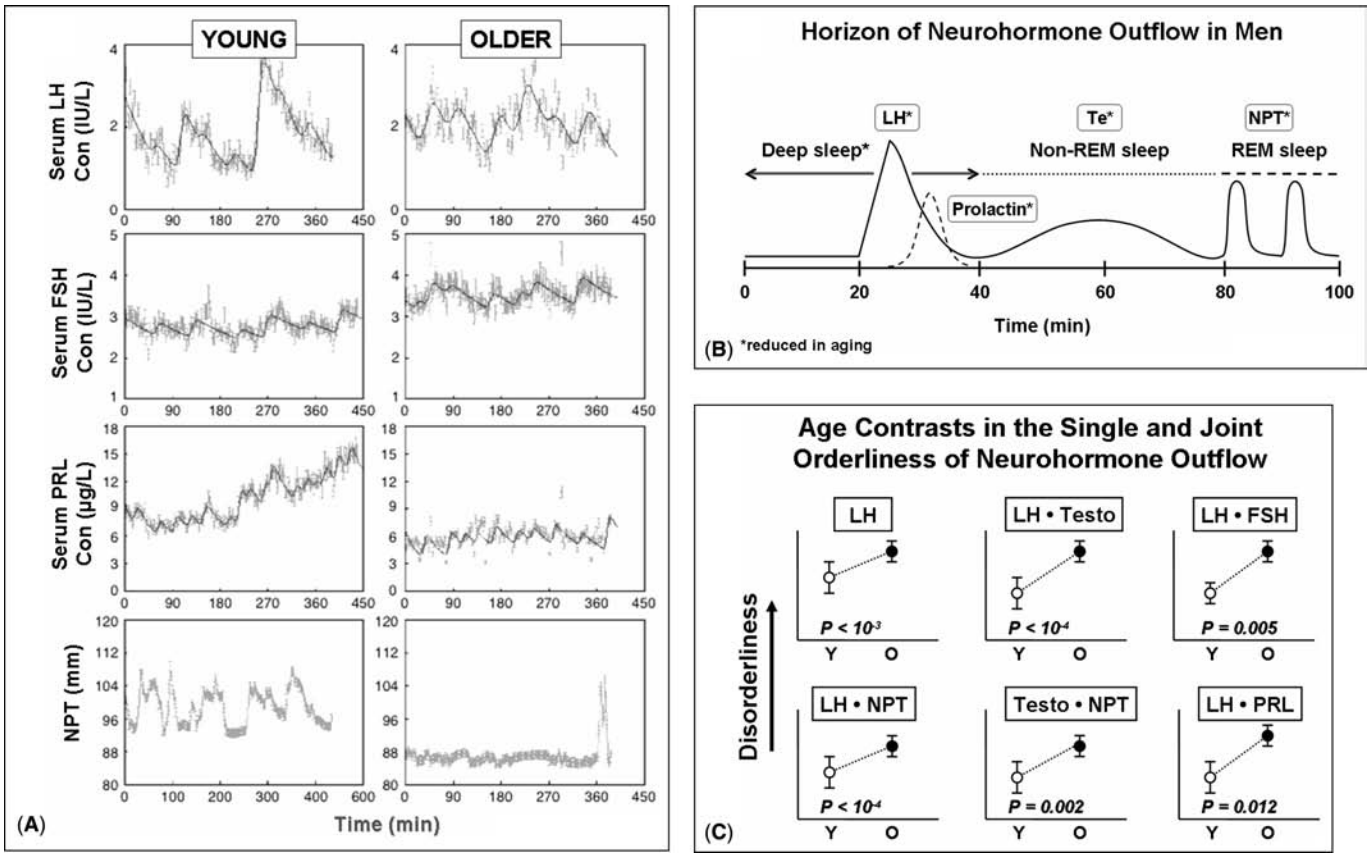


FIGURE 18 (**A**) Illustrative LH, FSH, and PRL concentration profiles and NPT oscillations in one young (*left*) and one older (*right*) man monitored every 2.5 minutes overnight (4,30,33). (**B**) Evolution of neurohormonal outflow during sleep in men. (**C**) Greater irregularity (disorderliness) of LH secretion (*top left*) and lesser synchrony of coordinate outflow of LH-Te, LH-FSH (*top middle* and *right*), LH-NPT, Te-NPT, and LH-PRL (*bottom row*) in older men than in young men. *, reduced in aging. *Abbreviations*: LH, luteinizing hormone; FSH, follicle-stimulating hormone; PRL, prolactin; NPT, nocturnal penile tumescence; Te, testosterone. *Source*: From Refs. 4,5.

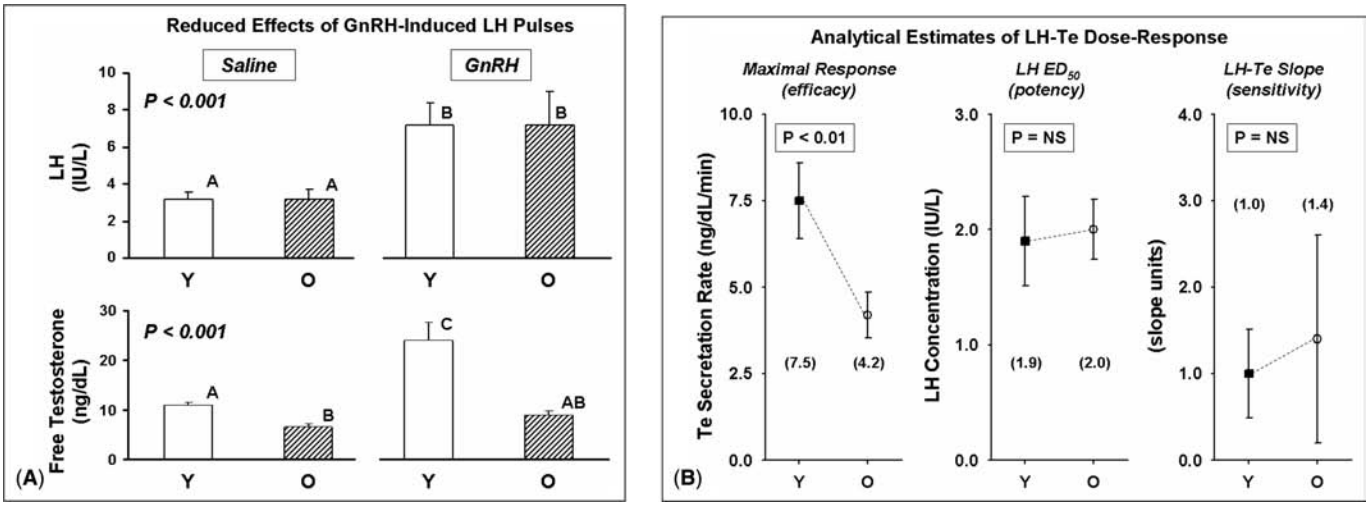


FIGURE 19 (**A**) Reduced free testosterone concentrations in older men compared with young men despite equivalent mean LH concentrations achieved by 14 days of intravenous pulses of GnRH. Responses are compared with those after saline infusion in the same subjects. (**B**) Age decreases the efficacy (maximal effect) but not the potency (one-half maximally stimulatory concentration) of endogenous LH pulses in driving testosterone secretion in healthy men. *Abbreviations*: LH, luteinizing hormone; GnRH, gonadotropin-releasing hormone. *Source*: **A**, from Ref. 2; **B**, from Ref. 16.

LH pulses (79). Thus, measuring (free) α subunit concentrations along with frequent blood sampling and deconvolution analysis should optimize reliable estimates of more rapid pulse frequencies (56,66).

Infusion of Recombinant Human LH Pulses as a "Pituitary Clamp"

Gonadotrope downregulation followed by intravenous injections of recombinant human LH provides a paradigm in which to test gonadal steroidogenesis without confounding by endogenous LH secretion. This stratagem is outlined in (58,59,93). Impaired responses of testosterone to biosynthetic LH in aging are illustrated in Figures 5 and 9. Whether the impairment is due to reduced LH efficacy, LH potency, or testis sensitivity is not yet known. However, analytical estimates of endogenous LH–testosterone feedforward forecast a 45% reduction in LH efficacy (maximal stimulation) in older men (16). Age does not change calculated LH potency (one half-maximally stimulatory LH concentration averages 2.0)IU/L) or testis sensitivity (dose-response steepness averages 1.2 slope units) (Fig. 19, panel B).

Graded Inhibition of Endogenous GnRH Action

Administration of a selective competitive GnRH-receptor antagonist rapidly suppresses LH and thereby testosterone concentrations. The degree of suppression is directly proportional to the dose of the antagonist and inversely related to the outflow of brain GnRH. Mathematical modeling of this paradigm predicts that age reduces hypothalamic GnRH outflow by 50% and negative feedback by a similar amount in healthy men (6,50,106). To illustrate this conclusion, Figure 20 depicts analytical estimates of LH secretion, virtual GnRH release, and endogenous testosterone feedback in young and older cohorts.

■ Paradigmatic Tests of Altered System Feedback Control

The precise mechanisms that mediate a reduction in LH secretory-burst mass, an elevation in LH pulse frequency, and more irregular LH secretion in aging men are not known. Relevant hypotheses can be explored by computer-assisted models of the GnRH-LH-testosterone ensemble (6,8,11,12, 16,19). One biomathematical construct incorporates reciprocal feedback and feedforward (dose-response) connections among GnRH, LH, and testosterone signaling (Fig. 21). The model allows one to test the impact of postulated effects of aging on the pulsatility, orderliness, and diurnal rhythmicity of GnRH, LH, and testosterone secretion. Although informative, model-based approaches confront several unresolved issues, as highlighted in Box 4.

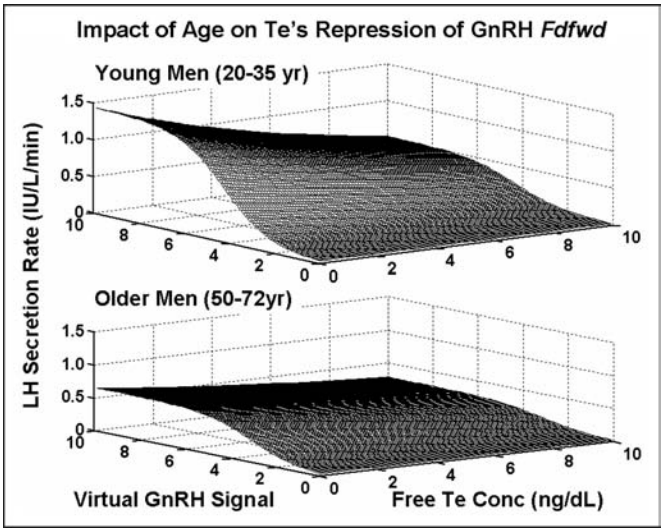


FIGURE 20 Three-dimensional response surface showing how unobserved brain GnRH drive (*oblique* axis) and Te negative feedback (*horizontal* axis) together control LH secretion (*vertical* axis) in young (*top*) and old (*bottom*) men. Aging limits both outflow of GnRH to pituitary gonadotropes (magnitude of virtual GnRH signal) and negative feedback by testosterone on GnRH drive. *Abbreviations*: LH, luteinizing hormone; GnRH, gonadotropin-releasing hormone; Te, testosterone. *Source*: From Ref. 6.

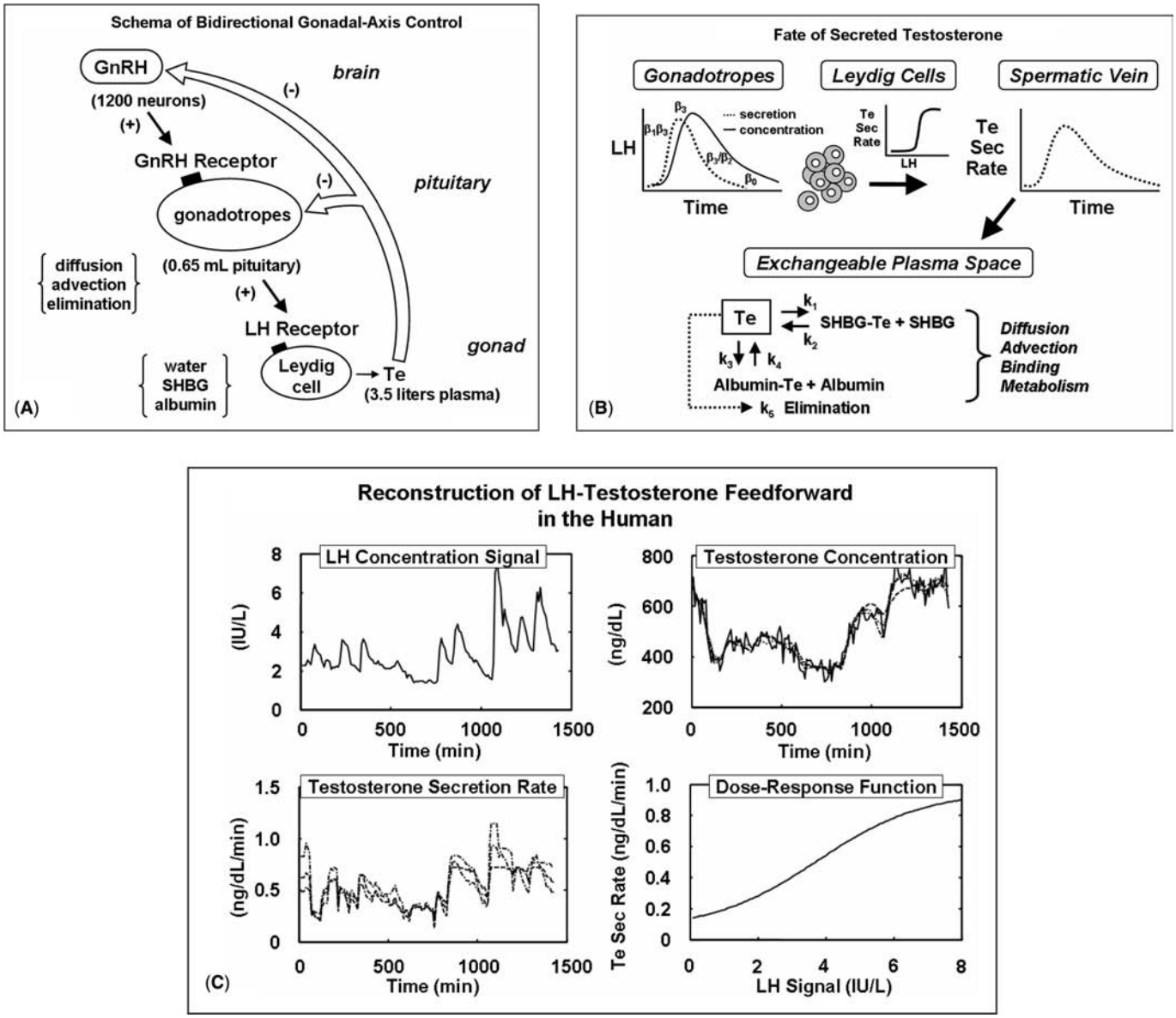


FIGURE 21 (**A**) Basic feedback and feedforward connections among GnRH (*top left*), LH (*middle*), and Te (*bottom right*). Equations can be written for primary feedforward (*short solid arrows*) and feedback (*broad open curved arrows*) connections in the male gonadal axis (6,19). (**B**) Schema of how LH secretory bursts (*top left*) act via a dose-response function (*top middle*) to stimulate Te secretion into the spermatic vein (*top right*). Secreted Te molecules undergo reversible diffusion (random dispersion), advection (linear motion due to blood flow), binding to SHBG or albumin, and irreversible elimination. (**C**) Analytical estimation of LH-Te dose-response function noninvasively in a young man. Data are measured and computed LH and Te concentrations (*top left* and *right*) calculated Te secretion rates (*bottom left*) and the estimated dose-response function linking LH concentrations to Te secretion rates (*bottom right*). *Abbreviations*: LH, luteinizing hormone; GnRH, gonadotropin-releasing hormone; SHBG, sex hormone–binding globulin; Te, testosterone. *Source*: Adapted from Ref. 16.

BOX 4 *Selected Neuroendocrine Modeling Issues in the Aging Male Reproductive Axis*

- In what order do putative regulatory deficits evolve in aging?
- Do feedforward and feedback fail equally in older men?
- Why does hypothalamic gonadotropin-releasing hormone (GnRH) outflow decrease with age?
- Which alterations in the aging male reflect primary pathophysiology vis-à-vis secondary feedback adjustments?
- What mechanisms account for greater intersubject variability in older than young cohorts?

BOX 5 *Alterations in the Aging Male Reproductive*

Hypothalamus
- ↓ Output of gonadotropin-releasing hormone (GnRH) to drive luteinizing hormone (LH)
- ↓ Suppression by testosterone feedback
- ↓ Sleep-related erections

Pituitary gland
- Normal total LH stores
- ↑ LH release after low doses of GnRH
- ↑ Follicle-stimulating hormone (FSH) secretion
- ↓ Prolactin secretion

Testis
- ↓ Testosterone synthesis
- ↓ LH efficacy to stimulate androgens
- ↓ Estradiol synthesis
- putatively preserved spermatogenesis

Blood
- ↑ Sex hormone–binding globulin (SHBG) concentrations
- ↓ Albumin concentrations
- ↓ Metabolic clearance of testosterone

Sexual organs
- ↓ Testes size
- ↑ Prostate size

Central nervous system (CNS)
- ↓ Libido and potentia
- ↓ Stages III and IV deep sleep

Target tissues
- ↓ Bone mass
- ↓ Muscle strength
- ↓ Aerobic capacity
- ↑ Visceral adiposity
- ↑ Insulin resistance

■ CONCLUSIONS

The reproductive system maintains fertility, sexual behavior, and physiological availability of sex steroids by adaptive signaling among the brain, endocrine glands, and target tissues. According to this general thesis, the male gonadotropic axis should be viewed as an ensemble. The ensemble comprises specialized cortical regions in the brain, which integrate visual, emotional, and stress-related cues; hypothalamic neurons, which secrete the decapeptide GnRH in discrete bursts; gonadotrope cells in the pituitary gland, which release distinct pulses of LH; the circulation, which delivers LH to the testes; Leydig cells in the testes, which produce testosterone in response to LH; SHBG and albumin in blood, which transport testosterone and estradiol; and target tissues, such as brain, pituitary, muscle, bone, fat, and sexual organs, which respond to sex steroids. The interlinked system as a whole, rather than any one locus of control, mediates reproductive health and androgen availability. Aging forces multilevel failure within the male gonadal axis (Box. 5). Prospective studies are needed to ascertain the precise tempo and order in which regulatory deficits emerge.

■ ACKNOWLEDGMENTS

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12

The Thyroid, Parathyroid, and Pineal Glands

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INTRODUCTION

The thyroid gland, a key organ in the prenatal and postnatal whole-body growth and differentiation and maturation of the central nervous system, continues to influence the whole organism as it ages, particularly with respect to vital functions such as basal metabolism and thermoregulation. As in the case of other neuroendocrine systems (Chapters 9–11), in this chapter, the thyroid will be examined, first, within the framework of a three level axis, the hypothalamo-pituitary-thyroid axis (HPT). Second, by virtue of their intimate anatomic association with the thyroid gland, changes with aging of the parathyroid cells secreting parathyroid hormone (PTH) and of the thyroid C cells secreting calcitonin (CT) are included in this chapter. Third, also included in this chapter is a discussion of the aging of the pineal gland and its role as a photoneuroendocrine transducer, as a synchronizer of the sleep-wake, and night-day cycles, and as a possible contributor to those homeostatic networks that regulate the life span.

THE THYROID GLAND

Thyroid Function and Rejuvenation?

The hormones of the thyroid gland, primarily thyroxine (T4) and triiodothyronine (T3), and, to a minor extent, reverse triiodothyronine (rT3), have traditionally engaged the interest of investigators seeking hormonal determinants of aging. Early studies in humans compared certain signs of aging to those of thyroid insufficiency or hypothyroidism. Hypothyroid individuals develop a number of signs that might be interpreted as "precocious senility," including: reduced metabolic rate, hyperlipidemia and accelerated atherosclerosis, early aging of skin and hair, slow reflexes, and slow mental performance (1–4). Because such patients improve markedly after hormonal replacement therapy, it was argued that these symptoms may represent effects secondary to thyroid involution with old age. The well-known action of thyroid hormones in controlling whole-body growth and brain development made it reasonable to suspect that these hormones might also control the rate or site of aging.

In the early decades of the twentieth century, researchers optimistically attempted rejuvenation or prolongation of life through hormone administration [analogous to the current hormone replacement therapy (Chapters 9–13)]. In later studies, while thyroid hormone administration to some animals (mice) seemed to shorten rather than prolong life (5), in others (fowl), it caused an apparent dramatic rejuvenation (6). Delayed and impaired growth and maturation associated with a significant prolongation of the life span were also reported in rats made hypothyroid at an early (first postnatal week) age (7,8) and mimicked the effects reported after calorie restriction (9) (Chapter 23). In contrast, life span was shortened when rats were made hyperthyroid (by injections of high doses of exogenous T3). However, the aging process was not significantly slowed or altered in most elderly euthyroid (with normal thyroid state) individuals and the life span did not appear to be affected by either hypo- or hyperthyroidism (10,11).

The capacity to maintain a euthyroid state continues into old age and is well preserved in centenarians despite a number of changes in various aspects of thyroid hormone production, secretion, and action (12–14). However, as for all other endocrines, normal thyroid function in the elderly may easily be endangered by repeated challenging demands and stress; given the physiologic importance of thyroid hormones, the ensuing dysthyroid (abnormal) state might lead to decreased overall functional competence, disease, and aging (15).

During development, the thyroid gland is necessary for whole-body and organ growth, and for development and maturation of the central nervous system. In adulthood, it has an essentially metabolic function, regulating tissue oxygen consumption, and thereby maintaining metabolic rate and body temperature. Although the thyroid gland is not essential for life, in its absence, there is poor resistance to cold, mental and physical slowing, and, in children, mental retardation and dwarfism. Reciprocally, in hyperthyroidism, metabolic, and behavioral alterations threaten well-being and survival (15). Some morphologic, structural, and physiologic characteristics of the thyroid gland and the HPT axis are presented in Figures 1 to 3, Boxes 1 and 2, and Table 1.

Structural Changes with Aging

From maturity to old age, the size of the thyroid gland decreases, although in some cases, the weight of the gland may remain unchanged or even increase. The weight increase is usually due to the presence of nodules; these are lumps or masses of hyperplastic (i.e., increase in cell number) and hypertrophic (increase in cell size) cells. Nodules may be small (micronodules) or large (clinically palpable) nodules and they may remain benign or may lead to cancer, especially in women who have nearly three times as many thyroid cancers as men (16,17). The pre-valence of both large and small nodules may increase or decrease with aging (Figures 4); the variability of this finding reflects:

1. Geographic differences (e.g., endemic goiter in regions with insufficient iodine in the drinking water)
2. Poor dietary habits
3. Failure to meet the increased metabolic demands of the organism that may occur during pregnancy or adolescence

An underactive thyroid (hypothyroidism) often leads to a mild endemic goiter, that is, an enlargement of the thyroid

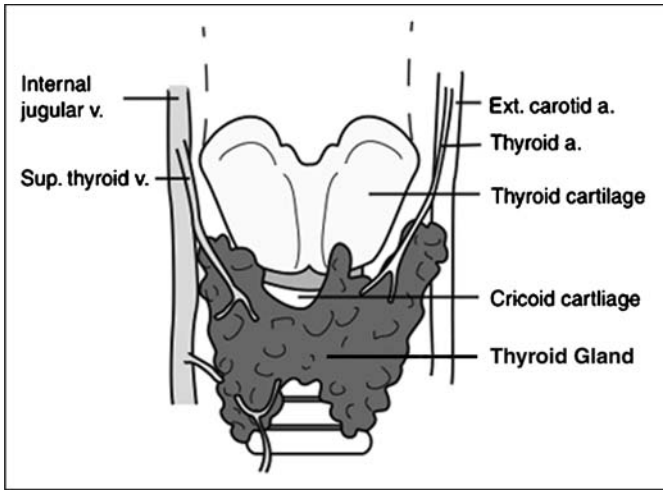

FIGURE 1 Diagrammatic representation of the thyroid gland. The thyroid and cricoid cartilages and the major blood vessels are included. Note that four parathyroid glands are imbedded in the superior and inferior poles of the thyroid gland. C, cells secreting calcitonin are dispersed throughout the thyroid gland. *Abbreviations:* a, artery; ext., external; sup, superior; v. vein.

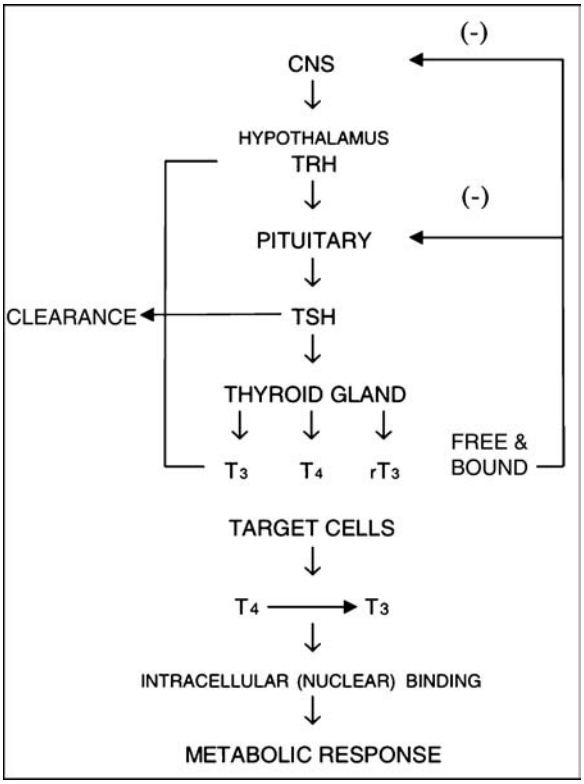

FIGURE 3 Diagram of the interrelations of the hypothalamo-pituitary-thyroid axis. *Abbreviations*: CNS, central nervous system; TRH, thyrotropin-releasing hormone; TSH, thyroid stimulating hormone; T3, triiodothyronine; T4, thyroxine; rT3, reverse triiodothyronine.

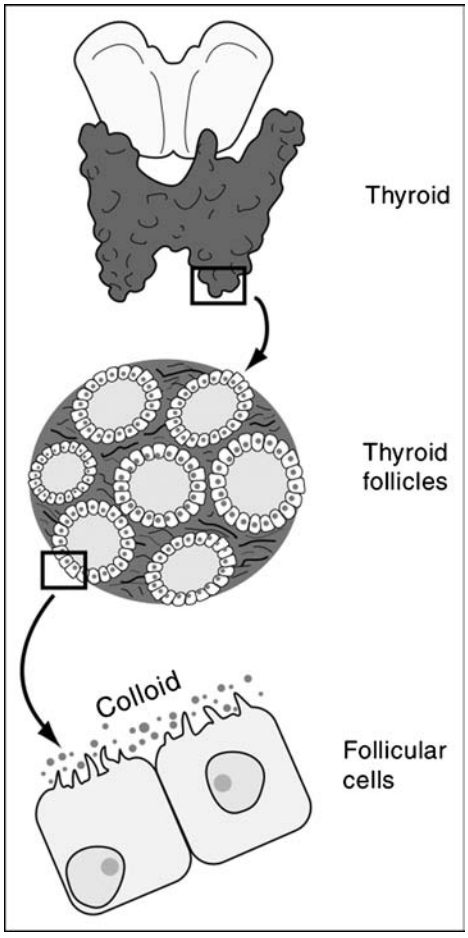

FIGURE 2 Diagrammatic representation of the thyroid (*top*), the thyroid follicles (*middle*), and the follicular cells (*bottom*): the microvilli from the follicular cells project into the follicular colloid.

gland, causing a swelling in the front part of the neck, usually due to iodine deficiency (18,19). Multinodular goiter is also associated with increased antithyroid antibodies (see below).

In the majority of elderly individuals, the general shape of the gland remains unchanged, although microscopic changes are frequent and suggest reduced function (Table 2). When the cells are inactive, as in the absence or deficiency of thyroid-stimulating hormone (TSH) or in some old individuals, follicles are large, more colloid is accumulated in the follicular cavity due to reduction of endocytosis, and the lining cells are flat and display signs of reduced secretory activity (Table 2). However, in acute illness, thyroid hormonal output may be greatly increased (see below) (15,20–22). When thyroid cells are active, e.g., following TSH stimulation, follicles are small and cells are cuboidal or columnar with signs of active endocytosis, i.e., transport of the colloid into the cell.

■ Changes with Aging in Thyroid Hormone Serum Levels

Changes with aging in the serum levels of thyroid hormones are ambiguous due to fluctuations in response to a variety of stimuli and diseases. Total and free T4 serum levels may be unchanged, slightly decreased, or increased depending upon age, sex, and general health. As for other functions, there is also a greater individual heterogeneity in thyroid activity among the elderly than among young subjects. Often, values in the elderly are in the lower normal range for T4 and T3 and in the higher normal range for TSH; borderline abnormal values are more usual in women than in men (Table 2) (23–25).

BOX 1 *Some Structural Characteristics of the Thyroid Gland and the Hypothalamo-Pituitary-Thyroid Axis*

The thyroid gland is a bilateral organ that bridges the lower larynx and upper trachea with a narrow isthmus. As one of the most vascularized endocrines, it receives blood from the superior thyroid arteries, branches off the external carotid artery, and is drained by corresponding veins into the internal jugular vein (Fig. 1). In normal individuals, vascularity, size and microscopic structures vary with thyroid stimulating hormone (TSH) levels, nutrition, temperature, sex, and age.

The functional units of the thyroid gland are multiple, variable-sized follicles (or cavities), filled with a colloid rich in a glycoprotein, thyroglobulin, which contains the thyroid hormones, triiodothyronine (T3) and thyroxine (T4), in precursor forms. Each follicle is lined by a single layer of epithelial cells resting on a basement membrane (Fig. 2). The cell surface lining the follicle is rich in microvilli that project into the follicular lumen where the colloid is secreted; hormones are secreted into the blood at the opposite basal cell pole adjoining the rich capillary net.

The function of the thyroid gland in the elderly, as in individuals of all ages, can be best viewed in the context of

- regulation by the hypothalamo-pituitary-thyroid (HPT) axis,
- thyroid hormone metabolism, and
- interaction of thyroid hormones with receptors at target cell.

These connected levels of integration (Fig. 3) can be briefly outlined as follows:

1. *At the level of the hypothalamus*, thyrotropin-releasing hormone (TRH), a tripeptide, is synthesized in neurons of the hypothalamus and secreted from their nerve endings into the primary capillary plexus of the pituitary portal system (Chapter 9). TRH is delivered by portal vessels to the thyrotropes (ant. pituitary cells secreting TSH) of the pituitary anterior lobe. TRH stimulates these thyrotropes to synthesize and secrete TSH. TRH synthesis and secretion are regulated by events in the nervous system and blood levels of thyroid hormones.
2. *At the level of the pituitary anterior lobe*, TSH, a glycoprotein secreted by the thyrotropes, stimulates, as its name implies, the cells of the thyroid gland to synthesize and secrete the thyroid hormones. Synthesis and secretion of TSH is regulated in a dual fashion: it is stimulated by hypothalamic TRH and inhibited by the thyroid hormones, in a so-called negative feedback loop (Chapter 9); i.e., the higher blood levels, of thyroid hormones, the lower TSH release and vice versa. TSH, in addition, maintains the integrity of the structure, growth, and vascularity of the gland. Thyroid hormones, their receptors, and function at target cells are summarized in Box 2.

The observation that free and bound T4 levels remain unchanged in old age is reminiscent of the case of the adrenocortical hormones; in the case of these hormones, a slower metabolism (in the liver and tissues) and excretion (by the kidney) of the hormones compensate for the reduced secretion and maintain the levels essentially constant (Chapter 9). A reported significantly reduced free and bound serum T3 in some aged individuals may reflect:

1. Impaired T4 to T3 conversion in tissues
2. Increased T3 degradation (breakdown or excretion)
3. Decreased T3 secretion from the thyroid gland due to either failure of stimulation by TSH or intrinsic, primary alteration of the gland

Based on the current data, it is impossible to distinguish among the foregoing possibilities. However, T4 to T3 conversion declines with age, at least in experimental animals (rat) (26). However, not only do observations in humans often differ from those in experimental animals, but, in the rat for example, there is also intraspecies variability: in the Sprague-Dawley rat, one of the most used animals for aging research, serum T4 rather than T3 is markedly reduced with aging whereas the reverse is true in other rat strains (27).

A certain number of elderly persons show elevated TSH with a T4 that falls within the "normal" range (as established by values obtained in the younger adult population). Under normal feedback conditions, TSH levels are elevated in response to low T4 values. In a good percentage of elderly, high TSH, in the presence of normal T4 levels, may indicate alterations in thyroid–pituitary–hypothalamus feedback (28–30). Abnormally high TSH blood levels may also suggest that a significant portion of the elderly are hypothyroid and may benefit from hormone replacement therapy. Inasmuch as hormonal levels are measured by immunologic assays, high TSH, despite normal T4 levels, may be due to altered TSH molecules that retain immunoreactivity but are less biologically active, thus requiring higher TSH levels to maintain normal T4 (see below).

■ Changes with Aging in the HPT axis

Despite a large number of studies, no consensus has been reached so far on the changes with aging in the hormones of the HPT axis or on the eventual health impact of subclinical thyroid dysfunction (31). Circulating TSH, the principal regulator of thyroid gland function, is regulated by the hypothalamic thyrotropin releasing hormone (TRH) and by direct inhibitory feedback of high-circulating thyroid hormone levels (Fig. 3). Other regulators of thyroid function include the following:

BOX 2 *The Thyroid Hormones*

At the level of the thyroid gland, under stimulation by the thyroid stimulating hormone (TSH) from the anterior pituitary lobe, the thyroid follicular cells synthesize and secrete, through their apical borders into the follicular lumen, the high molecular-weight glycoprotein, thyroglobulin. Iodide, trapped in the cells from blood capillaries by the basal cell membrane, then oxidized by the enzyme thyroid peroxidase (TPO), to the state of molecular iodine at the luminal cell surfaces, iodinates some of the tyrosyl residues of thyroglobulin. The magnitude of the iodide trap and the activity of TPO are augmented by TSH. Again, under the influence of TSH and TPO, some of the iodotyrosyl residues are coupled to iodothyronyll residues [incipient thyroxine (T4) and triiodothyronine (T3)]. Iodinated thyroglobulin is hydrolyzed in the follicular lumen, T4 and T3, liberated from peptide linkage, and secreted in the blood. Monoiodotyrosine and diiodotyrosine are deiodinated intracellularly and the resulting iodide recycled to iodinate thyroglobulin. Small amounts of T4, T3, and, to a much lesser extent, reverse T3 (rT3) are secreted from the gland. Binding of TSH to its receptors on the basal membranes of the follicular cells stimulates the enzyme adenyl cyclase and causes an increase in intracellular cAMP that mediates most of the stimulatory actions of TSH on these cells. Some thyroid actions of TSH are, in addition, due to stimulation of cell membrane phospholipids.

The major secreted hormonal product of the thyroid gland is T4; T3 is secreted only in small amounts and its concentration in the blood derives mainly from the peripheral deiodination of T4. One-third of circulating T4 is converted to T3 in peripheral tissues. Both hormones are present in serum bound to proteins or in the free state. T3 is less tightly bound to plasma proteins than is T4 and is therefore more readily available for cellular uptake. The free hormone is biologically active and interacts with specific receptors localized in the membrane, mitochondria, cytoplasm, and nucleus of responsive cells. T3 binds to nuclear receptors more tightly than T4, hence T3 is more rapidly and biologically active than T4. T3 and T4 are deiodinated and deaminated in the tissues. In the liver, they are conjugated, passed into the bile, and are excreted into the intestine. Conjugated and free hormones are also excreted by the kidney. Some critical aspects of thyroid hormone regulation are summarized in Table 1.

1. Direct autonomic inputs (thyroid follicular cells are innervated by the sympathetic nervous system and are sensitive to sympathetic signals)
2. Possible influence of the neurotransmitter, serotonin, perhaps involved in the regulation of circadian TSH periodicity
3. Possible involvement of the cytokines from the immune system (Chapter 14) that may be responsible for some of the thyroid changes in diseases not directly related to the thyroid
4. The inhibitory role of neuropeptide Y, a member of the pancreatic polypeptide family (Chapter 13) capable of inhibiting TSH secretion (26)

The major regulatory pathway remains the TRH–TSH axis: when TRH–TSH levels are low, the ability of the thyroid gland to secrete thyroid hormones is reduced, and vice-versa, when levels are high, the activity of the gland is increased.

TABLE 1 Some Critical Aspects of Thyroid Hormone Regulation

The major source of circulating T3 is not from thyroid gland secretion but from peripheral deiodination of T4
The negative feedback at the pituitary anterior lobe is mainly through T4 taken from the circulation and converted in the pituitary thyrotrope to T3 by thyrotrope T4-deiodinase
The peripheral deiodination of T4 depends on the physiological state of the organism. It allows autonomy of the response of the various tissues to the hormones
Deiodination can convert T4 (a less biologically active hormone) to T3 (a more active hormone). This conversion depends on activities of the various deiodinating enzymes

Abbreviations: T3, triiodothyronine; T4, thyroxine.

TRH–TSH action on the thyroid gland involves:

- Increase in iodide trapping and binding
- Stimulation of T3 and T4 synthesis
- Promotion of thyroglobulin secretion into colloid at the thyroid level
- Promotion of colloid endocytosis into thyroid cells
- Increase in blood flow at the thyroid level

Prolonged stimulation of the thyroid gland by TSH results in overall enlargement of the gland or goiter, due to cell hypertrophy and hyperplasia. TSH circulating levels are

TABLE 2 Some Morphologic and Secretory Changes in the Thyroid Gland with Aging

Morphologic
Distension of follicles
Variant color of colloid (after staining)
Flattening of the follicular epithelium suggestive of reduced secretory activity
Fewer mitoses
Increased fibrosis of interstitial connective tissue and parenchyma
Vascular changes of atherosclerotic nature suggestive of decreased transport of hormones between cells, blood, follicles
Secretory
Lower circulating T3 levels but generally within the normal (lower) range
Simultaneously decreased secretion and metabolic clearance of T4 with resulting essentially normal levels
Decreased peripheral conversion of T4 to T3
Failure of upregulation of T3 nuclear receptors
Elevated TSH levels in 10% of the elderly, associated with an increase in antithyroid antibodies, present even in the absence of manifestations of hypothyroidism

Abbreviations: T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone.

usually elevated when T4 or T3 circulating levels are lowered. *However, in rats, TSH, a glycoprotein, is present in several forms with different molecular weights and immunoreactivity. In old rats, there is a progressive increase in TSH polymorphism with prevalence of the quantitatively major but also biologically less active form of TSH.* The preponderance of less effective TSH forms in old age would be responsible for a decreased TSH ability to adequately stimulate synthesis and release of T4 and T3 from the thyroid gland and would drive the pituitary to secrete more TSH to compensate for decreasing TSH effectiveness (27). Polymorphism also occurs in other glycoproteinic hormones of the pituitary, such as the gonadotropins, in which the proportion of polymorphism increases with aging.

In addition to the polymorphism of TSH, there is also a polymorphism of other genes, such as the deiodinase gene, which is important for the expression of the enzyme for the conversion of T4 into T3. Failure of this enzyme would result in a relative decrease in the contribution of the deiodinase enzyme to serum T3 production (32).

In rats, not only are TSH levels and TSH polymorphism increased with aging but the typical circadian cyclicity of the hormone is abolished as well (27–30). The functional significance of TSH rhythmicity is still unclear; however, the loss of specific pulsatile signals may suggest a progressive failure with aging of fine tuning of thyroidal function (for neuroendocrine control of hormone pulsatility see Chapter 11). Such a failure is supported by the observation that in some species, the low T3 and T4 serum levels are associated with normal or reduced (rather than elevated) TSH levels. Inasmuch as the major amounts of pituitary T3 (effective in the negative feedback inhibition of TSH) may be derived from intrapituitary deiodination of T4, it is possible that the low circulating thyroid hormone levels are due to decreased systemic deiodination (without affecting the levels of pituitary T3) or that the thyroid hormones–pituitary feedback is impaired.

■ Thyroid Hormone Receptors

Thyroid hormone receptors are within the same family of nuclear receptors as those for glucocorticoids (Chapter 9). An analogy between the two classes of hormones may be useful: glucocorticoid levels do not change significantly in elderly individuals, and yet, the ability to withstand stress decreases, and the probability of the ensuing pathology (e.g., allostatic load) increases. Similarly, *the relative functional adequacy of the pituitary–thyroid axis into old age seems to belie the signs of altered thyroid state manifested by some elderly.* One possible explanation is that alterations with aging may occur primarily at the peripheral level, within the target cells of thyroid hormones, and involve the HPT axis to a much lesser extent. The study, then, of cellular hormone receptors, their eventual changes with aging, and their impact on target cell function may prove useful in elucidating intracellular alterations.

Thyroid hormone receptors have been identified in nuclei, mitochondria, plasma membranes, and cytosol of target cells (Fig. 5). T3 (more biologically active than T4) interacts with the nuclear receptors that bind to regulatory regions of genes (the thyroid hormone–response elements), thereby modifying gene expression and stimulating protein synthesis (28,33). There are two classes of nuclear T3 receptors, each subdivided into two subgroups, the α (α1 and α2) and β (β1 and β2) receptors located on chromosomes 17 and 3, respectively (33). They are expressed in almost all tissues, except for β2 receptors limited to the brain. Both α1 and β receptors, when occupied, activate a T3 response element in vitro, but they differ in potency; the α2 receptor does not bind to the T3 receptor and may inhibit binding of T3 receptors to DNA (34,35).

In general, the number of receptors appears to be inversely related to hormone levels, so that receptors increase in number when the hormone level is low, as in hypothyroidism (upregulation), and decrease in number when the hormone level is high, as in hyperthyroidism (downregulation). In vitro studies of T3 nuclear binding in rat liver and brain show that receptor number remains unchanged with aging, although low circulating T4 levels in vivo might have forecast an upward regulation. In vivo, the major source of intracellular T3 is local conversion from T4; thus, nuclear receptor binding is dependent on the availability of T4 and T3 to the cell, and hormone availability is decreased with aging (Fig. 6) (36).

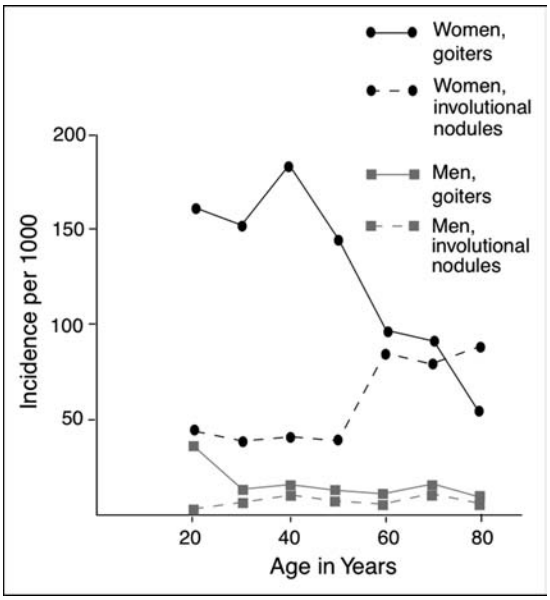

FIGURE 4 Incidence of goiter and involutional nodules in the thyroid gland as a function of age in women and men.

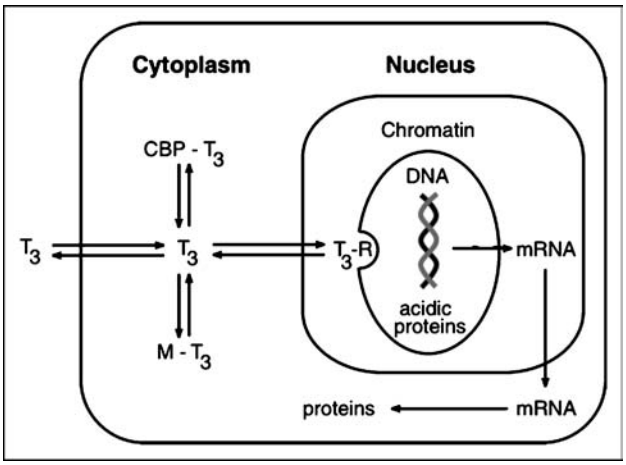

FIGURE 5 Model for T3 interaction with target cell. As T3 enters the cell, it may be bound to a CBP, in reversible equilibrium with a small pool of free T3 that can interact reversibly with T3 receptors in the nucleus, and perhaps also with receptors in the mitochondria. *Abbreviations*: CBP, cytosol-binding proteins; M, mitochondria; R, nuclear receptor; T3, triiodothyronine.

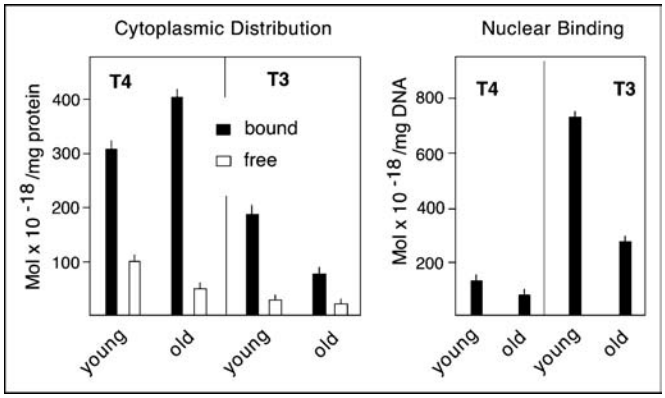

FIGURE 6 Changes with age in T3 and T4 cytoplasmic distribution and nuclear binding. Protein-bound cytosolic T4 and T3 is higher in the brain of old (24-month-old) than young (two-month-old) male Long-Evans rats. Lower T3 levels in older animals suggest depressed T4 conversion to T3 with aging. The reduced free-hormone availability is manifested in lower nuclear binding, particularly for T3. Similar results were also reported in the liver. *Abbreviations*: T3, triiodothyronine; T4, thyroxine. *Source*: From Ref. 36.

Changes with Aging in Metabolic and Thermoregulatory Actions

Of the major actions of thyroid hormones listed in Table 3, those that direct growth and development cease when adulthood is reached. In some instances, however, thyroid hormones appear to promote growth of adult tissues, even those that reach maturity early in life, such as the nervous tissue. For example, a facilitatory action of thyroid hormones has been proposed—although currently disputed—in the recovery of spinal cord and peripheral nerve injuries in humans and other animals. Catch-up whole-body growth and rehabilitation of some behaviors, impaired by thyroid deficiency at an early age, have been described in adult rats after return to the euthyroid state. Other actions of thyroid hormones that may be relevant to the aging process and that may affect the life span are summarized in Table 4. Metabolic rate, calorigenesis, and cholesterol metabolism will be considered here briefly.

Basal Metabolic Rate

Basal metabolic rate (BMR) is the rate at which oxygen is consumed and carbon dioxide is produced by the organism under conditions of physical and mental rest, 12 to 14 hours after the last meal and in moderate ambient temperature. BMR, significantly influenced by thyroid hormones, decreases progressively with aging, but the magnitude of the fall varies with the study and the criterion of measurement (body weight vs. lean body mass) (37). BMR is customarily calculated in terms of oxygen consumption in kilocalories per unit of time and of body surface. With this method, the value for a healthy (euthyroid) male weighing 80 kg and aged 20 to 30 years is 40 kcal per square meter of body surface and per hour.

TABLE 3 Major Actions of Thyroid Hormone

Thyroid hormones stimulate:
Calorigenesis
Metabolism
Brain maturation
Behavior
Growth and development

TABLE 4 Some Metabolic and Cardiovascular Actions of Thyroid Hormones

Stimulation of O_2 consumption (calorigenesis) in almost all metabolically active tissues (except brain, testes, spleen, others)
Stimulation of Na^+, K^+ ATPase activity: Increased Na^+ transport may be responsible, in part, for increased energy consumption
Stimulation by high doses of thyroid hormones of protein catabolism, body temperature, mentation (irritability, restlessness, perhaps mediated by neurotransmitter responses)
Stimulation of intermediary metabolism, particularly, cholesterol metabolism
Stimulation of cardiac output due to the increased sensitivity to catecholamine effects on cardiac rate and contraction strength
Decrease of peripheral circulatory resistance due to increased cutaneous vasodilation
Lowering of circulating cholesterol levels

With aging, from adolescence to old age, metabolic rate decreases progressively, in both men and women—in the latter, values are always slightly lower than in men—from 46 kcal in men and 43 in women at 14 to 16 years of age to 35 and 33, respectively, at 70 to 80 years. The cause of this progressive decline in BMR is not definitely known. It may be associated, at least in part, with the age-related declining levels of thyroid hormones, primarily T3, although, as described above, this decline appears generally slight and does not occur in all aged individuals.

The decreased BMR with aging has also been ascribed to a progressive increase in adipose body mass (less metabolically active) relative to lean body mass (more metabolically active). The metabolically active cytoplasmic mass decreases with aging, as shown by an age-related decline in total body potassium (the major intracellular electrolyte) and urinary excretion of creatine (a product of muscle metabolism).

Are Thyroid Changes with Aging Due to Altered Metabolism and Tissue Demand Rather Than Inadequate Secretion?

As previously discussed in relation to the adrenal hormones and as will be seen in relation to the pancreas and insulin, the peripheral metabolism of thyroid hormones and tissue demand for these hormones appears to be altered with aging. Thus, with old age, T4 and corticosteroid turnover rates decline, and insulin resistance develops. Several authors have concluded that it is the peripheral demand for the metabolic hormones that declines with increasing age, while the circulating hormone levels are maintained relatively constant. The most common explanation offered for this decline in demand would be the loss of metabolically active tissue mass with aging. However, this explanation has several flaws: (*i*) endocrine changes begin early in life before significant changes in body composition (*ii*), changes in body composition do not occur in all individuals, and (*iii*), when those changes occur, they may be secondary to the endocrine changes.

If a smaller lean body mass cannot account for the reduction in peripheral thyroid hormone metabolism, perhaps an explanation may be found elsewhere, for example, in some metabolic alteration in the target tissues, such as an overall decline in protein synthesis with aging. *Thus, endocrine changes that occur with aging may ultimately be associated with a decline in overall protein synthesis, which begins at the end of*

adolescence, when growth ceases, and persists throughout the duration of the life span. Thyroid hormones with their widespread actions on cell metabolism represent an ideal model to illustrate age-related changes in metabolic-endocrine interrelations. *Indeed, these metabolic alterations could result from both the changing thyroid hormone levels and the changes in body composition (31).*

Hypothyroidism as a "Protective" Response in the Elderly

Thyroid hormones, necessary for growth and development when energy requirements are high, may become detrimental when the energy needed is primarily for maintenance of homeostasis. A selective reduction of the general anabolic actions of thyroid hormones may occur with cessation of growth and subsequent aging without a concomitant slowing of catabolic effects and the building up of free radical accumulation (Chapter 5). For example, injections of high doses of T4 in young animals are tolerated quite well; the animals respond with increased appetite and more rapid growth. The same doses injected to the adult animal result in muscle wasting and weight loss. These and other findings have led to the suggestion that "*there is a homeostatic wisdom in the arrangement whereby conversion of T4 to T3 is inhibited when catabolism is already overactive*" (38). From this perspective, the age-related decline in thyroid hormone metabolism would reflect not so much a reduced demand for tissue utilization as an increased need for protection against the catabolic actions of these hormones (39). *Therefore, the reduced intracellular availability of T3 for nuclear binding and consequent effects on protein synthesis may represent a compensatory beneficial response to the decreased metabolic needs of the aging individual.*

Thyroid Status and Longevity

As noted previously, the lower circulating T3 in the old person and T4 in the aged rat, and the reduced conversion of T4 to T3 in target tissues may represent a beneficial compensation to the catabolic actions of the hormones, that is, a certain *"homeostatic wisdom," reflecting the changing metabolic needs of the organism.* This proposal is supported by studies in which rats made hypothyroid neonatally outlived the corresponding controls by about four months for males and two months for females (7,8). In these experiments, the mortality of hypothyroid rats was similar to that of controls until age 24 months but was markedly lower thereafter. Maximum life duration was 35 months for male hypothyroid and 31 for male euthyroid controls; it was 38 months for female hypothyroid and 36 months for female euthyroid controls. The sex difference may be related to the higher T3 and T4 levels in males than in females.

The life-extending effects of hypothyroidism resemble those found after pituitary ablation (hypophysectomy) (26). They also resemble effects produced by calorie restriction, for the body weight of hypothyroid animals is significantly reduced, and the inhibition of growth may act as an antiaging factor (Chapter 23). When the thyroid hormone levels are increased by administration of exogenous T4 over many months (12 and 22 months), the average life span is significantly shortened in the treated animals (7,8). If T4 treatment is initiated in rats, at an already senescent age (26 months), the life span is not affected. Thus, the life-shortening effects of excess thyroid hormones do not appear to be due to the direct action of the hormones to initiate or promote old age diseases that represent the direct cause of death; rather they might accelerate the aging process itself that might represent the consequence of a more rapid timetable of development. Thyroid hormones seem to act as pacemakers capable not only of controlling certain key events during development (e.g., the metamorphosis of tadpoles into frogs) but of intervening to initiate aging processes (26).

Calorigenesis

Thyroid hormones stimulate oxygen consumption in almost all tissues, with the exception of adult brain, testes, uterus, lymph nodes, spleen, and anterior pituitary. Increased oxygen consumption leads to increased cellular metabolic rate and this leads to increased heat production or calorigenesis.

The magnitude of the calorigenic effect of thyroid hormones depends on several factors such as interaction with catecholamines and initial metabolic rate—the higher the levels of catecholamines and the lower the metabolic rate at the time of T3 administration, the greater the calorigenic effect. The calorigenic effect of thyroid hormones contributes to the maintenance of body temperature together with a number of other metabolic and neural adjustments. With aging, thermoregulation is progressively impaired, and this decline may be due, in part, to alterations in thyroid function.

Thermoregulatory Changes

Thermoregulation, in homeothermic (warm-blooded) animals, including humans, involves a series of adjustments destined to maintain body temperature within optimal limits for most functions. Thermoregulatory adjustments maintain a balance between:

- Heat production (stimulated by muscular exercise, assimilation of food, and hormones regulating basal metabolism)
- Heat loss (induced by conduction and radiation heat loss through the skin and mucosae, sweat, respiration, urination, and defecation)

The balance depends on a group of reflex and hormonal responses that are integrated in the hypothalamus and operate to maintain body temperature within a narrow range, despite wide fluctuations in environmental temperatures.

The well-documented increased susceptibility of older people to hypothermia and heat stroke reflects the less efficient temperature regulation that is commonly associated with aging (Table 5). In the elderly, thermoregulatory inefficiency to cold or heat is usual and results from deficits at several levels of thermoregulation: in peripheral temperature sensation, at hypothalamic autonomic and neuroendocrine centers, and in higher cerebrocortical centers, which control temperature perception and coordinate the multiple inputs that determine the effectiveness of adaptative adjustments. The higher mortality of the elderly during "hot" or "cold waves" is well documented and may be attributed to physiologic decrements and to economic (inadequate diet, poor clothing, housing, etc.) and emotional and mental (depression, dementia, etc.) impairments.

TABLE 5 Thermoregulatory Insufficiency in the Elderly

In the elderly, thermoregulatory insufficiency results from the following:
Decreased heat production
Decreased body mass
Reduced muscle activity
Less efficient shivering
Reduced sweating response
Less efficient vasomotor responses
Decline in temperature perception

In rodents immersed in cold water, body temperature falls lower and takes longer to return to normal in old rats than in young animals. Dietary restriction, initiated at weaning, continued to one year of age and followed by a normal diet, improves thermoregulatory responses; the fall in temperature after cold exposure is less severe and the return to normal more rapid (40). This persistence of efficient thermoregulation in old animals has been interpreted as a benefit of the delayed aging induced by dietary restriction (Chapter 23).

Responses to heat are also impaired with aging. For example, the onset of sweating is slower in the elderly. As a consequence of the impaired thermoregulatory competence, the elderly also have a reduced fever response. Fever is the most universal hallmark of disease and depends on a "resetting" of the hypothalamic thermostat in response to a variety of agents, such as bacteria, responsible for the production of interleukin-1, which acts on lymphocytes (Chapter 14). Interleukin-1 enters the brain, where it stimulates the local production of prostaglandins, activating the fever response. Fever responses are dampened in old humans and old animals.

Cholesterol Metabolism

Serum cholesterol levels rise with aging in the human and in the rat, despite a fall in hepatic cholesterol synthesis, indicating a net reduction in overall turnover (Chapter 16). A similar situation is obtained in hypothyroidism, prompting several attempts to treat hyperlipidemia (excess lipids in the blood) with thyroid hormones and their analogs. Several studies suggest that a causal relationship exists between decreased thyroid activity and elevated serum cholesterol. In young rats, thyroidectomy results in reduced cholesterol turnover and elevated serum cholesterol levels, while in old rats, there is no significant postoperative change. Similar results have been observed in a wide variety of mammalian species. The implication is that a reduction in thyroid hormone secretion may be associated with a decrease in the sensitivity of cholesterol metabolism to thyroid hormones. In humans, a hyperbolic fall in serum cholesterol occurs with increasing serum T3 concentrations, and a similar inverse correlation is seen between declining serum T3 and age-related increase in serum cholesterol levels. Taken together, these results support the inference that age changes in cholesterol metabolism may be secondary to age changes in the thyroid axis (Chapter 16).

The specific defect in cholesterol metabolism that develops in the hypothyroid state would result from a reduction in turnover of the serum low-density lipoproteins (LDL), elevated in both hypothyroidism and aging, while metabolism of high-density lipoproteins (HDL) would remain unchanged. The significance of altered lipoprotein metabolism in the development of atherosclerosis (Chapter 16) suggests that early attempts by investigators to relate declining thyroid function to factors predisposing one to atherosclerotic lesions may not be entirely without foundation.

■ Abnormal Thyroid States in the Elderly

The precariousness of the euthyroid state in the elderly is translated into a greater frequency of thyroid disorders in old than young ages. This aging-related increase is often masked by the atypical manifestations of the disease. Thus, although modalities for treatment of thyroid disease are readily available and straightforward, the subtleties of diagnosis in the elderly often provide a challenge for the clinician. A diagnosis of thyroid disease in the elderly may be delayed or missed, because the possibility of thyroid disease is overlooked. In the elderly, signs and symptoms of thyroid disease frequently are minimal or atypical, and it is commonly assumed that the normal aging process or other diseases cause them.

At all ages, thyroid disease is three to four times more frequent in women than in men. Still unanswered are the questions concerning the cause(s) of this gender difference:

- Whether there are differences in TSH polymorphism (see above)
- Whether there are differences with aging in the sensitivity of TRH to the negative feedback by circulating thyroid hormones
- Whether (and eventually, how?) the relationship between TRH-TSH is more impaired with aging in women than in men

Abnormalities of thyroid function occur more often in older patients who are institutionalized or ill than in the healthier elderly who live in the community. Overall, in the elderly, the prevalence of hypothyroidism falls between 0.5% and 4.4% and that of hyperthyroidism between 0.5% and 3%. As already mentioned, in addition to individual variability, the thyroid state is greatly influenced by extrathyroidal factors, such as general health, disease, drugs, and nutrition, as discussed in the following section.

Effect of Nonthyroidal Disease on Thyroid Function

In case of illnesses of various etiologies, the most common finding is a lowering of free T3 serum concentration, whereas free T4 levels may be low, normal, or high, and total rT3 levels are high (10,41). Often, TSH levels, which would be expected to be elevated in response to T3 low levels, are decreased. Any nonthyroidal illness, that is, any systemic illness (e.g., liver disease, HIV infection), acute psychiatric condition, or postoperative state may modify the metabolism of thyroid hormones without implying primary thyroid dysfunction. In addition to medical conditions, a number of common medications (e.g., salicylates) may affect thyroid function. The presence of antibodies to the TSH receptor may also be diagnostically useful and reveal different forms of autoimmune thyroid disease.

TSH Antibodies and Thyroid Autoimmune Diseases

These diseases usually occur because the body's antibodies wrongly identify the thyroid tissue as foreign and destroy it (Chapter 14). The frequency of antithyroid antibodies, primarily directed against the cell-surface receptors for TSH on cells of the thyroid gland, increases with aging (42–45). This increased incidence may be due to

- the decline in the self-recognition ability of the immune system (Chapter 14) and
- the increased TSH polymorphism (reported above) (27).

The incidence of two autoimmune diseases of the thyroid—Graves' disease or toxic diffuse goiter and Hashimoto's disease or chronic lymphocytic thyroiditis—increases with increasing age (42–45). Graves' toxic goiter is associated with hyperfunction or hyperthyroidism (with low TSH and high T3 and T4) (Table 6). Hashimoto's thyroiditis is associated with hypofunction or hypothyroidism (with high TSH and low T3 and T4). Women are at a much higher risk than men for thyroid disorders. Hypothyroidism is more common than hyperthyroidism, especially, among older women. The risk increases with age; for example, about 6% of 40-year-old women have hypothyroidism, but 20% of those are over 75. TSH levels are not detectable in Graves' disease because of continuous

TABLE 6 Autoimmune Diseases of the Thyroid Gland

Characteristics	Signs of Graves' disease	Signs of Hashimoto's thyroiditis
Thyroid status	Hyperthyroid	Hypothyroid
TSH	Generally undetectable	Normal to elevated
T4, T3 (serum)	Above normal	Below normal
ABs	Stimulatory ABs compete with TSH at receptor sites Loss of TSH control over thyroid function	Some ABs block TSH actions
Autoantibodies against thyroglobulin, T3, T4, destroy thyroid microsomal and nuclear components	Generally present	Generally present
Lymphocytic invasion	Limited	Marked
Female:male ratio	As high as 10:1	As high as 10:1

Abbreviations: T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone; ABs, antibodies.

stimulation of the thyroid receptors by TSH antibodies and consequent high T4 and T3 secretion. TSH levels are either normal or elevated in Hashimoto's disease as a consequence of low T3 and T4 levels and TSH blockage by TSH antibodies.

Graves' and Hashimoto's diseases may represent the extremes of a continuum of signs and symptoms resulting from a deranged immune system with hyperthyroidism at one end and hypothyroidism at the other. Antibodies against TSH receptors or thyroid cell components are present in both disorders, although in different proportions; they may lead to cell death, which, in the case of Hashimoto's disease, occurs by apoptosis (Chapter 4) (44). TSH-receptor antibodies compete with TSH at the receptor site on the thyroid cell. Some of these antibodies stimulate T3 and T4 secretion as in Graves'; others block access of TSH at the receptor sites and reduce T3 and T4 secretion, as in Hashimoto's.

TSH-receptor antibodies are not the only antithyroid antibodies present in autoimmune disorders of the thyroid. The most common antibodies, important clinically, are those directed against thyroglobulin (the thyroid cell protein, precursor of T3 and T4), those against thyroidal microsomal and nuclear components, and those against T3 and T4. All these antibodies lead to cell apoptosis (Chapters 4 and 14) and, ultimately, to thyroid destruction; they are more frequent in Hashimoto's disease. Another feature of Graves' and Hashimoto's disorders is invasion of the thyroid tissue by lymphatic tissue. This infiltration is more aggressive in Hashimoto's, with extensive destruction of the thyroid gland.

Hyperthyroidism

Either Graves' disease (i.e., diffuse toxic goiter) or nodular toxic goiter may cause hyperthyroidism, or overactive thyroid: too much hormone is produced, and metabolism is revved up. Because the signs and symptoms in older patients often differ from those in the adult, they are attributed to another illness, hence, the introduction of the term "masked hyperthyroidism" (Table 7). Cardiovascular abnormalities are quite common in older patients with hyperthyroidism. In one study, 79% of subjects had an abnormal cardiovascular examination, 67% had

TABLE 7 Common Signs and Symptoms of Hyperthyroidism in the Elderly

Cardiovascular abnormalities
Congestive heart failure symptoms
Atrial fibrillation
Angina (coronary heart disease)
Pulmonary edema
Tremor
Nervousness
Weakness
Weight loss and anorexia (poor appetite)
Palpable goiter (may not be present)
Eye findings (may not be present)
Thyroid nodules (nonspecific)

symptoms of congestive heart failure, 39% were in atrial fibrillation (i.e., convulsive contractions of the atria), 20% had symptoms of angina, and 8% presented with pulmonary edema (Chapters 15 and 20). Tachycardia, or fast heartbeat, even in the presence of atrial fibrillation, is less impressive in older than in younger hyperthyroid patients. Typical signs and symptoms such as tremor, nervousness, and muscular weakness may be overlooked because they are considered common in the elderly. Weight loss, another common symptom in the elderly, may lead to an evaluation for gastrointestinal malignancy. A goiter may or may not be palpable, and the eye signs of Graves' disease may be absent. Because thyroid nodules are common in the elderly, it is difficult to arouse suspicion of significant thyroid disease solely on the basis of their presence. Laboratory diagnosis of hyperthyroidism includes assay of serum T4 and T3, and a radionuclide thyroid scan that can help differentiate Graves' disease from toxic multinodular goiter

Apathetic hyperthyroidism is another term used for a thyroid disease in the elderly, the characteristics of which could potentially be confused with a hypothyroid state. This condition is characterized by the following:

1. Blunted affect, i.e., withdrawal behavior with apathy and depression
2. Absence of hyperkinetic motor activity
3. Slowed mentation
4. Weakness of shoulder muscles
5. Diarrhea
6. Edema of the lower extremity
7. Droopy eyelids
8. Cardiovascular abnormalities

Treatment of hyperthyroidism includes medical, radiotlogical, and surgical approaches, singly or combined (19,46). In the medical treatment, the goal is to reduce thyroid hormone production. The most commonly used antithyroid medications are oral *propylthiouracil* and *methimazole*: both are thioureylenes that inhibit the formation of thyroid hormones by interfering with the incorporation of iodine into tyrosyl residues of thyroglobulin, perhaps by inhibiting thyroid peroxidase. The major disadvantage of these inhibitors of thyroid hormone synthesis is the recurrence of hyperthyroidism after treatment is stopped. *Administration of radioiodide* is usually the preferred treatment in the elderly. The isotope is actively collected by the thyroid gland, particularly in the regions of active proliferation, and the fast-growing and secreting tissue is destroyed. Radioiodide can be administered in conjunction with medical treatment because the effect of the former is gradual and not

usually complete until three months after treatment. Close follow-up is necessary in view of the possibility of postradioiodide hypothyroidism. Surgery of the thyroid gland may be necessary for large multinodular glands resistant to radioiodide (19).

Hypothyroidism

Thyroid hormone deficiency, or hypothyroidism, is caused by autoimmune thyroiditis or is a consequence of treatment for hyperthyroidism. Just as "masked hyperthyroidism" exists in the elderly, so does "masked hypothyroidism" (Table 8). Typical symptoms of hypothyroidism such as fatigue, weakness, dry skin, hair loss, constipation, mental confusion, depression, and cold intolerance may be attributed to old age instead of thyroid disease. In addition, the insidious onset and slow progression of hypothyroidism makes it more difficult to diagnose. An elevated TSH is one of the most reliable symptoms of hypothyroidism because T4 may or may not be decreased in mild cases.

Common signs and symptoms in hypothyroidism in the elderly are listed in Table 8. Of these, cardiovascular abnormalities and anorexia are common, muscular weakness and mild anemia are found in roughly one half of patients, depression and cold intolerance in 60% of patients, and joint pain may also be present.

Treatment of hypothyroidism is thyroid hormone replacement in the form of oral l-thyroxine or a combination of T4 and T3 (47). Follow-up determination that elevated TSH levels have returned to normal is the best indication that adequate replacement has been achieved. Over-replacement is dangerous in older patients, especially in those with cardiac disease. Safe replacement should be started with low initial doses. Maintenance dose in the elderly is generally lower than in younger patients, and once established, usually remains constant.

In view of the difficulties in clinically diagnosing thyroid disease in the elderly, the role of laboratory screening becomes important. Although arguments have been made that such screening, including a serum TSH, in the healthy ambulatory population of elderly is not worthwhile, certain guidelines can be established. Thyroid disease states, although potentially difficult to diagnose clinically in the elderly, can be detected if the possibility of such disease is entertained. The relative ease and success of treatment makes such detection well worthwhile.

■ THE PARATHYROID GLANDS AND THE THYROID C CELLS

The four parathyroid glands, in humans, are imbedded in the thyroid gland. They secrete the parathyroid hormone (PTH). The so-called C cells, dispersed throughout the thyroid gland, the parathyroids, and thymus secrete another hormone, calcitonin (CT). Both hormones play a significant role in the maintenance of calcium homeostasis (Chapter 21) (48,49). With aging, they do not appear to be consistently altered in their function or to undergo increased pathology. Major actions of PTH and CT are summarized in Box 3.

TABLE 8 Frequently Missed Common Signs and Symptoms of Hypothyroidism in Elderly Patients

Cardiovascular
Dyspnea (shortness of breath)
Chest pain
Enlarged heart
Bradycardia (slow heart beat)
Anorexia (poor appetite) and constipation
Muscular weakness
Mild anemia
Depression
Cold intolerance
Joint pain

■ Parathyroid Glands

Changes with Aging

Structural changes in the aging human parathyroids are few. Similarly, studies from laboratory animals reveal only minor changes such as the presence of degenerating cells containing colloid and of mitochondria showing bizarre patterns (50). *Studies of immunoassayable PTH levels show an increase or decline, depending on gender and race: they decline after 60 years of age in white men but not white women (51).* Increased PTH levels may reflect impaired hormonal renal clearance (52) or accumulation of immunoassayable but biologically inactive fragments. Both PTH and CT are derived by the action of proteases on larger preprohormones, which, in both cases, are less active biologically than immunologically. With aging, the processing of the precursor hormones may be altered, resulting in the secretion of less biologically active preprohormone molecules. Another interpretation relates PTH changes with aging to alterations in vitamin D. *PTH and vitamin D coordinate the regulation of calcium homeostasis by the intestine, bone, and kidney. Vitamin D receptors have been identified in parathyroid cells, and vitamin D metabolites reduce the synthesis of pre-pro-PTH mRNA (53–55).*

While serum calcium levels are maintained throughout the life span, the mechanism by which they are regulated changes markedly with aging. At young and adult ages, calcium levels are maintained by the calcium ingested (with the food) minus the calcium excreted (in feces and urine) without loss of bone mineral. At older ages, serum calcium levels are preferentially maintained by resorption of calcium from bone rather than by intestinal absorption of dietary calcium or renal retention of calcium (Chapter 20). With aging, possible mechanisms responsible for this shift include a decreased capacity of PTH to stimulate renal production of the biologically active form of vitamin D, which stimulates intestinal absorption of calcium and a decreased capacity of active vitamin D to simulate intestinal absorption of calcium (48).

Hormonal Regulation of Bone Metabolism

Bone remodeling is accomplished by the interaction of several growth factors and locally acting cytokines operating on diverse cell populations in a highly coordinated manner. Hormones may affect bone remodeling either by stimulating resorption or formation. Among those that affect resorption is PTH (see above). Bone changes with aging apparently occur without marked alterations in the parathyroid gland or in PTH levels (Table 9). However, in some old women, PTH levels increase with aging, but the contribution of this increase to bone loss may be minimal (56,57). Some racial and gender differences have been reported: black and Asian postmenopausal women have lower PTH levels and higher levels of calcium well into advanced age than white women, and men maintain lower levels coincident with a lower incidence and less severity of osteoporosis than women.

The increase in PTH levels with aging may represent a compensatory response to reduced intestinal absorption of calcium, and hence, lower plasma calcium levels. Reduced exposure to sunlight in the immobile and house-bound elderly will also impair vitamin D manufacture in the skin and

BOX 3 *Major Actions of Parathyroid Hormone and Calcitonin*

Parathyroid hormone (PTH), a polypeptide secreted in response to hypocalcemia, raises the concentration of plasma calcium by:

1. Increasing renal calcium resorption
2. Mobilizing calcium from bones by stimulating osteoclastic activity (i.e., destruction of bone cells, osteocytes)
3. Stimulating the absorption of calcium from the small intestine in the presence of adequate amounts of vitamin D
4. Lowering levels of plasma inorganic phosphate by inhibiting renal resorption of phosphate

In general, serum calcium remains unchanged well into old age despite a few reports of a slight decline. This maintenance of serum calcium is all the more remarkable in view of the decline in calcium dietary intake and intestinal absorption associated with old age (Chapter 20). One of the major regulators of calcium balance is PTH secretion: high concentrations of calcium inhibit PTH secretion and low concentrations stimulate secretion. The effects of PTH on mineral metabolism are mediated by the binding of the hormone to the PTH receptor in target tissues. The PTH receptor belongs to the family of receptors for light, odorants, catecholamines, and other peptides.

Another protein, PTH-related protein (PTHRP), is synthesized in cartilage and in other tissues (mammary gland, brain, renal glomeruli, etc.); it is particularly abundant in fetal life when it may function as a growth factor. Although PTHRP is not a true hormone, but a distant homologue of PTH, its local release activates the type 1 PTH receptor for which it has the same affinity as does PTH with a resulting inhibition of PTH action.

Calcitonin (CT), also a polypeptide, is secreted in response to not only increased calcium levels but also gastrointestinal hormones such a glucagon. CT receptors are found in bones and the kidneys. The major actions of this hormone involve regulation of plasma calcium levels by:

1. Lowering of plasma calcium and phosphate levels by inhibiting bone resorption and
2. Increasing calcium excretion in urine.

CT has also some minor action on water and electrolytes and decreases gastric acid secretion.

accentuate any dietary deficiencies of calcium. Reduced renal degradation and excretion of PTH may be another important factor, although it is still controversial whether or not such reductions occur and, if they do, how important they are.

Although best recognized for promoting bone resorption and elevating blood calcium levels, *PTH can also stimulate bone formation (58–60)*. There is some evidence that intermittent PTH administration increases mechanical strength and mass of intratrabecular bone, and that this action is mediated by transformation of the precursor cells into osteoblasts (61). PTH may also increase bone formation by preventing apoptosis of osteoblasts (62).

TABLE 9 Parathyroid Hormone Changes with Aging

Increased parathyroid hormone plasma levels
Causes
Decreased calcium intestinal absorption
Decreased production of vitamin D (skin)
Parathyroid tumors
Consequences
Increased bone resorption (osteoporosis)
Symptoms resembling cognitive disorders (e.g., Alzheimer's Disease)
Experimental progeria-like syndrome (rats)
Lesser increase of parathyroid hormone levels
Men
Black and Asian women
Lower incidence and severity of osteoporosis

Diseases of the Parathyroid Gland

Diseases of the parathyroid gland are infrequent at all ages; however, they bear mentioning here, for the symptoms associated with aging may mask parathyroid pathology (49). Indeed, if the disorder is recognized as a possible parathyroid dysfunction and is corrected, the symptoms described as aging changes may be ameliorated. Hyperparathyroidism presents with a variety of symptoms such as increased plasma calcium (hypercalcemia), renal calculi, peptic ulceration, and, in a few individuals, mental aberrations with psychotic components. The latter have been sometimes erroneously identified as senile dementia of the Alzheimer type (Chapter 7). In advanced stages, characteristic bone lesions are present. Hypoparathyroidism is quite rare at all ages and is easily recognizable, for it generally follows ablation of the glands during thyroid gland surgery.

■ Thyroid "C" Cells

Structural changes of C cells are rare, except for the finding, in old rats, of a higher ratio of C cells to thyroid follicular cells (50). Little is known of the changes with aging in CT and whether and how they affect the aging of bone (63). *A decrease in CT reported in humans is greater in men than in women*, but not in rats, in which there seems to be an increase. Because CT decreases bone resorption, its potential usefulness in the therapy of aging-related bone demineralization and osteoporosis has been explored but has not yielded significant preventive or therapeutic benefits (63). *CT inhibits bone*

resorption by blocking osteoclastic activity (Chapter 20). The major physiological role of CT is the fine tuning of extracellular calcium regulation, especially under specific conditions such as growth, pregnancy, lactation, stress, etc. However, over time, a resistance to the actions of the hormone can develop, due to loss of CT hormone receptors (64,65). Development of resistance may restrict the therapeutic use of the hormone.

■ THE PINEAL GLAND

The pineal gland (also called epiphysis) was believed by the seventeenth century philosopher, mathematician, and physician, René Descartes, to be "the seat of the human soul." This early role was followed by a wide variety of other equally imaginative functions ascribed to the gland. The pineal gland is now known to secrete melatonin, an indolamine synthesized from the neurotransmitter serotonin (Chapter 6) in the cells (pinealocytes) of the pineal gland and, to a lesser extent, other body tissues (66–71). Melatonin secretion is regulated by circadian rhythm (i.e., 24-hour daily cycles) and helps entrain and synchronize internal body functions with the day–night cycle and with annually changing seasons (with consequent shifts in respective length of light/dark cycles).

Melatonin influences various reproductive functions and developmental processes, especially pregnancy and the timing of reproductive aging in rats. In middle-aged female rats, the effects of pinealectomy (surgical removal of the pineal gland) are more prominent at the age of 60 to 80 days (i.e., shortly after puberty) and at the beginning of the cessation of cycles (72). *In recent years, numerous books aimed at the lay public have promoted melatonin as a "wonder drug," successful in treating, alleviating, or preventing a wide variety of human ailments, from lengthening of the life span, to strengthening of the immune system, to reducing the risk of cancer.* As with other hormones, the rationale for taking melatonin is to restore its decreased levels in old age; but, as is the case for "replacement therapy" with some other hormones (Chapters 10 and 11), melatonin benefits have not been conclusively validated, and the hormone's potential side effects have not been entirely excluded.

In the United States, melatonin is classified as a food supplement (Chapter 23) and, as such, can be purchased without need of a physician's prescription. Rigorous scientific studies in humans and other animals are needed to evaluate its therapeutic effects and eventual potentially adverse effects due to its indiscriminate use. Major characteristics of structure and function of the pineal gland are summarized in Box 4.

■ Changes with Aging

With aging, the human pineal size may increase due to calcium concretion accumulation (Box 4) and the presence of cavities that vary considerably among individuals and mammalian species (such enlargement does not appear to affect function negatively). Biochemical changes vary individually, although *both night and day melatonin levels decrease in most elderly, probably due to alterations (e.g., axon swelling) of sympathetic innervation (67,73).*

The functional importance of melatonin resides in its regulation or fine tuning of circadian rhythmicity. For example, exogenous melatonin may stimulate or inhibit gonadal function, depending on the animal species and the time of administration. This variability has been interpreted to mean that it is not the change in melatonin per se that causes the gonadal change but, rather, the consequent alteration in the timing signal that coordinates body function with the light–dark cycle in the environment (72). This interpretation may be correct, especially in seasonally breeding animals responsive to changes in day length. With respect to the gonadal action of melatonin in humans, pineal tumors may be associated with sexual precocity; but that occurs only when the tumors are large enough or localized in such a way as to produce hypothalamic damage, which then, would be responsible for the delayed onset of puberty.

BOX 4 *Structure and Function of the Pineal Gland*

Arising from the roof of the third ventricle, the pineal gland is an unpaired structure located almost at the center of the brain where it is connected by a stalk to other midbrain structures. The gland contains neuroglial cells and secretory cells (pinealocytes) and has abundant blood supply provided by highly permeable fenestrated capillaries. At early ages in young animals and infants, the gland is large with the cells arranged in alveoli. It begins to involute before puberty and to accumulate calcium concretions, called "pineal sand." The pineal synthesizes and secretes the indolamine melatonin, which derives from the neurotransmitter serotonin (Chapter 6) and its precursor, the amino acid *L*-tryptophan. Synthesis and secretion are high during the night period and low during the light period of the day. Melatonin is metabolized in the liver and excreted in the urine. Its actions are mediated by its binding to low- and high-affinity receptors. The high-affinity ones belong to the family of G-protein-coupled receptors; they are abundant not only in the pineal but also

1. in the hypothalamic suprachiasmatic nuclei, which contain the dominant pacemakers for many circadian rhythms in the body [e.g., rhythm in adrenocorticotropic hormone (ACTH) secretion (Chapter 9)],
2. in the retina, where they may relay photoperiod information, and
3. in other cortical and subcortical areas of the brain, where the receptors may be involved, in some species (e.g., humans) in mediating the sleep-inducing effects of melatonin.

In some fishes, amphibians and reptiles, the pineal gland has an extracranial component, a "third eye," that can act as a photoreceptor. However, in mammals, the pineal gland does not respond directly to light stimuli, but rather, indirectly via a multisynaptic pathway that originates in the retina.

Decline of melatonin levels at older ages may impair the orchestration of such rhythms that may be restored by its administration (e.g., effects of melatonin in improving sleep and alleviating jet-lag symptoms). The importance of this kind of biologic regulation for longevity has been questioned repeatedly, but its testing has been largely limited to insects, whose life span is shortened by disruption of light–dark cycles.

In humans, reduced activity of the aging pineal gland may play a role in the progressive impairment of immunomodulatory, metabolic, oncostatic, and autonomic functions (73,74). *There is now evidence that melatonin has a hypnotic (sleep-producing) effect:*

1. Peak melatonin concentrations coincide with sleep.
2. Melatonin administration in doses that mimic night levels can promote and sustain sleep.
3. Exogenous melatonin may also influence circadian rhythms, reduce fatigue, and reestablish the timing of sleep when night-light cycles are disrupted (75–77).

According to several studies, melatonin would be a potent scavenger of the highly toxic hydroxyl radical and other oxygen-centered radicals (Chapter 5), thereby possessing a protective action against oxidative damage (78–80). Although improvement of pineal function and, particularly, normalization of melatonin levels, may ameliorate the deficits of some aging-dependent functions, its actions in preventing or reducing a variety of aging-related diseases, as well as its contribution to longevity, await further confirmation.

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13

The Endocrine Pancreas, Obesity, and Diffuse Endocrine and Chemical Mediators

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INTRODUCTION

The well-established regulatory action of hormones on metabolism has been briefly reviewed in the preceding four chapters (Chapters 9–12). The many changes of this regulation with aging are well established; however, the onset, extent, and pathologic consequences of these changes vary with each individual endocrine gland considered. As already mentioned for the endocrine glands previously considered, *there is significant individual heterogeneity in the ability of the elderly to maintain metabolic balance in response to internal and external challenges disruptive of homeostasis.* One illustrative example of these aging-associated changes is the altered regulation of carbohydrate metabolism by the pancreatic hormones, insulin and glucagon. Carbohydrates, such as sugar and starch, are derived from aldehyde and ketone compounds. They represent, with proteins and lipids, the major energy-containing components involved in human nutrition (Chapter 23).

Interest in insulin, and its regulation of glucose metabolism in aging, arises from the relatively high number of individuals, 65 years and older, who have lost the ability to maintain glucose homeostasis. The mechanisms of this impairment in carbohydrate economy with aging have been extensively investigated, especially with regard to diabetes mellitus (DM). DM is a chronic disorder characterized by impaired metabolism of glucose, resulting in hyperglycemia (high blood sugar) and the late development of complications involving the cardiovascular, renal, and nervous systems. There are two different types of DM:

- DM type 1 starts at a young age, with the characteristic hyperglycemia resulting from insulin deficiency.
- DM type 2 typically starts in old age and is characterized, as is type 1, by hyperglycemia; it involves, initially, insulin resistance of the target cells and may progress into some degree of insulin deficiency over time (1–3).

Given the increasing evidence of a cause-effect relationship between obesity and diabetes, the role of obesity in increased morbidity and mortality and decreased function in the elderly will also be briefly discussed in this chapter, with focus on effectiveness of interventions for prevention and treatment.

In this chapter, the causes and consequences of insulin resistance and deficiency on carbohydrate metabolism (e.g., DM) and life span will be examined first. In this context, *a number of other hormones, termed "counter-regulatory," that oppose the metabolic action of insulin will be considered as well*. Second, we will discuss some changes with aging in another group of hormones with metabolic regulatory activity. These include (*i*) classical hormones (i.e., secreted by endocrine glands into the general blood circulation and acting on distant target cells), (*ii*) hormones with autocrine and paracrine secretions, and (*iii*) some neurotransmitters. They are all grouped under the term "diffuse endocrine and chemical mediators."

AGING OF THE ENDOCRINE PANCREAS

Several hormones participate in the regulation of carbohydrate metabolism. Four of them are secreted by the cells of the islets of Langerhans in the pancreas: two, insulin and glucagon, have major actions on glucose metabolism and two, somatostatin and pancreatic polypeptide, exert modulating actions on insulin and glucagon secretion. Other hormones affecting carbohydrate metabolism include epinephrine, thyroid hormones, glucocorticoids, and growth hormones (GHs). Some of their actions have been discussed in Chapters 9 and 12.

Major structure and physiologic characteristics of the endocrine pancreas are summarized in Box 1, Table 1 and Figure 1. The exocrine functions of the pancreas are concerned with digestion and are discussed in Chapter 19. Major actions of insulin and glucagon are listed in Tables 2 and 3; the well-recognized regulators of insulin and glucagon secretion are shown in Table 4, and a number of pancreatic and extrapancreatic causes for altered glucose metabolism with aging are shown in Table 5.

Morphological and Functional Changes with Aging in Pancreatic Islets

Morphological Changes

With aging, few morphologic changes have been reported in the endocrine pancreas in humans (4,5). Aging-associated changes include:

- A certain degree of atrophy
- An increased incidence of tumors
- The presence of amyloid material and lipofuscin granules (signs of abnormal cellular metabolism)

Contrary to the atrophy observed in old humans, islets are larger in old than in young rats and contain more B-cells and more insulin per B-cells. This increase in B-cells size has been interpreted as a possible compensatory mechanism for the decreased responsiveness of tissues to insulin (6). However, in some cases, despite the greater B-cell number in old rats, maximum glucose-stimulated insulin secretion is lower in both male and female old rats than in corresponding young controls when expressed in terms of islet weight. At either end of the age range, although female rats have smaller islets than male rats, the former secrete more insulin per unit amount of islet tissue, a gender difference still unexplained. *Given somatostatin's*

BOX 1 *Structure and Function of the Pancreas*

The pancreas lies inferior to the stomach, in a bend of the duodenum. It is both an endocrine and an exocrine gland. Four islet cell types have been identified, each producing a specific polypeptide hormone.

- A cells secrete glucagon
- B cells secrete insulin
- D cells secrete somatostatin
- F cells (also called PP cells) secrete pancreatic polypeptide

Of these pancreatic hormones, insulin is secreted only by the B cells whereas the other three are secreted also by the gastrointestinal mucosa, and somatostatin is also found in the brain.

Insulin and glucagon are important in the regulation of carbohydrate, protein, and lipid metabolism:

- Insulin, an anabolic hormone, increases the storage of glucose, fatty acids, and amino acids from ingested nutrients into cells and tissues.
- Glucagon, a catabolic hormone, mobilizes glucose, fatty acids, and amino acids from stores into the blood.

In the pancreas, somatostatin may regulate, locally, the secretion of the other pancreatic hormones; in brain (hypothalamus) and spinal cord, it may act as a neurohormone and neurotransmitter (Chapters 6, 7, and 9). Function and origin of the pancreatic polypeptide are still uncertain although the hormone may influence gastrointestinal function and promote intraislet homeostasis. A diagram of an islet of Langerhans is presented in Figure 1 and a list of the pancreatic hormones in Table 1. Each cell type secretes its hormone into the blood capillaries surrounding the islet, but also each hormone has direct access, in the islet extracellular space, to the other cell types and can influence the secretory activity of the others (paracrine effects). Somatostatin is the chief inhibitory paracrine mediator of the islets and locally inhibits both insulin and glucagon release. Some islet mediators, for example serotonin (discussed as a neurotransmitter in Chapter 6) contribute in B cells, to the regulation of insulin synthesis and release (thereby providing autocrine-type mediation).

inhibitory action on insulin secretion and the increase in somatostatin levels with aging, treatment of islets with antisomatostatin antibodies has been utilized with some success to remove the inhibitory action of somatostatin, and, thereby, to partially reverse impairments of the glucose-stimulated insulin response (7).

The totality of the reported morphologic changes in the pancreas with aging is, in most cases, modest and cannot account for the significantly frequent metabolic consequences in a number of elderly individuals in whom glucose homeostasis is impaired. Rather, several factors—changes in pancreatic hormones and in the responsiveness of extrapancreatic targets to insulin—have been implicated.

TABLE 1 Major Pancreatic Hormones[a]

Pancreatic site	Hormone	Alternate source
B cells	Pre-proinsulin	None
	Proinsulin	
	Insulin (+connecting C-peptide)	
A cells	Proglucagon	GI mucosa
	Glucagon (+glicentin)	
D cells	Somatostatin	GI mucosa
		CNS
F, D, or PP cells	Pancreatic	GI mucosa
	Polypeptide (PP)	

[a] A, B, and D cells are also called α, β, and γ cells. However, the use of Greek letters may lead to confusion as they refer also to other structures of the body, particularly adrenergic receptors (Chapter 9).
Abbreviations: GI, gastrointestinal system; CNS, central nervous system.

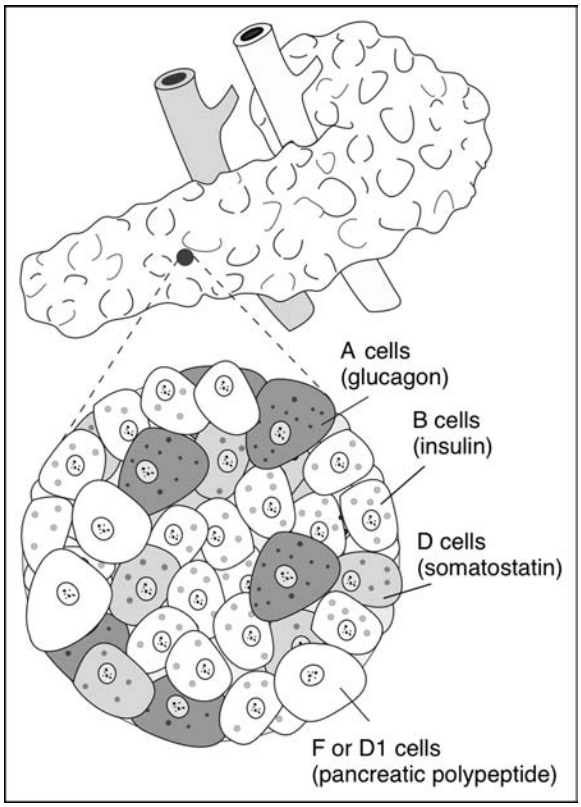

FIGURE 1 Diagrammatic representation of the pancreas with pancreatic cells.

TABLE 2 Major Actions of Insulin

Lowers blood glucose by facilitating transport of glucose into muscle and adipose cells
Simultaneously stimulates intracellular metabolic use of glucose
Increases glycogen synthesis (from blood glucose) and storage in liver and muscle cells
Slows down gluconeogenesis (synthesis of glucose from precursors such as amino acids, glycerol, or lactate) in liver
Facilitates intracellular transport of amino acids and lipids and promotes protein and triglyceride synthesis
Promotes overall body growth (general effect)

Insulin Secretion and Action

Insulin is synthesized in the B-cells as part of a larger (86 amino acids) single-chain polypeptide, proinsulin, which, in turn is derived from its pre-progenitor molecule. Cleavage of two pairs of dibasic amino acids of proinsulin, and removal of a so-called connecting strand (C-peptide) in secretory granules, converts proinsulin in to the double-chain polypeptide insulin (51 amino acids), the two chains being held together by two disulfide linkages. Upon appropriate stimulation of the B-cell, insulin and the C-peptide contained in the secretory granules are secreted, along with some remaining unprocessed proinsulin, into the portal circulation that drains venous blood from the gastrointestinal tract to the liver (Chapter 19).

The plasma concentration of glucose is the key regulator of insulin secretion. Glucose is first transported, by a glucose transporter (GLUT) protein (GLUT 2), into the B-cell, where it is phosphorylated and metabolized. Insulin secretion is stimulated when blood glucose concentrations are even slightly above the fasting level of 75 to 100 mg/dL and insulin secretion is reduced when blood glucose concentrations are low. Other stimulatory factors include several amino acids, intestinal hormones, glucagon, acetylcholine (parasympathetic stimulation), and others. Secretory inhibitory factors include somatostatin, norepinephrine (sympathetic stimulation), and others (Table 4). Circulating insulin is degraded in the liver and kidneys. Antibodies to components of islet B-cells are detected in a high proportion of patients with type 1 diabetes; these antibodies attack islet B-cells, leading to their extensive destruction and to insulin deficiency.

Insulin binds with specific membrane receptors forming an insulin-receptor complex that is taken into the cell by endocytosis (8,9). Insulin receptors are found in almost all cells of the body. The insulin-receptor is a heterodimer made up of two α and two β chains with disulfide bridges: the β subunit, a protein kinase, catalyzes the phosphorylation of two insulin receptor substrates (IRSs), named IRS-1 and IRS-2, located just inside the cell membrane (10–12). Thus phosphorylated, these two proteins may serve as "docking sites" for a number of other proteins, one of which is the "glucose transporter," the protein carrier of glucose into the cell. The second pathway activated by the IRS complex, is the RAS complex, a group of proteins that are very important molecular switches for a wide range of signal pathways that control several processes such as cell proliferation, apoptosis cell adhesion, and migration. The role of RAS in diabetes type 2 has been postulated but little studied to date.

TABLE 3 Major Actions of Insulin and Glucagon on Insulin-Sensitive (Muscle and Adipose) and Metabolically Important (Liver) Target Tissues[a]

Blood	Muscle	Adipose tissue	Liver
Insulin actions			
Glucose	Uptake of: ↑ Glucose, K^+, Amino acids, Ketones	Uptake of: ↑ Glucose, K^+	
	Synthesis of: ↑ Protein, Glycogen?	Synthesis of: ↑ Fatty acids	Synthesis of: ↑ Glycogen, Lipid, Protein
	↓ Gluconeogenesis, Protein breakdown	Activity of: ↑ Lipoprotein lipase	↓ Gluconeogenesis, Glucose output, Cyclic AMP, Ketogenesis
Glucagon actions			
Glucose	↑ Inotropic action on the heart	↓ Synthesis of: free fatty acids	↑ Glycogenolysis, Gluconeogenesis, Ketogenesis, Cyclic AMP

[a] *Ketogenesis:* formation of ketone bodies, metabolites derived from fatty-acid oxidation; *gluconeogenesis:* synthesis of glucose in the liver from precursors, such as amino acids, glycerols, or lactate; *lipoprotein lipase:* enzyme that catalyzes the hydrolysis of glycerides; *glycogenolysis:* breakdown of glycogen; cyclic *AMP*: adenosine 3', 5'- monophosphate important cellular regulator.

TABLE 4 Major Chemical Signals Regulating Insulin and Glucagon Secretion

	Insulin release by B cells		Glucagon release by A cells	
Source of signals	**Stimulation**	**Inhibition**	**Stimulation**	**Inhibition**
Nutrition	Glucose		Protein and amino acids	Glucose (hyperglycemia)
	Amino acids			Free fatty acids
	Fatty acids			Ketones
GI tract	GI peptide hormones (gastrin, secretin, CCK, etc.)		CCK and gastrin	Secretin
Pancreas	Glucagon	Somatostatin		Somatostatin Insulin
Autonomic signals	Acetylcholine cholinergic mediators	Catecholamine adrenergic mediators	Catecholamine adrenergic mediators	Acetylcholine cholinergic mediators
Local tissue autocrine and paracrine signals		Serotonin Prostaglandin E	Hypoglycemia Strenuous exercise	Catecholamines mostly α-adrenergic mediators

Abbreviation: GI, gastrointestinal; CCK, cholecystokinin.

TABLE 5 Some Factors Responsible for Glucose Intolerance[a] with Aging

Insulin alterations
Unchanged or elevated plasma levels of insulin
Alteration in insulin receptors and their internalization in target tissues
Decreased number of glucose transporter units in target cells
Alterations in activities of cellular enzymes involved in postreceptor cellular responses
Increased secretory ratio of proinsulin (less biologically active) to insulin (more biologically active)
Carbohydrate metabolism alterations
Decrease of body's muscle mass and increase in adiposity
Diminished physical activity
Increased fasting plasma free fatty acids that inhibit cellular glucose oxidation
Increased liver gluconeogenesis

[a] Glucose intolerance is measured by impaired ability to lower blood glucose after a standard dose of glucose. Fasting blood glucose levels remain unchanged or may increase with aging.

Because intracellular free glucose concentration is low (due to its rapid, efficient phosphorylation), some glucose enters the cells from the blood down its concentration gradient, even in the absence of insulin. With insulin, however, the rate of glucose entry is increased in insulin-sensitive tissues (muscle and adipose) due to facilitated diffusion mediated by the transporter. The insulin-receptor complex enters the lysosomes where it is cleaved, the hormone internalized, and the receptor recycled (13). Increased circulating levels of insulin reduce the number of receptors—downregulation of receptors—and decreased insulin levels increase the number of receptors—upregulation of receptors. The number of receptors per cell is increased in starvation and decreased in obesity and acromegaly; receptor affinity is decreased by excess glucocorticoids.

Changes in Metabolic Actions of Insulin with Aging

As the principal mediator of storage and metabolism of nutrient fuels (carbohydrate, protein, and fat), *insulin promotes the entrance of glucose and amino acids in insulin-sensitive cells, such as muscle and adipose tissue* (Table 3). After a meal, the increased blood glucose levels stimulate the release of insulin from the B-cells. Glucose is transported into cells by facilitated diffusion along an inward gradient created by the low intracellular free glucose and by the availability of specific carriers, the transporters. In the presence of insulin, the rate of movement of glucose into the cell is greatly stimulated in a selective (according to tissue) fashion. As a consequence of activated glucose transport from blood into cell, blood glucose falls to preprandial (or before meal) levels. If insulin is administered in inappropriately high doses, or at a time when glucose levels are low, then concentration of glucose in the blood falls below the normal limit (hypoglycemia) with severe consequences for glucose homeostasis and danger to survival (e.g., coma and neurologic and mental symptoms).

In the liver, insulin does not directly affect the movement of glucose across the cell membrane but facilitates glycogen deposition and decreases glucose output (Table 3). Consequently, there is a net increase in glucose uptake. Insulin directly and indirectly induces or represses the activity of many enzymes. For example, insulin suppresses the synthesis of key gluconeogenetic enzymes (i.e., forming carbohydrates from protein or fat) and induces the synthesis and increases the activity of key glycogenetic enzymes (i.e., forming glycogen) and of enzymes involved in lipogenesis (i.e., transformation of simple sugars or amino acids into body fats).

When insulin levels are low, as occurs several hours after a meal or during fasting, cellular availability of ingested nutrient fuels is depressed, blood glucose is low (hypoglycemia), and mobilization of previously stored fuels is accelerated. Maintenance of glucose levels within normal range includes the following:

- Decrease in glucose uptake by insulin-sensitive tissues (muscle and fat)
- Preservation of glucose uptake in nonsensitive tissues, primarily brain, critically dependent on glucose for energy metabolism
- Decreased liver glycogen synthesis
- Mobilization of glucagon, a counterregulatory hormone released in response to low insulin levels, to stimulate endogenous glucose release into the blood from glycogenolysis and gluconeogenesis

When blood glucose is high (hyperglycemia), during and soon after a meal, glucose balance is maintained by several adjustments:

- Insulin secretion is increased.
- Endogenous production of glucose is suppressed.
- Cellular uptake of glucose (generated from ingested food) is increased, primarily in muscle.
- Utilization (in muscle and adipose cells) and storage (in liver as glycogen) of glucose, fat, and amino acids arriving in the blood from the gastrointestinal tract are promoted.

A number of functional tests suggest impaired competence of glucose metabolism with aging (14,15). Many clinical studies indicate a slight (about 1 mg/dL/decade) aging-related increase in fasting blood glucose levels in healthy individuals—not significantly affected by gender. This aging-related effect may be less marked or does not occur in nonobese, physically active elderly (16,17). However, with age, there is often a striking change in response to a glucose challenge measured by the typical "oral glucose tolerance test" (Fig. 2) (18,19). In young adults, fasting

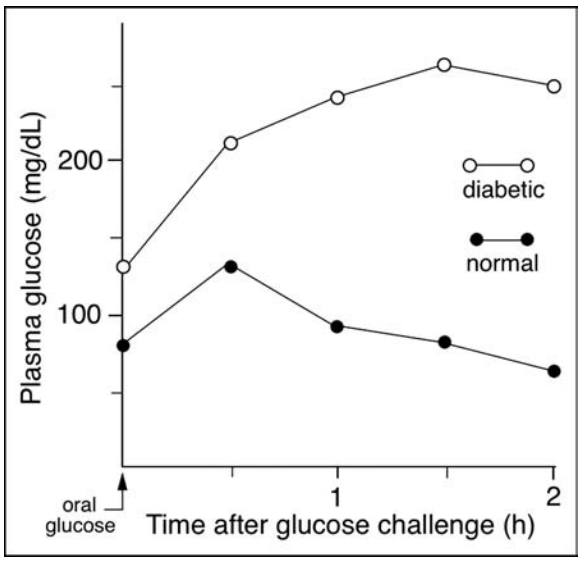

FIGURE 2 Normal and abnormal glucose tolerance tests. After measuring fasting blood glucose levels, the subject is given a glucose drink. In a normal subject, blood glucose level increases about 30 minutes after ingestion of the glucose. After about one hour, the blood glucose level is normal. In the diabetic, the fasting glucose level is higher and increases more after oral glucose than in normal controls. Furthermore, the blood glucose level remains significantly high two hours after ingestion.

plasma glucose levels range from 76 to 110 mg/dL; after oral administration of a standard glucose dose, glucose levels rise to a peak of about 120 mg/dL after 30 minutes but return to pre–glucose administration levels within one hour. In old individuals, blood glucose levels may be higher and take longer to return to normal compared to younger individuals. In persons with DM, fasting glucose is higher than 115 mg/dL and the 1, 1.5, and 2 hours post–glucose values are greater than those of young individuals.

The inability of a person to lower blood glucose after a standard glucose challenge, inability designated as "glucose intolerance," is used to diagnose DM. Considerable controversy in the literature has arisen from the apparent necessity, on the basis of this criterion, to label as diabetic more than half of the 65 years and older population. Glucose intolerance as measured above may be due to causes other than low insulin. Other mechanisms have been suggested to explain the decreased sensitivity of glucose metabolism to insulin with aging, taking into account insulin and carbohydrate metabolism alterations (Table 5).

Changes in Glucagon and Counterregulatory Hormones with Aging

Glucagon, secreted by the A cells of the islets of Langerhans, derives from a larger polypeptide precursor and is degraded in the liver. Factors affecting its release from A cells and its actions are, in general, antagonistic to those of insulin (Tables 3 and 4).

Glucagon:

- Promotes breakdown of hepatic glycogen (glycogenolysis) and lipids
- Promotes conversion of nonglucose molecules to glucose and ketone bodies (gluconeogenesis)
- Raises blood glucose levels
- Serves as a hormone of fuel mobilization through glycogenolytic, gluconeogenic, lipolytic, and ketogenic actions in the liver, while insulin serves as a hormone of fuel storage

Glucagon acts by binding to protein kinase receptors identified in various tissues. The increase in plasma glucagon reported in a number of pathologic conditions (e.g., trauma, infections, burns, and myocardial infarctions) has suggested a *"protective role" for glucagon as the hormone of insult or injury (e.g.,* increasing the force and energy of cardiac contractions or inotropic effects); however, this role and its possible relation to aging remain unclear.

The few studies investigating changes of glucagon actions in the elderly do not indicate any gross abnormality (20). In experimental animals and patients with type 2 diabetes, an apparently paradoxical rise in glucagon has been reported after glucose administration. This response may contribute to the complex arrays of alterations in glucose metabolism considered as part of the counterregulatory mechanisms.

Counterregulatory hormones (e.g., gastrointestinal peptides prostaglandins, GH, epinephrine, cortisol) serve relatively short-term and adaptive functions [for example, in response to stress, (Chapter 9)] and oppose the hypoglycemic action of insulin. They generally have a hyperglycemic action (i.e., increase blood glucose) and, in some older persons, may induce DM. Severe hypoglycemia may induce stress. In elderly diabetics, the response of counterregulatory hormones to hypoglycemia, often consequent to insulin administration, is impaired. Because the sensory and sympathomedullary responses to stress (Chapter 9) may also be compromised, the subject may be unaware of the life-threatening hypoglycemia and fail to undertake the necessary response (e.g., ingestion of glucose) for maintaining homeostasis.

■ Insulin Resistance in the Elderly

Insulin resistance refers to failure of insulin to stimulate glucose uptake by peripheral tissue. If an altered responsiveness to insulin exists in the healthy elderly, it cannot, by itself alone, account significantly for the observed impairment in glucose tolerance. Insulin secretion, its metabolism, hepatic extraction of insulin, insulin half-life, insulin clearance, etc., are not significantly changed in the elderly. In fact, in some cases, the higher blood glucose levels in the elderly are associated with higher insulin levels than in younger subjects. What, then, are the mechanisms of the loss of glucose tolerance with aging? The answer has been sought at:

1. The pancreas level, where insulin secretion may be depressed
2. The peripheral level, where resistance of target tissues to insulin may be increased due to a defect in insulin receptors
3. A disruption of the delicate balance between insulin production and tissue responsiveness to insulin involving primarily the liver

The fact that glucose metabolism is compromised in old age despite normal insulin secretion and metabolism is a cogent argument that peripheral tissues become resistant to the actions of insulin with aging. For this reason, a number of investigators have proposed that the decreased glucose uptake (by approximately one-third) in the usually insulin-responsive target cells of some elderly, compared to young persons, is due to a decreased number of insulin receptors. In contrast, other investigators have stated that insulin resistance is not due to an "insulin receptor problem" (21–24). In experiments with knockout mice, it appears that tissue resistance to insulin may result from a defect in the signaling pathway linked to the *IRS-2* genes (see above) and that stimulation of this pathway by appropriate compounds may effectively abolish the insulin resistance that is the hallmark of diabetes type 2 (10–12).

Other explanations of the defect or defects in peripheral glucose uptake have focused on postreceptor reactions, including the involvement of a number of cytoplasmic processes such as receptor-mediated phosphorylation and dephosphorylation events or the generation of intracellular mediators following receptor-ligand interactions (25). The observation that minimal receptor occupancy is required for proper insulin action further supports the suggestion that postreceptor defects may be responsible for the apparent "resistance to insulin."

A corollary to a decreased insulin secretion hypothesis with aging is a proportional increase in the secretion of unprocessed proinsulin, which has considerably less biologic activity than insulin (26,27). Plasma proinsulin levels in the basal state do not differ significantly in the elderly compared to younger controls, but, after glucose loading, the amount of proinsulin relative to that of insulin is greater in some older individuals. Considerable attention has been directed to the enzymes that process prohormones (26,27). Enzymes for cleaving proinsulin to insulin and C-peptides might become less efficient with aging; hence, the high circulating levels of the prohormone. *Alternatively, the increased resistance of peripheral tissues to insulin with aging may create a greater demand for insulin with consequent insufficient time before secretion for cleavage of the prohormone.* Irrespective of its causes, the elevated proinsulin to insulin ratio with aging appears to be too small to account for the observed alterations in glucose metabolism.

In conclusion, although the mechanisms underlying glucose intolerance with aging are still a subject of debate, the prevalent view is that they reflect a major reduction in peripheral tissue responsiveness to glucose and insulin. The possible existence of defects in the cascade of postreceptor reactions is being actively investigated.

■ Additional Mechanisms Responsible for Glucose Intolerance in the Elderly

Current research, in the main, supports the concept that, with aging, defects in glucose homeostasis (also referred to as glucose intolerance) may involve a multiplicity of factors (Table 5), including:

1. Loss of hepatic sensitivity to insulin and reduced glycogenesis
2. Increased glucagon levels
3. Changes in diet and exercise regimen
4. Loss of lean muscle mass and impaired insulin-mediated glucose uptake in skeletal muscle
5. Increase in adipose tissue (e.g., obesity), and impaired insulin-mediated glucose uptake in adipose tissue

The increase in adipose tissue may contribute to the decreased ability of insulin to facilitate cell glucose uptake (Table 6). With aging, while muscle cells undergo sarcopenia, adipocytes increase in number and in size (Chapter 20). Cell enlargement reduces the concentration of receptors on the cell surface (28). This, coupled with a possible reduction in the absolute number of receptors (suggested above), could lead to reduced insulin binding and decreased cell response to the hormone (29). A comparison of the metabolic characteristics in aging, obesity, and diabetes type 2 reveals the complexity and the subtlety of the changes and underlies the specificity of the changes (29). In addition, this comparison may explain why obesity contributes or predisposes to diabetes type 2. The relationship of obesity to diabetes is reflected in the high incidence of the two conditions in specific ethnic groups, such as the Pima Indians of the state of Arizona (30).

Calorie (dietary) restriction in rats reduces adipose tissue mass and adipocyte size and maintains responsiveness to insulin and glucagon into old age. However, caloric restriction also retards the aging process, in general, and persistence of normal insulin response may merely reflect this antiaging effect, also involved in the maintenance of small-size adipocytes (Chapter 23).

TABLE 6 Characteristics of Diabetes Mellitus

Decreased glucose uptake	Hyperglycemia
	Decreased glycogenesis
	Increased hepatogluconeogenesis
	Glycosuria
	Polyuria
	Polydipsia
	Polyphagia
Increased protein catabolism	Increased plasma amino acid
	Increased gluconeogenesis
	Weight loss, growth inhibition
	Negative nitrogen balance
Increased lipolysis	Increased free fatty acids
	Ketosis
	Acidosis
Vascular changes	Microangiopathies

Despite the close relationship between aging, obesity, and insulin resistance, it must be realized that adipose tissue is responsible for the removal of less than 5% of administered glucose in humans and rats. Liver and muscle are the primary sites of insulin-dependent glucose uptake. It is in these tissues that the age-related defect in transport and intracellular metabolism develops even though several factors must intervene simultaneously to explain the increased insulin resistance of the elderly.

It must be kept in mind that, in addition to alterations of the endocrine pancreas and insulin receptor abnormalities, hyperglycemia and obesity may be caused by a number of endocrine disorders (e.g., Cushing's syndrome, acromegaly). This multifactorial etiology may be profitably studied in animal models such as the obese ob/ob mouse, which shows hyperinsulinemia and insulin resistance. The etiology of the syndrome has been related to a number of neuroendocrine defects involving the hypothalamic satiety center, the neurotransmitter serotonin, temperature regulation, and alterations in glucocorticoid and thyroid hormones. Despite these metabolic and endocrine disorders, the life span of the ob/ob mouse is little affected, and, as the animal ages, there is a remission of symptoms. Spontaneous remission with aging of diabetic or prediabetic symptoms is also a common finding in humans.

■ Diabetes Mellitus (DM) Type 2

Prevalence and Classification of DM Types

DM is a widespread health problem in humans: according to l999 statistics, it affects almost 7% of the population in the United States, with a health-care cost of $100 billion. In the last 10 years, its prevalence has been rapidly escalating due to changes in demographic age distributions (Chapter 2), with an increasing proportion of obese and elderly in the population. It has been predicted that the prevalence of type 2 diabetes will double by the year 2010. In the United States and Western Europe, the disease affects about 16% to 20% of those aged 65 and over, and it is likely that the size of the diabetic population will increase.

In countries with more limited food availability, the number of diabetics is much lower (as low as 2%). These low rates have been attributed to a variety of factors, among which the diet is most important, but exercise, lifestyle, and heredity may also contribute to the low risk and reduced severity of the disease. In all cases, this disabling disease has a tremendous impact not only on the immediate health of the affected individuals but also on their long-term viability. DM has been classified into several types and subtypes according to a variety of etiologies—endogenous or genetic and exogenous or environmental. An abbreviated classification (31), restricted to the most frequent types, considers the following four categories:

- Type 1 DM is due to little or no secretion of insulin. It is primarily found in children (hence the alternative name of early-onset DM) but is also known to occur occasionally at other ages. It is considered an autoimmune disease (Chapter 14) due to its association with specific immune-response (*HLA*) genes and the presence of antibodies that destroy the islets cells.
- Type 2 DM is found in over 90% of the diabetic patient population. Insulin-secreting capacity is partially preserved, but plasma insulin levels, sometimes quantitatively high, are inappropriately low in relation to the magnitude of insulin resistance and hyperglycemia. Type 2 diabetes generally appears after the age of 40 years; hence, its alternative name of "late-onset diabetes."

- Gestational diabetes occurs, as the name indicates, in women during pregnancy.
- Maturity-onset diabetes of the young (MODY) (types 1, 2, and 3) is characterized by genetic defects of B-cell function (e.g., mutations of several genetic loci in chromosomes 12, 13, and 17).

Of these classes, late-onset DM (type 2), the most frequent form of the disease as well as the one related to aging, is the only one that will be briefly considered here.

Pathogenesis of DM Type 2

As indicated above, DM type 2 is the most common form of diabetes. The onset of the disease typically occurs years before symptoms are recognized. Therefore, it is important to screen high-risk individuals systematically (e.g., every three years). Well-established risk factors to screen candidates for type 2 diabetes include the following:

- Increasing age (usually 40 years and older)
- Reduced physical activity
- Obesity, especially in those individuals with central or upper body obesity and with genetic susceptibility (100% concordance rates in identical twins), the expression of which is modified by environmental factors (see below)
- Certain ethnicities: In the United States, DM type 2 occurs at an earlier age (in some cases during adolescence), and it is more frequent in Native Americans (e.g., Pima Indians 30), Mexican descendents, and blacks. Worldwide, there is a propensity for people of Asian descent, Polynesians, and Australian Aborigines to develop the disease when they migrate to westernized surroundings (underlying the importance of changes in diet and degree of physical activity).

DM types 1 and 2 share similar clinical and laboratory characteristics, as summarized in Table 6. Some characteristics specific to type 2 are compared with those of aging and obesity in Table 7. In general, type 2 is characterized by the following:

- Levels of insulin that are usually normal or increased but low, relative to the severe hyperglycemia, and these insulin levels further decline with the increasing severity of the hyperglycemia
- Impaired insulin action that may be due to increased insulin resistance and extra-insulin aging-associated changes (see above)
- Given the failure of insulin to control hyperglycemia, the consequent "glucotoxicity" that may impair B-cell response to glucose, perhaps through the glucose metabolite, glucosamine, which would interfere with insulin-mediated translocation of GLUT to the cell membrane

As already discussed, insulin action is complex and involves multiple steps, and most of these are affected in diabetes. At present, it appears unlikely that a single pathogenic mechanism may be responsible for type 2 diabetes (32,33).

TABLE 7 Diabetes and Accelerated Aging

Diabetes	Aging
Microangiopathy	
Cataracts	Cataracts
Neuropathy	Neuropathy
Accelerated Atherosclerosis	Atherosclerosis
Early decreased fibroblast proliferation	Decreased fibroblast proliferation
Autoimmune involvement	Autoimmune involvement
Skin changes	Skin changes

Consequences of DM Type 2

A variety of "diseases of old age" (e.g., coronary heart disease, glomerulonephrosis, retinopathy, limb gangrene, stroke, and cataract) are consequences or complications of diabetes and major causes of ill health and mortality (2,3,32,33). While some individuals with diabetes live long lives with little indisposition, in general, the rate of disability of diabetics is two to three times greater than that of nondiabetics. For example, in diabetics, blindness is about 10 times (34) and gangrene (with consequent limb amputation) about 20 times more common, (35,36) and 14% of diabetics (usually the elderly) are bedridden for an average six weeks per year (37).

Types 1 and 2 DM are major risk factors for the pathologic consequences of atherosclerosis (38,39). For unclear reasons, the risk increase is greater in women than men (Chapters 15 and 16). *Diabetes induces pathologic changes in the arterial wall, a specific type of microangiopathy (40), which aggravates the vascular aging-related atherosclerosis (Chapter 15).* Atherosclerosis involving the arteries of the heart, lower extremities, and brain is the major cause of death from diabetes (38–40). In diabetics, the atherosclerotic process is undistinguishable from that affecting nondiabetics, but it begins earlier and, often, may be more severe. Diabetes increases the severity of hypertension and promotes higher low-density lipoprotein (LDL) levels (Chapter 16), thereby, worsening the atherosclerotic lesions (41). The causes of microangiopathy, hypertension, and high LDL remain poorly understood: clinical studies indirectly support the hypothesis that hyperinsulinemia (high blood levels of insulin) either due to endogenous causes, such as in type 2 diabetes, or to exogenous administration of insulin for diabetes therapy, may contribute to microangiopathy, perhaps by stimulating vascular smooth-muscle proliferation (Chapters 15 and 16). Another hypothesis focuses on the high levels of glucose, transitorily present even under excellent conditions of therapeutic management. Under these conditions, glycosylation of cellular polypeptides and proteins (i.e., covalent attachment of a carbohydrate molecule to a polypeptide or polynucleotide) might modify their enzymatic and structural properties and cause widespread cellular alterations that could contribute to long-term complications (42,43).

Relationship between DM and Aging

Historically, while research has focused on the increased incidence of diabetes with aging, recent years have witnessed a reversal of this focus, the question asked being whether there might not be an acceleration of aging in diabetes (Table 7). Patients with diabetes display an increased incidence of several features commonly associated with aging: cataracts (Chapter 8), microangiopathy (Chapter 15), neuropathy (Chapter 7), dystrophic skin changes (Chapter 21), and accelerated atherosclerosis (Chapter 15). Accelerated atherosclerosis is a major feature of the various genetic syndromes reported to resemble premature aging, and all of these syndromes include abnormal glucose tolerance (44) (Chapter 3). Further, in normal aging as well as in patients with progeria or diabetes, the proliferative capacity of cultured fibroblasts is reduced, perhaps due, in part, to a reduced response to insulin and to growth factors. Insulin resistance has been reported in cells from patients with Werner's syndrome and progeria (Chapter 3). In addition, in both juvenile-onset and maturity-onset diabetics, the rate of collagen aging (the aging of collagen having been represented as the fundamental aging process) is accelerated. Lastly, the putative autoimmune etiology of type 1 diabetes, observations of immune dysfunction in aging and in type 2 diabetes, and the reports of increased pancreatic amyloidosis in senile humans and animals (5) have excited the interest of proponents of a

chronic inflammatory component of diabetes type 2 (45–47). Collectively, these studies point to the possibility of an intriguing relationship between diabetes and aging.

The great deal of genetic variability in human populations with respect to the life span also applies to predisposition to diabetes. Studies of twins have demonstrated a high degree of concordance in late-onset diabetics, which would make the incidence of a putative recessive diabetic gene greater than 40% (48). However, genetic factors in diabetes are at present poorly understood, probably owing to the considerable heterogeneity and complexity of this disorder. Type 1 diabetes has been recently linked to several specific major histocompatibility complexes (HLA) phenotypes that may predispose selected individuals to viral infection or autoimmune reactions (Chapter 14), but the role of genetic factors in type 2 diabetes remains unresolved. The apparent high heritability of late-onset diabetes may indicate a pathology complicated by the effects of "normal aging," or alternatively, diabetes may represent an acceleration of basic aging processes in a large, genetically predisposed percentage of the population.

Whichever the genetic and pathological implications in the causes and consequences of diabetes, it is perhaps significant in this regard to note that the *currently recommended treatment for type 2 DM is based on a carefully restricted diet and regular exercise*. While both diet and exercise are commonly considered to be the normal healthy individual's best defenses against atherosclerosis and senility, the current population in the United States and other industrialized countries is increasingly more overweight and sedentary. *Thus, with advancing age, the increasing prevalence of diabetes, often associated with increases in body fat and obesity, is considered a key risk factor for the disease as briefly discussed in the following section*.

Relationship between DM and obesity

Obesity is defined as "an excess of body fat, which increases the risk of medical illness and premature mortality." Obesity impairs optimal function of tissues, organs, and body systems at all ages of life. Moreover, in older persons, obesity facilitates the occurrence of medical complications and exacerbates their seriousness. *Currently, prevalence of obesity is increasing in all age groups, including persons about 65 years old and older*; however, it decreases in later old age because most of those affected die prematurely of the complications of aging and obesity. From an overall perspective, obesity appears to be one of the major public health and medical problems facing us at the beginning of the twenty-first century (49–51).

Worldwide, obesity is measured according to the body mass index (BMI); BMI represents the ratio between body weight (in kilograms) and body height (in square meters) (Table 8) (52,53). Underweight, normal, and overweight values for young, adult men, and women are presented in Table 8. Differences in BMI values between men and women may reflect the different fat content (e.g., higher in women) and distribution (e.g., higher in the buttocks) in women. A study using the National Health and Nutrition Examination Survey (NHANES) data (54) calculated the lifetime risk of diabetes according to BMI for men and women, 18 to 84 years of age:

- For a normal 18-year-old man, the average risk of diabetes is 19.8%; the risk increases to 29.7% for overweight men, to 57% for obese men, and to 70.3% for very obese men.
- For an 18-year-old, normal weight woman, the average lifetime risk of diabetes is 17.1%; for overweight women, it is 35.4%; for obese women, 64.6%; and for very obese women, 74.4%.
- For men 65 years and older, there is a much lower risk of diabetes with increasing BMI compared to normal weight men at this age. Overweight men have 3.7% higher risk, obese men have a risk of 18.8% higher, and very obese men a risk of 23.9%.
- For older women, the increased risk is likely higher in each weight category but still much lower than for younger women.

Other, more sophisticated techniques are available for measuring obesity but are less frequently used because they are more invasive and costly and less readily available outside of research settings. After 20 to 30 years of age, fat-free mass

TABLE 8 Body Mass Index

Body weight/height^2
Step 1: Body weight (lb) × 0.4536 to convert weight to kilogram
Step 2: Height (in) × 0.0254 to convert height to meter
Step 3: Multiply Step 2 by itself to obtain square meter
Step 4: BMI = Step 1/Step 3, i.e., weight/height^2
BMI values
Underweight <18.5
Normal 18.5–24.9
Moderate obesity 25–29.9
Massive obesity 30–39.9
Morbid obesity 40+

Abbreviation: BMI, body mass index.

TABLE 9 Causes, Consequences, and Benefits of Obesity in Old Age

Causes
Progressive BMR decrease (Chapter 12). From 20 years on, BMR decreases by 2–3% every decade. Primary cause of BMR decline is FFM. Reduction due to sarcopenia (Chapter 20)
Muscle and FFM decreased due to reduced physical activity
Physical activity reduction accounts for 50% of decline in TEE
Hormonal changes include
Decrease in growth hormone secretion (Chapter 9)
Reduced tissue responsiveness to T3, T4 (Chapter 12)
Decreased testosterone secretion (Chapter 11)
Increased leptin resistance (Chapter 23)
Adverse effects
Decrease of life expectancy/increase of life mortality due to health complications
Health complications include
Metabolic syndrome
Diabetes mellitus type 2
Hypertension
Dyslipidemia [low HDL cholesterol (Chapter 16)]
Arthritis with decreased mobility (Chapter 20)
Pulmonary abnormalities: hyperventilation, obstructive sleep apnea, decreased respiratory compliance (Chapter 17)
Urinary incontinence (Chapter 18)
Increased incidence of injury
Cataracts (Chapter 8)
Increased risk of cancers occurring more commonly in older than young adults, e.g., prostrate, pancreas, bladder, colon
Beneficial effects (due to presence of estrogens)
Increased BMD and decreased osteoporosis (Chapter 20)
Slow bone loss after menopause due to conversion in adipose tissue of adrenal steroid precursors to estrogens (Chapter 10)
No hot flashes
Less CNS complications

Abbreviations: BMR, basal metabolic rate; FFM, fat-free mass; TEE, total energy expenditure; BMD, bone mineral density; CNS, central nervous system; HDL, high-density lipoprotein.

progressively decreases [mainly due to loss of muscle (Chapters 20 and 24)], whereas fat mass increases. Fat-free mass decreases by about 40% from 20 to 70 years of age: it reaches peak value at about 20 years in contrast to fat mass that reaches peak value at 60 to 70 years. Both fat-free mass and fat mass decrease after 70 years.

Balance between energy intake and energy expenditure is a determinant of body fat mass. Indeed, in the absence of changes in energy intake, decrease in basal metabolic rate and calorigenesis (Chapter 12), decrease in muscle mass [or sarcopenia (Chapters 20 and 24)], reduction in duration and intensity of physical exercise (Chapter 24), and hormonal changes (Chapters 9–11) represent some of the main causes of obesity (Table 9). From a clinical standpoint, the health complications that accompany obesity lead to considerable morbidity and impaired quality of life and represent serious risk factors for several diseases, among which DM and cardiovascular disease are a prime example. About 25% of the elderly population has features of the metabolic syndrome strongly advocated in the last three decades (and still utilized as a diagnostic tool) (55,56). However, different clustering of the three key factors of the syndrome—obesity (primarily excess abdominal fat), insulin-resistance, and cardiovascular disease—has led to somewhat different versions. Rather, it has been proposed that the syndrome be diagnosed only in insulin-resistant persons and that the clinical emphasis should be in treating effectively any cardiovascular risk factor that is present (Chapters 15 and 16). In addition to the causes of obesity, both the numerous adverse consequences of obesity and the few beneficial effects are listed in Table 10 and the reader is referred to other chapters dealing with a similar topic.

The treatment of obesity closely resembles that of DM and involves lifestyle changes involving diet, physical activity, and behavioral modification. Treatment also involves pharmacotherapy, with the warning of the dangers of polypharmacy: older individuals are affected by several diseases simultaneously, and, therefore, take concomitantly a large number of hormones, drugs, and dietary supplements that may add or detract from the effect of each single medication and, in combination, may lead to toxic and, even lethal reactions (Chapter 22). *A third approach to the treatment of obesity is surgical, that is, bariatric surgery (the branch of medicine that deals with the causes, prevention, and treatment of obesity) (Table 10)*. Bariatric surgery (usually, gastric bypass or laparoscopic adjustable gastric band procedures) is the most effective weight loss therapy for morbid obesity in patients with a low probability of success with nonsurgical therapy. Result in patients 60 years and older show a greater morbidity and perioperative (i.e., period before and after surgery) mortality higher than in younger individuals, with lower weight loss and slower improvement of obesity-related medical complications (57–59).

Management of Type 2 Diabetes: Diet, Exercise, and Pharmacologic and Transplantation Interventions

Treatment of DM, in general, and of type 2 diabetes, in particular, involves the following:

TABLE 10 Some Guidelines for Treatment of Obesity

Lifestyle interventions
Diet (Chapter 23)
Physical exercise (Chapter 24)
Pharmacological agents
Danger of polypharmacy (Chapter 22)
Surgery
Safety and efficacy of procedures in the elderly still under discussion

- Changes in the lifestyle, which can be viewed as the cornerstone of treatment, especially in the early stages of the disease
- Pharmacologic interventions that include oral glucose-lowering agents and insulin therapy; irrespective of the medication used, the goal of the therapy should be to lower blood glucose as close to normal levels as possible
- Transplantation of entire pancreas or of some pancreatic islets (60)

As stated above, in diabetes type 2, changes in lifestyle—diet (61–63) and physical exercise (64–68)—are the cornerstone of treatment, especially in the early stages of the disease. Pharmacologic interventions represent a secondary therapeutic strategy and include not only insulin but also a variety of agents capable of stimulating insulin secretion or reducing tissue resistance to insulin (69). Success in treatment requires careful followup and rapid interventive responses to the continuously progressing dysfunction of glucose homeostasis. For most diabetics, glucose control deteriorates over time, thus necessitating more intensive pharmacologic interventions (69). The synopsis of treatment presented in Box 2 is not intended to give practical information on the complex management of diabetes but rather to emphasize some of the major characteristics of the disease by pointing out the sites and mechanisms of therapeutic interventions. Current advances in transplantation of pancreatic islets and of whole pancreas have rekindled interest for this type of intervention. Despite the many clinical and research challenges still raised by these interventions, a number of key refinements in pancreas transplantation justify the eventual choice of this therapeutic approach in the not too distant future (60).

■ DIFFUSE ENDOCRINE AND CHEMICAL MEDIATORS

A diverse group of mediators, less important than insulin and glucagon, contributes to regulation of nutrient metabolism. Some of these change in level and significance in older age. The so-called counterregulatory hormones have been discussed. A disparate group of similar mediators, some of them opposing the hypoglycemic actions of insulin, is discussed briefly here. Among these are hormones of the diffuse endocrine system of the gastrointestinal tract, generally peptide in nature: *gastrin*, involved in stimulation of gastric acid and pepsin secretion; *secretin*, involved in the stimulation of the secretion of pancreatic digestive enzyme and of gallbladder contraction and *cholecystokinin*, which stimulates delivery of pancreatic digestive enzymes and bicarbonate to the lumen of the intestines and of the bile by contraction of the gallbladder. Others such as *gastric inhibitory polypeptide* potentiate insulin release mediated by glucose or amino acid. The concentration of some of these hormones changes with aging, with fasting, and during glucose tolerance tests (70,71).

Other local intercellular mediators include *prostaglandins*, a large class of bioactive lipids with multiple actions (e.g., inhibition of platelet aggregation, increase in vascular permeability, and promotion of smooth-muscle contraction) (72). Some prostaglandins elicit a selective decrease in the counter-regulatory response of glucagon to hypoglycemia. Calcium ion is an important regulator of the cellular actions of insulin. Physiologic insulin concentrations augment intracellular calcium concentrations, and experimental lowering of intracellular calcium compromises some of the metabolic actions of insulin (73,74). Insulin regulates the enzyme Ca/(++) Mg(++) adenosine triphospate synthase (ATPase), which

BOX 2 *Treatment of Diabetes Type 2*

Lifestyle changes

- Weight loss, especially in obese persons reduction of total food intake; diet rich in fruits and vegetables, low in fat (Chapter 23)
- Regular exercise, aerobic physical activity especially in sedentary/obese persons (Chapter 24)
- Na intake to be reduced, but adequate intake of K, Ca, Mg maintained (Chapter 23)
- Smoking cessation (Chapter 17)
- Reduced alcohol (Chapter 23)

Pharmacological measures

- Oral glucose-lowering agents:
  Sulfonylurea drugs act by stimulating B cells to produce more insulin.
  Benzoic acid derivatives have the same mechanism as sulfonylurea drugs.
  Biguanides decrease liver glucose production and body weight.
  Thiazolidinediones reduce insulin resistance, may increase high-density lipoprotein (HDL) and lower blood pressure (by activation of nuclear receptor regulating transcriptions of several insulin responsive genes).
  α-glucosidase inhibitors decrease carbohydrate absorption from the intestine.
- Insulin administration.
  Once or twice daily, injection of Lente (intermediate) or Ultralente (long) acting insulin.

Transplantation of pancreatic islets or whole pancreas

is also an important regulator of intracellular calcium ion. The activity of this enzyme is decreased in several tissues of diabetic or obese rats and in human diabetics (74). Stimulation of this ATPase lowers the increased insulin resistance characteristic of type 2 diabetes. These and other data suggest that lowered insulin sensitivity of target cells is related to a decrease in intracellular calcium ion. A large number of other factors that influence the actions of calcium and possible relations to insulin action have not yet been evaluated in aging.

It may be useful to recall here that the *role of nutrients is not only to satisfy the metabolic needs of the cells under steady-state conditions but also to provide greater energy under conditions of increasing demand, such as during the period of growth or under the challenge of stress*. Thus, carbohydrates, absorbed primarily as glucose, are used, immediately, for energy through aerobic pathways and, secondarily, for lipoprotein synthesis, conversion to fat, and for storage as glycogen. The actions of most hormones, including insulin and glucagon, the major hormonal regulators of carbohydrate metabolism, as well as those of the counter-regulatory hormones, glucocorticoids, thyroid hormones, and GHs, may occur in a time frame ranging from seconds to hours; for example, insulin may initiate stimulation of glucose transport in cells in a few seconds, but it may require minutes and hours before the full extent of its actions are completed. Under conditions of acute stress, when an immediate and robust response is indispensable for survival, the rapid activation of neurotransmitters and local hormones with paracrine action may serve to provide the metabolic energy needed to support the necessary adjustments for adaptation (Chapter 9).

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14

The Immune System

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INTRODUCTION

Understanding the mechanisms of the immune function and the influence of aging thereon is not a simple matter. Immunological reactions are highly complex (Table 1). The immune system has the enormous task of protecting us from disease (Table 2) and that which is foreign, while avoiding harm to that which is self. It requires the participation of numerous humoral factors, cell types (Table 3), tissues, and organs (Table 4). Immunological reactions are under the sensitive control of a variety of chemical mediators (Table 5) and molecular interactions. To help appreciate the dynamic effects of aging on the immune system, we list some key terms and concepts in Table 6.

HOW DOES THE IMMUNE SYSTEM FUNCTION?

The immune system exerts defense functions that can be differentiated into specific or acquired, and innate responses, which are closely interconnected. Innate immunity provides broad, nonspecific host defenses that do not require antigenic specificity or immunologic memory. The specific immune responses are mediated by T- and B-lymphocytes, are clonally distributed, are capable of specific reactions with single epitopes of a given antigen, and display adaptive immunity.

T-Lymphocytes

T helper (Th) cells function as key regulatory elements in the immune response, whereas cytotoxic T-lymphocytes (CTL) function mainly as effector cells to eliminate the cells of the host infected by viruses. Progenitor T cells, derived from the bone marrow, must first enter the thymus and rearrange the genes encoding components of the T cell receptor (TCR) for antigen. As a result, each T cell expresses a unique receptor made up of a combination of DNA regions, selected from a multitude of different copies available. Thereafter, newly generated T cells must undergo positive selection for antigen reactivity and negative selection for reactivity to autoantigens. T cells that survive these sequential processes are then released from the thymus as naïve cells, and may persist for many years until stimulated by a specific antigen. These mature, selected, antigen-specific T cells are then required to recognize antigenic peptides together with self–major histocompatibility complex (MHC) products, circumscribed by the immunological rule of "self-restriction."

Because only very small numbers of T cells expressing each of the multitude of possible clonotypic antigen-specific receptors can exist in each individual, a successful immune response is absolutely dependent upon the rapid production of larger numbers of T cells of the same antigenic specificity. This process, called "clonal expansion," is dependent upon specific antigenic stimulation, nonantigen-specific costimulation, and the production and utilization of cytokine growth factors such as interleukin-2 (IL-2) (1). At the termination of the response, the excess cells must be removed from the system by programmed cell death (Chapter 4).

A small fraction of the antigen-specific cells must, however, remain intact to function as memory cells in the event of antigen reexposure occurring at a later date. There are three major subsets of memory cells, all characterized by different receptor expressions (2,3):

- Central memory
- Early effector memory
- Late effector memory

Some memory cells are also maintained in the body in a constant state of activation and slow turnover. The balance between immunity and tolerance is important for maintaining

TABLE 1 Complexity of the Immune System and Immunologic Senescence

Components are multiple and include the following:
Organs and tissues
Cells
Secretory factors
Differential reactions depend on the following:
Immunogen (composition, route, dose, half-life)
Histocompatibility genes
Prior humoral, psychologic, and nutritional state
Antigenic history
Age
Immunologic senescence does not equally affect all components and activities of the immune system
Differences in experimental approaches and subjective interpretation of data often lead to contradictory conclusions

TABLE 2 Major Functions of the Immune System

To prevent disease from infection
Bacterial
Viral
Fungal
Parasitic
To prevent cancer
To prevent immunological destruction of self (autoimmunity)

TABLE 3 Cell Types of the Immune System

Stem cells
Pluripotent cells in bone marrow that differentiate into all other leukocytes (white blood cells) involved in immune function
Lymphocytes
T cells (originating in thymus)
Th (receptor CD4), differ in part based on cytokines
Th0-form by naïve T cells activation; features in common with Th1 and Th2; mature into Th1 and Th2 upon stimulation with antigen
Th1 participates in cell-mediated immunity, provides help for CD8-mediated effector function and IgG2α antibody; often involved in response against intracellular antigens, e.g., Listeria and *Mycobacterium tuberculosis*
Th2 efficient in aiding B-cells to produce IgA, IgE, and IgG1 antibodies (may be involved in allergic responses)
Th3 are Treg lymphocytes exerting suppressive capacity via cytokines such as TGF-β
T-killer-CTL, CD8+ subset, with Fc receptors for Igs, destroy malignant and viral infected cells
B cells
Mature in bone marrow, migrate to other tissues, multiply, and further differentiate (upon appropriate interaction with antigen presenting cells) to form plasma cells; each B-cell is genetically programmed to secrete one specific antibody
Naïve (T or B) cells
Long-lived quiescent cells never yet activated by antigen interaction; important in preventing new opportunistic infection
Memory (T or B) cells
Upon initial exposure of naïve cells to antigens within lymphoid organs, these cells undergo clonal expansion, generating memory cells with various cytokine secretion, responses, and effects
Thymocytes
Immature pre-T cells located in the thymus; stimulated by thymosins (from thymic epithelial cells) to further differentiate into mature T-lymphocytes
Natural killer cells
Lyse target cancerous cells or infected by virus; considered a component of the innate immune system and comprise 5–15% of leukocytes; release cytotoxic substances upon interaction with abnormal cells
Neutrophils
Also called PMN leukocytes; most common leukocytes (50–70%); may be considered: the first line of defense against infection; possess a dense lobed nucleus and short life; first to migrate and enter infected site; cytoplasm contains granules with lysosomal (degradative) enzymes that lyse engulfed bacteria along with release of oxidizing agents; involved in immune reactions against bacteria, fungi, parasites, viral infections, and tumor cells
APC
Accessory cells that engulf antigen, degrade it into fragments, and present a portion on the cell surface in association with membrane receptors; examples include: macrophages, large cells (derived from monocytes) with high quantities of degradative enzymes; DC, involved in primary immune responses within lymphoid organs; type DC1: derived from monocytes and, upon interaction with T helper cells, induce the Th1 cell phenotype; type DC2: derived from lymphocytes, and upon interaction with T helper cells, induce the Th2 cell phenotype; Langerhans cells: specific DCs within the skin

Abbreviations: APC, antigen processing cells; CTL, cytotoxic T-lymphocytes; DCs, dendritic cells; PMN, polymorphonuclear; TGF, transforming growth factor; Treg, T regulatory; Th, T helper.

TABLE 4 Major Structures of the Immune System

Lymph nodes
Gland-like structures, arranged in groups, interspersed throughout the lymphatic circulation. They consist of a fibrillar network in which lymphocytes are organized, mature, and interact. They serve as sites where antigens are trapped and destroyed. Major lymphoid organs include adenoids, appendix, Peyer's patches, spleen, and tonsils
Bone marrow
Meshwork of connective tissue and stem cells contained within bone cavities. Stem cells are multipotent and differentiate into leukocytes and reticulocytes
Lymphatic vessels
Thin-walled vessels that direct the flow of lymph in a particular direction using valves. The lymph is pumped, upon muscle contraction and osmotic pressure, through the lymphatic system, into the lymphatic duct, and then into the large subclavian veins
Reticuloendothelial system
Phagocytic cells contained in reticular tissues located in lymph nodes and liver
Spleen
A relatively large lymphoid organ situated in the upper quadrant of the abdominal cavity. Like the thymus, it has a cortex and medulla. The cortex contains densely packed lymphocytes and germinal centers. The medulla encapsulates the cortex (unlike other lymphoid tissues) and has a variety of leukocytes. Some regions are particularly rich in B-lymphocytes and appear to be important for B-lymphocyte storage and activation
Thymus
A lymphoid organ under the sternum. It consists of a network of epithelial cells that secrete various polypeptide factors important for the maturation of thymocytes (which are also contained within the thymus) into T-cells and immune function in general. Thymus is therefore a primary lymphoid organ, able to generate mature T-cells that colonize secondary lymphoid organs, and is therefore essential for peripheral T-cell renewal

immune homeostasis. Several mechanisms are in place to ensure that the immune response is controlled, such as T-cell anergy, apoptosis, and immune ignorance. Another mechanism of peripheral tolerance is the active suppression by regulatory or suppressor T cells. Several types of T regulatory (Treg) cells are described, including natural (cluster of differentiation CD4+ CD25+ T cells) and adaptive (Th3 and Tr1 cells) Tregs (4–8).

■ B-Lymphocytes

The main characteristic of B-lymphocytes is their capability to differentiate into plasma cells and secrete immunoglobulin (Ig) proteins called antibodies. Igs are receptors for antigen on the B-cell surface, and mediators of humoral responses when secreted in a soluble form. B-cells derive from a bone marrow precursor that becomes a virgin, mature B-lymphocyte through a DNA rearrangement of the genes encoding for the Ig variable region, with a process similar to that involving the TCR specificity generation. The variable region can be further subdivided into regions where variability is greatest, called hypervariable or complementary determining regions; these regions are truly responsible for antigen binding and antibody diversity (9). However, the most potent process in the generation of antibody diversity is somatic mutation, which occurs in germinal centers following specific antigen stimulation. There are five classes of Ig detectable in serum: IgG, IgA, IgM, IgE, and IgD. Each Ig class or isotype differs by the sequence of its constant region (Table 7), and this difference translates into a distinct functional capability (Table 8).

Innate Immunity and Specific Adaptive Immunity

The capability to cope with pathogens, cancer cells, and other threatening agents resides not only in specific immune mechanisms, but also in innate immune reactions, mainly mediated by polymorphonuclear leukocytes, macrophages, and natural killer (NK) cells. The natural defense mechanisms include the following:

TABLE 5 Chemical Mediators or Modulators

Cytokines
Influence proliferation, differentiation, and survival of lymphoid cells; have numerous actions on other body cells; comprise the following:
IL
Family, different proteins from IL-1 and up; numerous effects on lymphocytes and other cells with IL receptors
TNF
TNF-α: cytotoxic against malignant and inflammatory cells; produced primarily by macrophages
TNF-β: cytotoxic against malignant cells; enhances phagocytosis; produced primarily by T-cells
IFN
IFN-α,β: produced by many cells; antiviral actions
IFN-γ: synthesized by activated NK and T-cells; involved in activation of macrophages and inflammation
CSF
Glycoprotein regulating white blood cell production, activity, and survival
GM-CSF
Regulates hematopoiesis, affects phagocyte function and angiogenesis
Surface receptors
CAM
Surface receptors mediating cell–cell and cell–matrix interactions
Influence maturation, circulation, and homing
Involved in inflammatory response and signal transduction
Mediate interaction of killer cells with target cells
CD receptors
Designate dozens of human surface molecules on leukocytes (CD4, CD8, CD28, etc.)
Perform different functions, e.g., adhesion, migration, stimulation, activation, proliferation, and cell death
CTLA
Stimulation inhibits T-cells with opposite effects than CD28
Selectins
Family of membrane components on lymphocytes for adhesion, migration, and homing to capillary endothelial cells
Mediators involved in apoptosis
Fas (CD95)
Membrane receptors involved in triggering apoptosis upon interaction with Fas-ligand (membrane receptors on natural killer and activated T-cells)
Bcl-2
Protein family derived from chromosome 18; involved in apoptosis and prosurvival (i.e., preventing cell death from various stresses)
Caspases
Protein families (intracellular cysteine proteases) act on apoptosis and inflammation
ICE
Triggers apoptosis by overproducing a family of caspases
Thymic secretory factors
FTS
Small nonapeptide involved in pre-T-cell maturation
Thymostimulin and thymopoietin
Small proteins involved in T-cell maturation and immune regulation
Thymosins
Small polypeptides secreted by thymic epithelial cells; regulate maturation and proliferation of pre-T-lymphocytes
Thymosin α1 is the most studied with regard to therapeutic potential toward infectious disease (hepatitis, HIV, and cancer)

Abbreviations: IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; NK, natural killer; CSF, colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; CAM, cell adhesion molecules; CTLA, cytotoxic T lymphocyte antigen; Bcl-2, B-cell leukemia; ICE, interleukin 1β converting enzyme; FTS, factor thymique serique; CD, cluster of differentiation.

TABLE 6 Key Terms and Concepts

Humoral immunity	Aspect of immune function related to the secretion of antibodies by B-cells or plasma cells
Cell-mediated immunity	Immune reaction elicited by cytotoxic lymphocytes
Epitope	Specific region (also called antigenic determinant) on a molecule(s) recognized and bound by an antibody
MHC	Represents a large cluster of genes, which code for many of the cell surface glycoproteins that play a role in defining the interactions between lymphocytes and macrophages during an immunological reaction
HLA	Represented by gene clusters on chromosome 6. The gene cluster contains three loci called class I, II, and III
An individual's HLA genotype has been linked to predisposition to certain diseases	Among the functions of genes encoded are: Defining self: cell surface recognition glycoproteins present on almost every body cell and involved in rejection of allografts (coded as class I loci, i.e., specifically designated HLA-A, B, and C) Cell interaction: cell surface molecules on B-lymphocytes and macrophages important in cellular interactions related to immune function (class II at loci HLA-D or DR, D-related) Complement-mediated lysis: blood proteins known as complement (i.e., class III loci), whose major function is lysis of infected cells or bacteria. They are sequentially activated in a cascade-like reaction resulting in cell lysis and other activities required for normal immune function

Abbreviations: HLA, human leukocyte antigen; MHC, major histocompatibility complex.

- Chemotaxis
- Phagocytosis
- Natural cytotoxicity
- Cell–cell interactions
- Cell–matrix interactions
- Production of soluble mediators

Innate immunity is nonspecific, and repeated exposure to the same pathogen will result in the same response. In contrast, adaptive immunity is characterized by specificity and immunologic memory. Thus upon reexposure to a given pathogen, memory cells will be recruited rapidly and generate a rapid and directed immune response.

The innate immune system is intermixed and collaborates with clonally distributed T- and B-cells and represents a first line of defense against different pathogens. An example of this strict collaboration is the "antigen recognition process" that requires the presentation of the epitope to T-lymphocytes on the surface of antigen-presenting cells (APCs), such as macrophages and dendritic cells (DCs), in association with the MHC class II (in the case of Th cells) or class I (for CTL) molecules (10).

TABLE 7 Some Characteristics of Ig Classes

IgG
- Most abundant antibody (80%)
- About half in the blood
- Consists of four subgroups: IgG1–IgG4
- Found in mammary glands
- Can cross placental barrier providing prenatal Ig protection
- IgG4, primary antibody to enter tissues and become involved in Ig reactions
- IgG1 class is the major Ig produced by B cells
- IgG class can also trigger complement-mediated lysis

IgM
- Largest Ig (multivalent pentamer comprising 5–10% of total Ig)
- Secreted upon initial exposure to antigen
- Like IgG, it can trigger complement-mediated lysis

IgA
- May exist as a monomer or dimer and has two subclasses (IgA1 and IgA2)
- Can tolerate adverse conditions
- Commonly found in the respiratory tract, tears, intestine, and stomach
- Produced by plasma cells localized in these tissues
- Predominant Ig in milk, thereby helping to provide postnatal immune protection

IgD
- Present in very low concentration in serum
- Serves as a highly specific membrane receptor on B-cells
- Interaction of antigen with IgD receptors can trigger the mechanism of antigen processing and presentation

IgE
- Often produced in response to exposure to parasite antigens
- Receptors on mast cells (called Fc receptors).
- Binds the IgE class and binding of antigen to IgE on mast cells can trigger the release of the vasodilator histamine causing redness and inflammation (allergic reactions)

Abbreviation: Ig, immunoglobulin.

TABLE 8 Some Key Ig Characteristics

- Polypeptides (with small amounts of carbohydrate on heavy chain)
- Bivalent: two identical sides
- Four-chain monomers consisting of two identical heavy chains (each about 50,000 MW) and two identical light chains (each about 25,000 MW)
- IgM is a pentamer
- IgA is a dimer
- Each heavy chain is linked to a light chain by disulfide linkages, and the two heavy chains are bound to each other by disulfide linkages
- Constant region (relatively conserved amino acid sequence) that defines functional capabilities and distinguishes the five different heavy chain classes, IgG, IgA, IgM, IgE, IgD, and two types of light chains k and l
- Fab has a variable amino acid sequence near the amino terminus, while the Fc is near the carboxy ends
- CDRs are portions of heavy and light chains with hypervariable amino acid sequence responsible for determining antigen-binding specificity

Abbreviations: CDRs, complementary determining regions; Fab, antigen-binding portion; Fc, constant region; Ig, Immunoglobulin; MW, molecular weight.

The adaptive immune system is dependent upon the functional integrity of the innate immune system, without which APCs cannot be primed to present antigens in a stimulatory form to T cells. Humoral immunity is, in turn, dependent upon intact T-cell responsiveness for B-cell generation, differentiation, and antibody production. Moreover, signals delivered through innate immune receptors in response to pathogen-associated molecular patterns affect adaptive immune responses via modulation of Treg regulatory function. The interplay between Treg and antigen-responsive T cells is modulated by DCs. Mature DCs, activated through toll-like receptor (TLR) pattern-recognition receptors, produce proinflammatory cytokines, including IL-6, which render the responder T cells refractory to the suppressive effect of Tregs (11).

The different cell types of the innate immune system interact with each other and influence the quality and strength of an immune response. The final outcome of a response to microbial infection may greatly vary as a result of the interactions occurring between different pathogen-derived products and different cell types of the innate immune system. These interactions also determine the quality and strength of the subsequent adaptive responses (12).

Cytokines are responsible for differentiation, proliferation, and survival of lymphoid cells. Other soluble mediators are proinflammatory agents; these play an important role not only in specific immune responses but also in inflammation. Cytokines include ILs, colony-stimulating factors (CSFs), chemokines, and others, such as tumor necrosis factors (TNFs). These molecules constitute a complex network and act by binding to specific membrane receptors. The immune orchestra depends on a network of these humoral mediators (13).

Cell adhesion molecules (CAMs) are surface receptors mediating cell-cell and cell-matrix interactions. Cell adhesion is fundamental in lymphocyte functions including the following:

- Maturation
- Circulation
- Homing
- Generation of inflammatory responses
- Interaction of killer cells with their targets

There are different families of CAMs, and many of them also have regulatory functions and signal transduction properties. The modification of the number of CAMs on the cell surface, in addition to alterations of their affinity and avidity, provides the molecular basis for the interaction between different cells. Chemokines are chemotactic small cytokines that orchestrate leukocyte trafficking in tissues, thus playing an important role in the regulation of immunological processes.

■ IMMUNE FUNCTION AND LONGEVITY

The optimal functioning of the immune system has crucial importance for survival and aging (14). There are several links between longevity and the genes involved in determining immune responsiveness, mainly histocompatibility complex. For example, an increased frequency of human leukocyte antigen (HLA)-B16 and DR7 and a decreased frequency of HLA-B15, B8, and DR4 have been demonstrated in elderly subjects (15).

The causes of death in the very old often involve infectious agents (e.g., pneumonia, influenza, gastroenteritis, bronchitis) (16). This suggests that failing immunity primarily contributes to the increased incidence of those diseases that the immune system is designed to protect. In a longitudinal study of the very old, nonsurvival was predicted by the clustered parameters of poor T-cell proliferative responses, high CD8 (cytotoxic/suppressor) cell fraction, and low CD4 (helper/inducer) and CD19 (B) cells. In a cross-sectional study, it was demonstrated that CD4 lymphopenia in the oldest resulted in an increased mortality risk over the first two years following diagnosis (17–19).

A variety of theories exist that explain the immune modifications occurring during aging (Chapter 5). The immune system is supposed to collapse with age, and several changes have been considered as paradigms of a defective responsiveness, such as the following:

- Increased susceptibility to infectious diseases and cancer
- Increased levels of auto antibodies
- Higher incidence of autoimmune manifestations
- Decreased antibody production to nonself antigens
- Defective NK activity
- Thymus involution
- Decreased T-lymphocyte proliferation

However, aging is not simply the cause or the result of immune deterioration, but it depends also on a network of interconnected cellular defense mechanisms (Chapters 3–5), including DNA repair, antioxidants, production of heat, shock, and stress proteins, and apoptosis (20). All of these antiaging tools, acquired during evolution, are variably regulated in different species and in different individuals of the same species. Accordingly, it has been hypothesized that individuals who have survived in good health to the maximum life span are equipped with optimal cell-defense mechanisms.

In the past, the great majority of studies simply compared immune parameters from young and old subjects, including people over 65 years; however, the human life span is potentially longer, that is, around 100 years. Furthermore, the effects attributable purely to aging can be difficult to dissect from the effects that are secondary to exogenous factors, such as underlying diseases, which are frequent in aging, or the use of medication (Chapter 22), which might influence the immune system (21). For this reason, the use of strict biochemical and clinical inclusion and exclusion criteria for immunogerontological studies in humans, known as the Senieur protocol, has been proposed (22). Thus, studies with healthy centenarians reveal a great capacity of the immune system to maintain its defensive function in even the very old (23). Centenarians are, therefore, the best examples of successful aging because they have escaped major age-related diseases and have reached the extreme limit of human life.

A complex reshaping of the immune system occurs with age. The remodeling theory of aging suggests that immunosenescence is the result of the continuous adaptation of the body to the deteriorative changes occurring over time. According to this hypothesis, body resources are continuously optimized, and immunosenescence must be considered a dynamic process. Some immune parameters decline and deteriorate in the elderly, including centenarians, while many others remain unchanged or even increase.

Furthermore, accumulation of memory T cells, decrease and exhaustion of naïve T cells, and marked reduction of T-cell repertoire, mostly CD8+ T cells, are apparently some hallmarks of immunosenescence in humans (Table 9) and may be considered potential candidates for predicting morbidity and mortality (24). Immunosenescence, as a manifestation of clonal exhaustion, may also occur in young individuals as a result of chronic antigenic stimulation, and as such, is not a problem of only the elderly. Therefore, research on immunosenescence, already important in the context of increasing numbers of the elderly in society, is also critical in the context of chronic infection, organ transplantation, and possibly cancer, in younger individuals. The evidence of interdisciplinarity research on immunosenescence underlines the necessity for an integrative approach to age-associated clinical disease.

TABLE 9 Hallmarks of Immunosenescence

Atrophy of the thymus
Decreased size
Decreased cellularity (fewer thymocytes and epithelial cells)
Morphologic disorganization
Decline in the production of new cells from the bone marrow
Decline in the number of cells exported by the thymus gland
Decline in responsiveness to vaccines
Reduction in formation and reactivity of germinal center nodules in lymph nodes where B-cells proliferate
Decreased immune surveillance by T-lymphocytes and NK cells

Abbreviation: NK, natural killer.

■ IMMUNOSENESCENCE: REMODELING OF THE IMMUNE SYSTEM

The classical concept of "immunosenescence" considers a generalized, age-related, unidirectional decline in immune responses, leading to increased susceptibility to infections as well as to inflammatory and degenerative diseases, enhanced autoreactivity, and frequent occurrence of tumors. However, recent research, highlighting the complexity and multifaceted effects of aging on the immune system, has led to a reformulation of these original concepts. Aging rate and longevity, as well as the incidence of the great majority of age-related diseases with an inflammatory background, are determined by both environmental and genetic factors that are able to counteract antigenic stress as well as the deleterious effects of reactive oxygen species (ROS) production (Chapter 5). Individuals who are genetically equipped with strong immunological defense mechanisms and are concomitantly characterized by efficient mechanisms for the control of inflammatory reactions, could be potentially destined to become healthy centenarians. The quality and quantity of the lifelong antigenic load, conditioning inflamm-aging, are major determinants of immunosenescence, aging rate, and longevity, as well as of quality of life in advanced ages. The plasticity of the immune system, the lifelong antigenic load, and the oxidative stress, which dynamically interact over the life span, configurate the individual's immunological history (Chapter 5). Longevity largely coincides with successful immune system remodeling. Healthy centenarians, taken as an example of successful aging, are not necessarily the more robust individuals at the beginning, but rather those individuals capable of an intermediate response to stress. The phenotype of centenarians is the result not only of the starting genetic makeup, but also of the chronic stimulation of the immune system and the remodeling process occurring lifelong (25). It is now well established that immunosenescence does not mean immunodeterioration (26). Although the capacity to cope with major immunological stresses, such as chronic and acute infections, declines, basal levels of immune function are maintained. Changes in the expression of functionally important cellular receptors can contribute to the remodeling of immune function characteristic of the elderly (27).

Several changes in antigen expression that characterize the elderly are shared by various pathological conditions, raising the question of the relationship between the aging process and the pathogenesis of such diseases, which are particularly frequent in the elderly. For example, the CD3 downregulation is a feature of several T-lymphoproliferative disorders (28), the CD20 overexpression characterizes various infectious diseases, and the high density of CD5 on B-cells is a feature of B chronic lymphocytic leukemias (29). Changes in lymphocyte function are not necessarily always toward a lowering of response. For example, aging of immune cells is associated with increased expression of several CAMs, which could likely result in an augmented capability to adhere. Another example is the CD50 overexpression on T cells during aging, which may represent an effort of the immune system to partially supply the decreased number of monocytes bearing its ligand LFA-1,

which could result in compromised recognition and presentation processes (30).

Immunosenescence therefore represents a new and unique reequilibrium of the immune system, in which several changes in the representation and phenotype of lymphocyte subsets are implicated. On the whole, data on immunosenescence indicate that changes occurring over time might be considered the result of global reshaping, where the immune system continuously looks for possible stable points for optimal functioning (31).

■ THE CYTOKINE NETWORK

The cytokine network undergoes profound changes with age. Some of these shifts are noted in Table 10; however, additional shifts in cytokines and other immune factors are listed in Table 11. Immunologic aging includes a shift toward a type 2–dominant state (32). The cellular and humoral components of the immune response are regulated by cytokines produced by two general subsets of helper cells known as Th1 and Th2 (13). Th1 cells tend to provide help for CD8-mediated cellular effector function and the IgG2a class of antibody. Th2 cells are more efficient in providing help for B-cells and the IgA, IgE, and IgG1 classes of antibodies. Type 1 cytokines include IL-2, interferon (IFN)-γ, IL-12, and IL-15. Type 2 cytokines include IL-4, IL-5, IL-6, IL-10, and IL-13.

Newborn mice and infant humans exhibit impaired cellular-mediated immunity but strong humoral immunity, a state in which type 2 responses dominate. Soon thereafter, a type 1 state becomes dominant and persists in healthy mice and humans until mid- to later life, at which time a dominant type 2 cytokine profile may again emerge (33). The reduced production of cytokines such as IL-2 and perhaps IL-3 by aged naïve T cells will lead to less expansion during effector generation. This cytokine-reduced production may also lead to altered properties of the effectors due to a decreased susceptibility to cell death (34). It is possible that, with aging, the increase in tumor incidence, the increased rate of infections, and the reappearance of latent viral infections are the result of decreased cellular immune surveillance due to this cytokine imbalance.

A progressive increase of proinflammatory cytokines is a major characteristic of the aging process. Lifelong antigenic stress not only induces an enormous expansion of memory T cells, but also increases their functional activity, exemplified by a high frequency of cells positive for proinflammatory cytokines (35). Age-dependent modifications of type 1 and type 2 cytokines within virgin and memory CD4+ T cells have also been documented (36).

The capability of mononuclear cells to produce proinflammatory cytokines such as IL-1, IL-6, and TNF-α increases with age (37). The abnormal IL-1 and TNF-α secretion observed in old subjects may be relevant to a number of abnormalities associated with aging. For example, IL-1 increases the secretion of serum amyloid A protein and other acute-phase proteins by the liver and stimulates bone resorption; IL-6 induces fever, activates T- and B-lymphocytes, and modulates hepatic acute-phase protein synthesis. Moreover, high levels of IL-6 have been referred to as the most powerful predictors of morbidity and mortality in the elderly. IL-6 is one of the pathogenetic elements in inflammatory and age-related diseases such as rheumatoid arthritis, osteoporosis, atherosclerosis, and late-onset B-cell neoplasia.

TABLE 10 Some Aging-Related Shifts in Cytokines

Increased proinflammatory cytokines IL-1, IL-6, TNF-α
Increased cytokine production imbalance
Decreased IL-2 production
Increased production of IL-8, which can recruit macrophages and may lead to pulmonary inflammation
Increase in dysfunctional IL-8
Decreased secretion of IFN-γ
Altered cytokine responsiveness of NK cells, which have decreased functional abilities
Increased levels of IL-10 and IL-12 upregulated by antigen processing cells

Abbreviations: IFN, interferon; IL, interleukin; NK, natural killer; TNF, tumor necrosis factor.

TABLE 11 Additional Aging-Related Shifts in Immune Functions

Altered membrane fluidity
Increased apoptosis perhaps due to decline in CD28 expression and IL-2 production
CD20 overexpression on lymphocytes
Increased CAMs expression on lymphocytes
Old cells may have greater levels of messenger RNA for three mitotic inhibitors
Decreased number of HLA class I and II antigenic sites on lymphocytes
Increase in activated T-cell–expressing DR molecules
Decreased proportion of T-, B-, and NK cells expressing CD62L and increased density per cell of this adhesion receptor expression
Upregulation of L-selectin per T-cell
Shift in lymphocyte population to contain more CD3-NK cells and CD3+CD56+ T-cells
CD3 downregulation and CD50 upregulation on T-cells affecting activation and proliferation
Increased T-cell death by Fas/Fas-ligand–mediated response in presence of IL-2
Heightened density of CD5 on B-cells
Decreased number of monocytes with LFA-1
Decreased ability of dendritic cells to stimulate T-cell secretion of IFN-γ and IL-2
Increased proportion of granulocytes and monocytes lacking CD62L
Downregulation of CD50 intensity expression on monocytes and granulocytes

Abbreviations: CAMs, cell adhesion molecules; IFN, interferon; LFA, lymphocyte function associated antigen-1; IL, interleukin; NK, natural killer.

■ THE PROINFLAMMATORY STATUS

Inflammation is not per se a negative phenomenon: it is the response of the immune system to the invasion of viruses or bacteria and other pathogens. During evolution, the human organism was set to live 40 or 50 years; today, however, the immune system must remain active for a much longer time. This very long activity leads to a chronic inflammation that damages several organs: this is a typical phenomenon linked to aging and it is considered the major risk factor for age-related chronic diseases. Age-related diseases and senescence are the price we pay for a lifelong-active immune system (Chapter 3). Proinflammatory genotypes are related to unsuccessful aging and, reciprocally, controlling inflammatory status may allow a better chance of successful aging. Inflammatory genotypes are an important part of the normal host responses to pathogens in early life, but the overproduction of inflammatory molecules might also cause immune-related inflammatory diseases and eventually death (38).

However, the proinflammatory status is seemingly characterized by successful and unsuccessful aging. Centenarians have lived in good shape and without disability until very old

age, despite the fact that in most of them, the biochemical parameters related to inflammation can reach high values. The new theory of "inflamm-aging" involves an interplay between genetic and environmental components. The capability to mount a strong inflammatory process can contribute to fitness and survival, and people characterized by such a capacity have been positively selected. According to the antagonistic pleiotropy theory of aging, natural selection has favored genes conferring short-term benefits to the organism at the cost of deterioration in later life (Chapters 4 and 5) (25). Cytokines interact in networks in which the functions of one cytokine are modified, modulated, or substituted by another one. For example, IL-10 and TNF-α have complex and predominantly opposing roles in the inflammatory responses. IL-10 limits and ultimately terminates inflammatory responses, whereas TNF-α determines strength, effectiveness, and duration of inflammatory reactions. Interperson differences in the regulation of IL-10 and TNF-α production may be critical with respect to the final outcome of an inflammatory response. TNF-α is an independent prognostic marker for mortality in persons aged 100 years. Mooradian et al. (39) studied 129 elderly nursing-home patients and found that a detectable serum level of TNF-α was associated with death within 13 months. Plasma levels of TNF-α are correlated linearly with IL-6 and C-reactive protein in centenarians, indicating an interrelated activation of the entire inflammatory cascade in the oldest old. Roubenoff et al. (40) report, on 525 ambulatory, free-living participants in the Framingham Heart Study, aged 72 to 92 years at baseline, that TNF-α and IL-6 are associated with increased mortality, whereas insulin-like growth factor 1 (IGF-1) levels had the opposite effect. In the Women's Health and Aging Study, a three-year cohort study with five-year mortality follow-up, Cappola et al. (41) report that women with IGF-1 levels in the lowest quartile and IL-6 levels in the highest quartile have significantly greater limitation in walking and disability in mobility tasks and instrumental activities of daily living than those with neither risk factor. Women with both risk factors are at greater risk for death. The combination of low IGF-1 and high IL-6 levels confers a high risk for progressive disability and death in older women, suggesting an aggregate effect of dysregulation in endocrine and immune systems (Chapters 3 and 9).

Genetic variations located within the promoter regions of proinflammatory and regulatory cytokines could influence inflamm-aging and the susceptibility to age-related diseases (Chapters 3–5). Current data are compatible with the conceptualization of aging as the result of chronic stress (Chapter 9) impinging upon the macrophage as one of the major target cells in this process. Human immunosenescence can, therefore, be envisaged as a situation in which the most evolutionarily recent, sophisticated defense mechanisms deteriorate with age, while the most evolutionarily ancestral and gross mechanisms are preserved or negligibly affected and, in some cases, almost upregulated. The prolonged attrition exerted on the immune system by antigens leads to the production of memory T cells, indicating that the body reacted successfully (42). However, these physiological responses at the same time lead to a progressive accumulation of clones of memory cells, which fill the entire "immunological space." Together with thymic involution and the consequent age-related decrease of thymic output of new T cells, this situation leaves the body practically devoid of virgin T cells, and thus, likely more prone to a variety of infections, such as bacterial (e.g., *Escherichia coli*, *Streptococcus pneumoniae*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*) and viral (e.g., herpes virus, influenza virus), as well as noninfectious (e.g., atherosclerosis, diabetes, osteoporosis and osteoarthritis, dementia, autoimmunity) diseases, where immunity and inflammation play major roles. Aging is associated with increased circulating levels of proinflammatory cytokines. Increased levels of inflammatory serum markers in the elderly are associated with dementia (Chapter 7), Parkinson's disease (Chapter 6), atherosclerosis (Chapter 15), type 2 diabetes (Chapter 13), sarcopenia (Chapters 20 and 24), osteoporosis (Chapters 10 and 20), functional disability (Chapter 3), and high mortality risk (Chapter 2) (43–45). Therefore, through inflammation and its mediators, the immune system not only influences the immunological defense reactions, but also exerts detrimental effects on muscle, bone, cardiac function, hemopoiesis, and cognition. With regard to atherosclerosis and coronary artery disease in particular, there have been greater insights into the mechanisms and role of inflammation (46–48). The blood levels of various biomarkers of inflammation such as C-reactive protein and lipoprotein-associated phospholipase A2 correlate well to atherosclerosis and heart disease. Cytokine release by white cells can substantially enhance the secretion of C-reactive protein. Likewise, activation of certain white blood cells such as macrophages lead to their increased secretion of lipoprotein-associated phospholipase A2 (Chapter 16). Thus, inflammatory reactions and inflamm-aging, which results in cytokine release, appear to be exceptionally important contributory factors in atherosclerosis and coronary artery disease leading to heart attack and stroke (Chapters 15 and 16).

■ HUMORAL IMMUNITY

There has been the observation, in clinical practice, of an increased frequency with aging of pathological processes involving B-cells and antibody production. Examples of such pathological conditions include the following:

- Chronic lymphocytic leukemia
- Presence of autoantibodies or monoclonal gammopathies
- Common occurrence of amyloidogenesis

Moreover, a consequence of the altered antibody response may be a marked propensity for the following:

- Infectious diseases, particularly pneumonia
- Recurrent infections
- Poor responses to vaccines

Such changes result in increased morbidity and mortality in elderly subjects. Although the presumption has been that much of this change is due to decreased Th-cell function rather than an intrinsic primary B-cell deficit, aging-associated alterations in B-cell repertoire expression and in the generation of primary and memory B-cells have been documented (49). Thus, the decreased humoral responsiveness of aged individuals is apparently due not only to alterations in the environment and ancillary stimulatory mechanisms, but also to alterations in B-cells per se (Table 12).

TABLE 12 Some Aging-Related Changes in B Cells

Decreased number of circulating and peripheral blood B cells
Alteration in B cell repertoire (diversity)
Decreased generation of primary and secondary memory B cells
General decline in lymphoproliferative capacity

■ B Cells

Alterations in B cell development include the following:

1. Skewing of V-gene utilization
2. A decrease in the generation of various developmental B cell subsets
3. A decrease in the number of pre-B cells (50)

A reduction in newly emerging cells from the bone marrow is consistent with the alterations cited above (51).

Bone marrow stromal cell contact and IL-7 produced by stromal cells are essential for B-lymphopoiesis. The pre-B-cell receptor (a functional heavy chain with the surrogate light chain) has a ligand on stromal cells that may play a role in inducing the secretion of IL-7. The stromal cells from aged mice are unable to correctly process the signals from the developing B-lineage precursors and are unable to efficiently secrete the IL-7 (49).

Some studies describe a significant decrease with aging, including in centenarians, of peripheral B-cells and of those B-cells coexpressing the CD5 molecule. CD5+ B-cells are specifically involved in the development of autoimmune and lymphoproliferative disorders. Although B-cells coexpressing CD5 are decreased in the elderly, they exhibit a higher expression of CD5 molecules at a cellular level, and this finding is probably implicated in the dysfunction or hyperfunction of these cells, probably contributing to the increased susceptibility of old subjects to oncological diseases and autoimmunity (27). More recently, an expansion of CD5+ B1 cells has been described in the elderly. They respond in the absence of cognate T-cell antigen recognition, produce low-affinity IgM antibodies, and do not develop memory. Oligoclonal expansions of the B1 subset are frequent (52). Defects in B-cell activation and signal transduction are also apparent. The B-cell antigen CD20, increased during B-cell stimulation (44), is also overexpressed in the elderly, probably reflecting a condition of chronic B-lymphocyte activation that leads to the above-mentioned immune manifestations. Recent observations support the hypothesis of an age-associated decline in B-lymphopoiesis within the bone marrow, which ultimately limits the output of new B-cells to the periphery. Lack of competition for space in the peripheral niches allows environmental/self-reactive B-cells, which would normally be silenced, to survive. These self-reactive B-cells, as well as antigen-experienced B-cells (CD5+ B1 like, marginal zone, and memory) accumulate in and dominate the peripheral B-cell compartment. Cytokine dysregulation helps maintain this skewing of B-cell population. The decline in humoral immunity reflects the forced reliance on antigen-experienced B-cells, rather than naïve, follicular B-cells, to respond to new immunologic insults; lack of appropriate T-cell help and defective follicular DC function probably also play a role (53).

■ Immunoglobulins

The paradox of an increase in Ig serum level and a concomitant decrease in peripheral blood B-lymphocytes is observed in the elderly (14). Both IgG and IgA serum levels significantly increase with age, whereas IgM does not (Table 13). A number of possibilities can explain the aging-related changes in the level of autoantibodies and B-cells, including the following:

- An increased number of B- and plasma cells in organs other than peripheral blood
- An increased life span of B- and plasma cells in germinal centers

TABLE 13 Aging-Related Shifts in Antibodies

General decrease in humoral responsiveness: decline in high-affinity protective antibody production
Increased autoantibodies: organ-specific and non–organ-specific antibodies directed to self
Increased serum levels of IgG (i.e., IgG1 and IgG3) and IgA; IgM levels remain unchanged

Abbreviation: Ig, immunoglobulin.

- An increased production of Igs per cell (30)
- Profound changes in cytokine network

The amount of antibodies with low-affinity self-reactivity increases and the amount of antibodies with high-affinity reactivity with foreign antigens decreases.

A large fraction of elderly people have low-affinity autoantibodies in their serum, and the prevalence of autoantibodies associated with systemic autoimmune diseases increases with age. Although the frequency of subjects with detectable serum levels of autoantibodies increases with age, organ-specific autoantibodies (antithyroperoxidase and antithyroglobulin) are practically absent in the plasma of healthy centenarians; in contrast, nonselected elderly people show an age-related increase in these autoantibodies. Nonorgan-specific autoantibodies (e.g., anti-dsDNA, antihistones, rheumatoid factor, anticardiolipin) seem to follow a different trend by increasing in healthy aged donors and centenarians (14).

A widely observed change in the in vivo immune response of aged humans or experimental animals is the diminished ability to generate high-affinity, protective antibody responses to immunization against infectious agents or experimental antigens (54). It is proposed that a signaling disequilibrium from the aged T cells, which provide less efficient help in quantitative terms, supports the growth of low-affinity B-cells. Although age-related declines in IL-2 production and T-cell expansion may contribute to the poor help for humoral responses, aging also impairs other aspects of the interaction between T cells and B-cells, in particular, the contact-mediated help. The ligand for CD4O (CD4OL or CD154), expressed in Th cells, is a crucial antigen in T-B cooperation. This receptor stimulation develops a cascade of events including B-cell CD23 expression, which is important in regulation of Ig synthesis. CD4OL expression and its activation pathway are clearly impaired by aging (9).

The formation of germinal centers during T-dependent antibody responses provides an apparently critical environment for the selection of high-affinity antibodies through processes including somatic hypermutation of the Ig variable regions and the subsequent selection of B-cells expressing high-affinity antibodies. The effective response and the faster neutralization of the pathogen after a secondary challenge is a consequence of this phenomenon. Reduction of germinal center reactivity is a landmark of immunosenescence and contributes to immunological dysfunction in the elderly (55,56).

■ CELLULAR IMMUNITY

Although cellular and humoral immune responses are modified with advancing age, the loss of effective immune activity is largely due to alterations within the T-cell compartment (Table 14), which occur, in part, as a result of thymic involution (57). Substantial changes in the functional and phenotypic profiles of T cells have been reported with advancing age (58).

TABLE 14 Some Aging-Related Changes in T-Cells

General decline in cell-mediated immunological function
T-cell population is hyporesponsive
Decrease in responsiveness in T-cell repertoire (i.e., diversity of CD8+ T-cells)
Decline in new T-cell production
Increase in proportion of memory, effector, and activated T-cells, while naïve T-cells decrease
Diminished functional capacity of naïve T-cells (decreased proliferation, survival, and IL-2 production)
Senescent and dysfunctional T-cells accumulate due to defects in apoptosis
Increased proportion of thymocytes with immature phenotype
Shift in lymphocyte population from T-cells to NK/T cells (cells expressing T-cell receptor and NK cell receptors)
Increased number and decreased activity of Treg cells
Increased clonal expansions and filling of the immunological space

Abbreviations: IL, interleukin; NK, natural killer; Treg, T regulatory.

■ T-Lymphocyte Subpopulations and the Memory/Naïve Unbalance

One of the most consistent changes noted in T cells with advancing age is the decrease in the proportion of naïve T cells with a concomitant increase in T cells with an activated/memory phenotype (31). Virgin, unprimed, naïve T cells are CD45RA+ CD62L+ CD95− T cells. They require a costimulatory signal, such as CD28, to optimally proliferate after CD3 stimulation. CD95 antigen is expressed on peripheral blood T cells after TCR/CD3 stimulation. Memory/activated T cells are CD45RA+ CD95+ CD28− lymphocytes. CD8+ CD28− T cells, which are increased in the elderly, have phenotypic and functional features of terminally differentiated, armed effector T-lymphocytes. The differences in activation requirements and response characteristics of naïve versus memory T-lymphocytes may in large part explain the changes in immune responsiveness that occur with advancing age (59).

The age-related unbalance of virgin and memory cells is found among CD4 and CD8 cell subsets, but it is deeper within the CD8+ T cells. Class I–restricted CD8+ T cells play a major role in infectious diseases caused by pathogens living inside cells, and they constitute an important effector arm for immune surveillance against tumors. Infectious diseases, such as influenza and pneumonia, and cancer are major health problems in older people and represent leading causes of death in this population. A very low number of CD95− T cells correlates with a shorter life expectancy. Concomitantly, the progressive expansion of CD28− T cells can be interpreted as a compensatory mechanism. During aging, when the ability to replenish the naïve pool via thymopoiesis is reduced, the immune system tries to compensate for the progressive loss of naïve T cells by increasing thymic-independent pathways, such as the peripheral expansion of mature CD28− T cells, especially within the CD8+ subset (60).

The age-related increase of T-lymphocytes that lack CD7 is consistent with the increase of HLA-DR+ and CD45RO+ T cells. CD3+ CD7− T-lymphocytes are in fact mainly activated and memory cells and may represent T-lymphocytes at a fully differentiated maturational stage. Both the absence of CD7 and the downexpression of CD3 probably contribute to their lower proliferative response compared to CD7+ T cells (27).

■ T-Cell Dysfunctions

T-cell clonality is a common characteristic of healthy aged persons with clonal expansions found in the CD4+ as well as in the CD8+ memory population (61). T-cell expansions may derive from latent or repeated infections. They do not represent malignant clonal T-cell expansions. Once expanded, it is possible that these T-cell clones persist, because mechanisms that regulate clonal homeostasis, such as apoptosis, are defective in the elderly (Chapter 4).

The T-cell population from aged individuals is hyporesponsive (62). Unlike the antigen-stimulated memory cells generated in young subjects, which proliferate vigorously and often produce high levels of IL-2 and other cytokines, the memory cells found in aged subjects are hyporesponsive. Naïve cells from aged mice respond to antigenic stimulation with decreased cell survival, decreased proliferation, and markedly reduced IL-2 production.

Overall decreases in responses of older organisms to a new antigenic challenge may therefore reflect both

- a diminished residual population of available antigen-specific cells and
- a diminished functional capacity of the remaining naïve T-cells (34).

The age-related defects in naïve CD4+ T cells are due to the aged environment from which they come as well as to intrinsic defects that are caused by homeostasis and their long life span (63,64). Also, perturbations of TCR signal transduction pathways with aging could contribute to the decline of cell-mediated immune function with age. T-cell hyporesponsiveness due to defects of signaling through the TCR results in an impaired ability to mount efficient immune responses and to maintain responsiveness to foreign antigens. These defects result in a modification of the activation of transcription factors involved in *IL-2* gene expression leading to decreased IL-2 production (65).

CD4+ CD25+ Treg cells prevent T-cell–mediated autoimmune diseases. Their suppressive activity declines with age, leading to the increased incidence of autoimmune manifestations. The establishment of tissue-specific tolerance essentially stems from the promiscuous expression of tissue antigens by thymic epithelial cells. Age-related thymic involution could explain Treg dysfunction in the elderly, whereas their expanded number could be linked to the age-related chronic inflammatory status (66,67).

Most of the above-mentioned deficits are a consequence of the so-called "replicative senescence," leading to a sort of clonal exhaustion, partially sustained by telomere shortening (Chapter 4) (68). While healthy T cells remain below the threshold of activation, senescent T cells integrate a number of signals and are able to respond to TCR stimulation with low-affinity antigens. Cumulative replicative stress encourages the emergence of such cells. Replicative stress is a normal physiologic challenge but may turn into a disease risk factor when extensive and beyond the compensatory reserve mechanisms of the immune system. The accumulation of senescent CD4+ T cells is a risk factor for several clinical conditions of tissue injury (69).

■ IMMUNOSENESCENCE AND THE THYMIC FUNCTION

During the physiological thymus involution that accompanies aging, the organ diminishes in size and cellularity, and its structure becomes disorganized. Accompanying the morphologic changes of the thymus are changes in its biochemistry and physiologic function.

The number of T cells exported decreases. There may be a developmental block resulting in an increased frequency of

thymocytes with an immature phenotype (Table 14). The effect of age-related involution on the kinetics of thymocyte differentiation could depend on an intrinsic defect within the thymocyte population or a deficiency in the ability of stromal cells to support differentiation or defects in lymphokine-driven thymocyte proliferation. Administration of factors such as FTS, thymostimulin, thymopoietin, and thymosin-1 reduces various aspects of age-related immune dysfunction (70).

The Thymus and the Peripheral Microenvironment

A complete naïve T-cell repertoire is produced within the first year of life, and naïve T cells survive for the life of the host and maintain that repertoire in the absence of a thymus (71). As individuals age, the composition of their T-cell compartment changes, accumulating antigen-experienced or memory cells, while losing naïve cells. This phenomenon is believed to occur gradually over time as a consequence of (*i*) antigen-driven clonal expansion and maturation, as well as (*ii*) a decline in the output of newly differentiated naïve T cells from the thymus. These processes would result in an immune compartment that has shifted toward memory-like responses, able to respond to previously encountered antigenic determinants but much less competent to respond to novel antigens. Probably, the aged peripheral microenvironment causes an accelerated maturation of newly produced T cells to the memory state. The capacity of the host environment to present Ag, as well as cytokine production, appears to play a role in this process (72).

Immunosenescence is associated with a loss in the diversity of the T-cell repertoire. The T-cell repertoire is created within the thymus (70). With aging, antigen-driven clonal T-cell expansion, as well as the decreased availability of naïve T cells, is likely to compromise the broad diversity of the T-cell repertoire seen early in life (61).

The functional competence of the T-cell system is determined not only by its size but also by its diversity. Homeostatic control mechanisms have to secure sufficient T-cell replenishment while preventing loss of clonal diversity. Major homeostatic challenges include profound expansion and shrinkage of T-cell clonotypes upon antigenic triggering and, more importantly, age-related changes in T-cell generation. The ability of the thymus to rebuild a diverse repertoire declines with advancing age. Failure of T-cell homeostasis appears to result from cumulative defects of T-cell generation (71).

Thymus in Centenarians

Recent studies showed that, unexpectedly, age-related changes in the T-cell compartment in healthy centenarians are much less dramatic than might be predicted. Centenarians who have retained immune responsiveness at levels usually observed in young individuals also show better retention of thymic structure and function (20,60).

The presence of a great number of naïve T-lymphocytes within the CD4 and the CD8 T-cell subsets in the peripheral blood of centenarians poses the problem of their origin when the thymus has undergone profound involution (73). Thymic remnants or other lymphoid organs may be able to produce T-cells continuously, until the extreme limit of human life (74). Despite the replacement of the perivascular space with fat, the remaining cortical and medullary tissue in the aging thymus is histologically normal. The phenotypes of all of the expected thymic T-cell intermediates are present, there is evidence of cell proliferation, and there is evidence of ongoing *TCR* gene rearrangement. Episomal DNA fragments representing the excisional products of the *TCR* gene rearrangement process are produced during thymopoiesis. These fragments are called TCR rearrangement excision circles (TRECs). They are stable, do not duplicate during mitosis, and are therefore diluted with each cellular division, and have been used to successfully quantify thymus function. TREC frequency in peripheral T cells decreases with age (75). Furthermore, the continued presence of TREC-containing cells within the peripheral blood of elderly subjects could reflect these cells' longevity but might also reflect the sustained output of recent thymic emigrants from the thymus.

T-CELL REPLICATIVE SENESCENCE

Replicative senescence describes the characteristic of all normal somatic cells to undergo a finite number of cell divisions before reaching an irreversible state of growth arrest (Chapter 4). The so-called Hayflick limit (76) might be particularly devastating for lymphocytes because the capability of rapid clonal expansion is essential to their function (10).

Because resting T cells are long lived, they are susceptible to the processes of postmitotic senescence relevant to nonreplicative cells. In contrast, activated T cells proliferate in response to antigens, and these clonally expanding T cells suffer replicative senescence that might lead to clonal exhaustion. Concomitantly, they alter their functional phenotype and surface markers.

Telomere Shortening and Clonal Exhaustion

Telomeres are the repetitive DNA sequences at the end of eukaryotic chromosomes and are critical for genomic stability, the protection of chromosome ends from exonucleolytic degradation, and the prevention of aberrant end-to-end fusion (Chapter 4). An additional aspect of telomere biology is the so-called "end-replication problem." The ends of linear chromosomes cannot be fully replicated during each round of cell division. Thus, somatic cells lose telomeric DNA during replication. A critical telomere length may signal cell-cycle arrest (68). Therefore, telomeres function as a mitotic clock, and telomere shortening with age may contribute to immunosenescence. Lymphocytes from centenarians and individuals with Down's syndrome and progeria (models of accelerated aging) have telomere lengths in the same range as senescent T-cell cultures. CD4+ memory cells that have experienced several rounds of cell division show consistently shorter telomeres than naïve cells, and this difference is the same when the cells are isolated from young or old donors.

The characteristics of replicative senescence observed during aging may also be present in other diseases involving chronic antigenic stimulation, such as HIV infection, and other infectious diseases, such as cytomegalovirus (CMV) and Epstein–Barr virus infections, or chronic inflammatory diseases, including rheumatoid arthritis (42) and Crohn's disease (60). These examples illustrate situations in which T-cell replicative senescence may play a part in immune system dysfunction independently from the age of the host. The effects of this type of clonal exhaustion in the elderly may simply be more noticeable than in the young because of thymic involution, which would prevent effective generation of naïve T cells, and because T cells present in the old may already have undergone many rounds of division. Therefore, in an aged or exhausted immune system, clonal expansions of antigen-specific, memory, senescent, dysfunctional T cells fill the immunological space, whereas thymic involution brings to a contraction the T-cell

repertoire. Clonal expansions of virus-specific CD8+ CD28− CD95+ memory effector cells and decreased numbers of CD45RA+ CD62L+ CD28+ CD95− naïve cells and TRECs are hallmarks of both senescence and chronic infections. Large numbers of dysfunctional CD8+ T-lymphocytes bearing receptors for a single dominant CMV epitope in the very old have been described (77). The presence of a large fraction of the entire CD8+ cell subset with one single viral epitope may contribute to the increased incidence of infectious diseases in the elderly by shrinkage of the T-cell repertoire available for responses to other antigens. Therefore, CMV infection, whose prevalence is 60% to 90% worldwide, induces specific T cells to extreme differentiation (57). Persistence of CMV as a chronic stressor is a major contributor to immunosenescence and associated mortality (78). In addition to the number of cell replications, other mechanisms could modulate telomere length. For example, telomerase is a reverse transcriptase that elongates telomeres. Telomere loss is proposed to be a consequence of the downregulation of telomerase activity with age (Chapter 4). Because optimal telomerase induction requires optimal stimulation via CD3 and costimulatory receptors, age-associated defects in costimulation may contribute to suboptimal telomerase induction. The introduction of a constitutively expressed telomerase catalytic subunit into cells with a limited life span is sufficient to stabilize their telomeres and extend their life span indefinitely without inducing changes associated with neoplastic transformation (51).

■ HIV Infection: A Model for Immunosenescence

Advanced age, characterized by lack of adaptive immune response to new intracellular pathogens, shares similarities with persistent and chronic stimulatory conditions of the immune system by infectious agents such as HIV (79–81).

AIDS has characteristics in common with an accelerated aging of the immune system. For example, in HIV-infected patients, the TREC number is very low, similar to that characteristic of centenarians, and the proliferative capability of T-lymphocytes is greatly decreased. Immunosenescence and AIDS also share the same cytokine pattern, that is, the predominance of the Th2 profile, and the same receptor modulation on the lymphocyte membrane, for example, CD3 downregulation on naïve and memory cells and increased CAM expression on leukocytes, concomitantly with increased markers of immune activation (79). Such a chronic stimulation results in clonal exhaustion, which leads to immunodeficiency marked by telomere shortening.

The loss of naïve T cells, particularly within the CD8+ T-cell compartment, represents a hallmark of immunosenescence as well as AIDS and could provide a useful biomarker in both conditions. In particular, the progressive decrease of naïve CD8+ T cells, the expansion of CD8+ CD28− T cells, and the restriction in the CD8+ T-cell repertoire suggest a typical perturbation of the CD8+ T-cell subset that occurs in HIV disease and advanced aging (60).

Aging is an important predictor of progression in HIV infections. The more rapid progression of the disease appears due to an inability of older persons to replace functional T cells that are being destroyed. The additive effects of HIV infection and age on the immune system contribute to a decreased length of survival as well as more rapid disease progression (81).

■ INNATE IMMUNITY

Innate immunity remains relatively intact in the elderly, whereas acquired immunity is primarily affected. However, there are changes in the ability of APCs to communicate with T cells (82). The upregulation of nonspecific proinflammatory responses and downregulation of specific immunity may reflect a compensatory event. Because of the limited capacity of T-cell modulation due to the dramatic involution of the thymus, the potential to modulate innate immunity at the APC–T-cell interface could be a key for reversing impaired immune competence, even in the face of multiple external factors and chronic illness (10).

■ The Interface Between Innate and Adaptive Immunity: Antigen Presentation

The complex process of immune activation is dependent on the close participation of T cells and APCs. APCs are responsible for uptake, processing, and presentation of antigen to T cells. The ability of APCs to stimulate T cells is impaired in the elderly. Expression of costimulatory molecules that assist in the efficiency of cell-to-cell communication may be altered in old subjects and thus alter cytokine production by APCs, which regulates downstream T-cell effector functions (42). However, some studies have shown enhanced antigen presentation by APCs from healthy elderly, associated with increased levels of IL-10 and IL-12. It is hypothesized that this upregulation in IL-12 production by APCs may be compensatory to an inherent age-related decline in T-cell function to maintain immunocompetence (83).

Antigenic presentation is a very complex phenomenon involving the formation of the immunological synapse via the activation of the TCR and coreceptors. This interaction determines whether the interacting T-cell becomes tolerant or proliferates and differentiates into a functional effector T-cell. The capacity for immune synapse formation between APC and T-lymphocytes is altered with age. This may be partly due to an alteration in the membrane properties and costimulatory molecules of the cells of the innate immune system with aging. The innate immune system also influences the adaptive immune response through the timing, type, and strength of cytokines produced. Aging is associated even in healthy persons with a nonspecific increase in the production of proinflammatory cytokines originating from macrophages (65).

■ NK Cells

NK cells, originally identified as a population of large granular lymphocytes, are cytotoxic cells that play a critical role in the innate immune response against infections and tumors. NK cells lyse target cells (tumor cells or virus-infected cells) upon initial encounter without the need of prior antigenic sensitization (distinct from cytotoxic T cells) and without MHC restriction. Target-cell lysis takes place by a secretory mechanism via exocytosis of cytoplasmic granules containing perforins that damage the membrane, and granzyme that damages DNA, or by a nonsecretory mechanism via Fas–Fas ligand interaction.

Aging-associated alterations in the number and function of NK cells have been reported (Table 15). There is a general consensus that a progressive increase in the percentage of NK cells occurs in the elderly, which is associated with an impairment of their cytotoxic capacity when considered on a "per cell" basis (32). Furthermore, there is a major shift in lymphocyte population from conventional T cells to NK/T cells with senescence (27). These cells acquire NK receptors and, in particular, express killer-cell Ig-like receptors (KIRs), exert cytotoxic activity, and produce proinflammatory cytokines. The increased percentage of NK cells observed in the elderly is

TABLE 15 Aging-Related Changes in NK Cells

General decline in cell function
Good correlation between mortality risk and NK cell number
Increase in proportion of cells with high NK activity (i.e., CD16 , CD57)
Progressive increase in percentage of NK cells
Impairment of cytotoxic capacity per NK cell
Increase in NK cells having surface molecule CD56dim subset

Abbreviation: NK, natural killer.

mainly due to the increase in the mature CD56+ dim subset (84). An age-related increase in cells with high NK activity (i.e., CD16+ CD57−) has also been described. In contrast, changes in cells with intermediate (CD16+ CD57+) or low (CD16− CD57+) NK activity are minor.

Decreased NK cell function in the very old is associated with an increased incidence of infectious diseases (85). Old subjects with low numbers of NK cells have three times the mortality risk in the first two years of follow-up than those with high NK cell levels. Furthermore, other evidence supporting the significance of NK cells in healthy aging comes from the studies in centenarians. In fact, centenarians have a very well-preserved NK cell cytotoxicity (20). It can be speculated that well-preserved NK activity can help in becoming a centenarian. The shift in lymphocyte population from conventional T cells to CD3− NK cells and CD3+ CD56+ T cells, possibly of extrathymic origin, is probably a consequence of the age-related thymic involution (19).

A main function of NK cells is the capacity to synthesize and release a broad range of cytokines, such as IFN-γ or TNF-α, that participate in the initiation of the Th1-dependent adaptive immune response. The secretion of IFN-γ by activated NK cells is significantly lower in the elderly compared to young individuals (19).

Antibody-dependent cellular cytotoxicity (ADCC) mediated by NK cells when triggered via CD16 is comparable between young and aging subjects (86). However, NK cells from elderly donors are defective in their response to cytokines, with a subsequent decreased capacity to develop lymphokine-activated killer cells able to kill NK-resistant cell lines. Decreased NK cytotoxic capacity is associated with defective phosphorylase kinase C–coupled transmission signals. Deterioration at the molecular level (perforin and granzymes) in the lytic mechanism may, at least in part, be responsible for the decline in cell-mediated cytotoxicity. This is an important mechanism of tumor control in vivo, mediated by both CTL and NK cells, and both types of cells predominantly use the perforin-dependent pathway. It is possible that the high incidence of tumors in old age could partly be a result of a compromised early spontaneous cytotoxicity mediated by perforin (87). CD4+ CD25+ Treg cells are able to inhibit both T CD8+ and NK lymphocyte cytotoxic activities (88). Despite their decreased function, the number of peripheral blood CD4+ CD25+ high Treg cells increases with age, leading to a decrease in the cytotoxic activity of CD8+ T- and NK cells and a decreased production of IL-2 (89).

■ Phagocytic Cells: Granulocytes and Macrophages

Macrophages and polymorphonuclear (PMN) cells or neutrophils are important components of the first line of defense because they are the first inflammatory cells recruited to tissue sites in response to inflammation or infection. The function of macrophages and granulocytes in the elderly is impaired (Table 16) (90).

The function and phenotype of monocytes and granulocytes in the elderly is consistently remodeled. Age-related changes in adhesion-molecule expression on granulocyte and monocyte surfaces are responsible for immune dysfunction during senescence. The increased proportion of granulocytes and monocytes lacking CD62L and the downregulation of CD50 intensity expression on both cell types suggest a state of in vivo activation (91). CD50 and CD62L shedding from the cell surface of activated granulocytes and monocytes could be interpreted as a mechanism to counteract the dangerous effects of an excessive chronic inflammation. However, the increased proportion of CD62L-negative granulocytes leads to an impairment in cell adhesion which is the first line of response to acute inflammatory stimuli. This phenomenon likely contributes to the increased susceptibility to acute infections in elderly people.

Studies on polymorphonuclear neutrophil (PMN) function in older persons have yielded conflicting results. Normal or impaired phagocytosis, chemotaxis, degranulation, and nitroblue tetrazolium reduction, and a relatively preserved intracellular killing activity in PMN from the elderly compared to younger individuals have been documented (92). The flow cytometric analysis of cell surface marker expression in PMN displayed a higher level of CD15 (adhesion with endothelial cells and platelets) and CD11β (receptors to C3bi). Aging is associated with an impairment in the insertion of CD69− containing vesicles into the plasma membrane of human PMN. This impairment in the externalization of CD69−containing vesicles is likely to be related to the impairments in PMN phagocytosis, bacteriacidal activity, and release of ROS, seen with increasing age (93).

Cytokines, such as IL-2, and bacterial products (including lipopolysaccharide), rescue PMN cells from undergoing apoptosis or programmed cell death and continue to produce the superoxide anions needed to kill pathogens that have been engulfed; this cascade of PMN cell functions has been found to be suppressed in older individuals (91).

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a regulator of granulopoiesis and of granulocyte and mononuclear phagocyte function. GM-CSF is not able to prime granulocytes from elderly subjects for the activation of several parameters, including superoxide production, intracellular calcium flux, ADCC, and intracellular killing mechanisms.

Macrophages perform several functions, from phagocytosis and killing to cytokine production. Some macrophage functions seem to decline with age, while others remain apparently fully intact. For example, the capacity of macrophages to respond to a virulent intracellular bacterial infection

TABLE 16 Influence of Aging on Macrophages and Granulocytes

General functional impairment of macrophages and granulocytes
GM-CSF is unable to activate granulocytes from elderly subjects (e.g., superoxide production and cytotoxic abilities)
Polymorphonuclear neutrophils appear to possess higher levels of surface markers CD15 and CD11β and lesser vesicles containing CD69, which leads to the impairment observed to destroy a bacteria
In elderly subjects, the monocyte phenotype shifts (i.e., expansion of CD14dim and CD16bright subpopulations, which have features in common with mature tissue macrophages)
Macrophages of aged mice may produce less IFN-γ, less nitric oxide synthetase, and less hydrogen peroxide

Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon.

does not seem to be influenced by the increasing age of the host (94,95). However there appears to be diminished (50%) hydrogen peroxide production from macrophages of aged BALB/c mice compared to those from young mice in response to stimulation with bacterial products. Also, the monocyte phenotype is consistently modified in the elderly. There is a significant expansion of CD14dim CD16bright subpopulation of circulating monocytes in elderly subjects, which may indicate a state of in vivo monocyte activation.

The secretion of IL-8, which recruits macrophages, appears to be dysfunctional in the elderly. The overproduction of IL-8 in elderly men may be detrimental. For example, the recruitment of massive numbers of immune cells to the lungs could result in increased pulmonary inflammation, which may well increase morbidity and mortality. In addition to cytokine dysregulation, hormonal imbalances in the elderly may also affect functioning of the constitutive immune response (Chapters 9–13).

■ AUTOIMMUNITY AND AGING

In addition to predisposition to infections and neoplasia, another possible consequence of the progressive aging of the immune system is the increase in autoimmune phenomena. The clinical expression of systemic autoimmune diseases in older patients has specific characteristics:

- Frequent atypical presentation
- Higher morbidity and mortality
- Frequent association with neoplastic processes (96)

The increase in autoimmune phenomena in the elderly involves multiple causes. Although B- and T-lymphocytes lose the ability to respond to antigenic stimulation with age, it has been noted that the frequency of autoreactive antibodies is higher in older individuals. This increase in antibodies specific for self-antigens could be due to changes in the B-cell repertoire and/or to differences in the mechanisms responsible for generating immune tolerance in primary responses.

Memory B-cells that are self-reactive may be harbored within an organism as it ages and the potential exists that they become reactivated at a later time, resulting in a vigorous autoreactive recall response. This may occur preferentially in older individuals due to several factors, including the following:

- Deficiencies in immune tolerance with aging
- Progressive aging-associated loss of tissue integrity yielding neoself antigens
- Possible reexposure to an infectious agent, which induces an autoimmune memory response through molecular mimicry (97)

Autoimmunity occurs preferentially in individuals in whom the processes of T-cell repertoire formation are compromised. Thymic T-cell production declines rapidly with advancing age. Multiple mechanisms, including antigen-driven clonal expansion and homeostasis-driven autoproliferation of postthymic T cells, impose replicative stress on T cells and induce the biological program of cellular senescence.

T-cell immunosenescence is associated with profound changes in T-cell functional profile and leads to accumulation of CD4+ T cells that have lost CD28 but have gained KIRs and cytolitic capability and produce large amounts of IFN-γ. In patients with rheumatoid arthritis, T-cell immunosenescence occurs prematurely, probably due to a deficiency in the ability to generate sufficient numbers of novel T cells (98). Senescence of the immune system is a risk factor for rheumatoid arthritis, with chronic inflammation resulting from the accumulation of degenerated T cells that have a low threshold for activation and utilize a spectrum of novel receptors to respond to microenvironmental cues. The process of immunosenescence is accelerated in rheumatoid arthritis and precedes the onset of disease. Naïve CD4+ T cells are contracted in diversity and restricted in clonal burst. Senescence of effector cells is associated with the loss of CD28 and the de novo expression of *KIR2DS2*, *NKG2D*, and *CXCR1*, all of which function as costimulatory molecules and reduce the threshold for T-cell activation (71). Also, alterations of the immune receptor signaling machinery underlie the higher incidence of autoimmune phenomena in the elderly. Aging is associated with alterations in several components of the signaling complex in B-cells, memory, and naïve T cells, and a reduced activation of several lipid rafts-associated proteins (52,99).

■ LYMPHOCYTE PROLIFERATION AND APOPTOSIS

Cell proliferation and cell death are two physiologically active phenomena closely linked and regulated (Chapter 4). A failure of these mechanisms leads to profound dysregulations of cell homeostasis, with major consequences in immune functioning and the onset of autoimmune diseases and cancer, which increase in old subjects (100). An important function that has been suggested to deteriorate with age and to play a major role in the aging process is the capability of cells in aged subjects to respond to mitogenic stimuli and, consequently, to undergo cell proliferation (Chapter 4).

Cell growth, immunosenescence, and longevity are strictly interconnected and strongly related to programmed cell death or apoptosis. The cellular equilibrium between cell survival and proliferation on one hand, and programmed cell death on the other, seems to be unbalanced with advancing age, although in each type of immune cell, it could be differentially modulated, resulting in a variety of clinicopathological consequences.

The impairment of lymphoproliferative capacity commonly associated with senescence has been attributed to several mechanisms, including the following:

- Reduction in the number of functional cell precursors
- Reduction of cell responsiveness to activation signals
- Alteration of intracellular signaling
- Increased tendency of activated aged lymphocytes to undergo cell cycle arrest
- Expansion of the memory cell compartment
- Expansion of other lymphocyte subsets that may be functionally restricted
- Altered pattern of cytokine production
- Endocrine changes affecting the hormonal milieu within the organism
- Altered expression of receptors on T-cell surface
- Decreasing telomerase induction
- Failure to maintain telomere lengths
- Cessation of proliferation

A current hypothesis suggests that telomere attrition may trigger growth arrest by activating DNA damage limitation programs. Old, resting, or stimulated cells have more messages for three mitotic inhibitors (*p16*, *p21*, *p27*) than young cells (101,102).

The cells of the immune system undergo two different kinds of apoptotic processes:

- Activation-induced cell death (AICD), peculiar to immune cells

- Damage-induced cell death (DICD), a more generalized phenomenon in response to a variety of cellular insults, mainly oxidative metabolism by-products

AICD is geared toward the elimination of unnecessary lymphocytes following clonal expansion that results from antigenic stimulation, whereas DICD is particularly important for preventing the onset of neoplastic proliferation.

Apoptotic deletion of activated mature lymphocytes is an essential physiological process implicated in both the regulation of the immune response and the control of the overall number of immunocompetent cells. Closely interrelated signaling mechanisms convey activation or death messages, achieving the necessary equilibrium between cell proliferation and cell deletion. During the course of aging, there are numerous alterations to these signaling pathways that may shift the balance toward cell death, including the following:

- Diminished synthesis of growth and survival factors
- Transmembrane signaling defects
- Default in the expression of particular genes implicated in the control of cell proliferation
- Inability to cope with oxidative stress

CD28 costimulation may protect against apoptosis in several ways, including increased IL-2 production. The increased apoptosis with aging may be at least partly explained by the decreased CD28 expression and decreased IL-2 production. However, even in the presence of exogenous IL-2, old cells show increased susceptibility to AICD, which is mediated by Fas/Fas-ligand interactions (103).

The increased susceptibility to apoptosis of T-lymphocytes in elderly humans is associated with increased expression of functional Fas receptors. However, T-cell senescence may also be associated with defective apoptosis. In some cases, cells from centenarians were more resistant to apoptosis than were cells from young donors or aged subjects. Resistance to apoptosis could contribute to cellular longevity and possibly to organismic longevity. The data concerning the resistance of lymphocytes from centenarians to undergo apoptosis are consistent with the observation that the intracellular levels of bcl-2 in centenarians are similar to those present in cells from young donors. This protein plays a critical role in protecting cells from several stresses, including oxidation, as well as from cell death (102).

Although the precise role of apoptosis in the aged immune system remains to be identified, investigators are pursuing at least three lines of inquiry:

1. Senescent T cells accumulate due to acquired defects in apoptosis.
2. Older T cells may undergo apoptosis at an early stage of activation more rapidly than younger T cells, thus explaining the proliferation defect noted in T cells of older subjects.
3. Defective costimulation through CD28 may result in reduced T-cell responses with aging (104).

In senescent T cells, due to their reduced capacity to execute DNA repair, the frequency and quantity of cells undergoing apoptosis can be increased (105). For example, the IL-1β–converting enzyme family of caspases, the downstream apoptosis-related proteolysis enzymes, are induced after DNA damage, and these proteases are responsible for the breakdown of DNA-repair proteins. However, only cells with functional apoptosis capability would be deleted by this process, whereas certain mutant cells not able to undergo apoptosis can escape from this deletion process and further proliferate into tumor or autoimmune phenotypes. All these apparently discordant data on apoptosis in the elderly could be explained in the light of a recently proposed hypothesis on age-related apoptosis changes. Both oxidative stress and chronic antigenic load, which impinge heavily on the immune system, induce decreased lymphocyte susceptibility to DICD and a proinflammatory status leading to increased AICD. The effect of the lifelong exposure to oxidative stress results in cells in aged individuals developing a sort of oxidative stress adaptation and becoming less prone to DICD. As a consequence, senescent cells persist and accumulate, contributing to decreased stress responsiveness, increasing resistance to spontaneous apoptosis, and accumulation of memory lymphocytes, which fill the immunological space, making immune responses less efficient. Chronic immune stimulation, through the hyperproduction of proinflammatory cytokines, increased production of activated cells, and overexpression of death receptors on lymphocytes, induces AICD upregulation. Because AICD intervenes in the downmodulation of clonal expansion following antigenic stimulation, the increased susceptibility of lymphocytes to AICD results in decreased immune responsiveness, less efficient clonal proliferation, and impaired memory cell generation and survival (106). Moreover, increased AICD plays an important role in the pathogenesis of chronic inflammation during aging by two mechanisms: (*i*) a defective clearance of apoptotic cells as a result of poor phagocytosis of apoptotic cells by aged macrophages results in secondary necrosis and releases endogenous ligands for TLRs to activate APC to differentiate into more mature phenotypes and secrete proinflammatory cytokines (e.g., TNF-α and IL-6); and (*ii*) increased number of apoptotic lymphocytes in aged humans may directly trigger caspase-1–mediated IL-1β and IL-8 release from dying cells (107). This could contribute to explaining the paradox of immunodeficiency and inflammation in aging.

■ IMMUNOSENESCENCE: CLINICAL CONSEQUENCES AND THERAPEUTIC APPROACHES

Greater numbers of individuals are living to older ages. A major concern is how to live these years at a high functional level. It appears that the very old who have retained good health are those with relatively well-functioning immune systems. Manipulations designed to prevent or delay immunosenescence might, therefore, allow susceptible individuals to achieve their potential life span while remaining in good health (108). If we had physiological markers to identify those at risk for progressive functional decline and impending death, therapies could be targeted towards these individuals to prevent adverse outcomes. Inflammation markers are synthetic measures of the individual immunological history: they give us a measure of the lifelong attrition and oxidative stress that individuals have undergone as a consequence of both their lifestyle and behavior and the genetic tendency to develop an inflammatory phenotype. Such markers are the most powerful predictors of frailty and mortality in the elderly actually available (Table 17). They are the epiphenomena of the chronic antigenic load and inflammation impinging upon the individual genetic background (45). The analysis of immunological changes during senescence and age-related markers of chronic inflammation could therefore provide a window to the individual immunological history as well as useful prognostic markers of morbidity and mortality. The characterization of elderly subjects with higher risk factors might allow for preventive and/or

TABLE 17 Main Predictors of Frailty and Mortality in the Elderly

Low CD4+ and high CD8+ T-cells
Low CD19+ B-cells
High NK and NK/T-cells
Decreased virgin T-cells
High central/effector memory cells
High neutrophil count
Decreased lymphocyte telomere length
Decreased number of TREC
Poor proliferative T-cell responses
CMV and EBV seropositivity
Increased clonal expansion of CMV and EBV specific T-cells
High C-reactive protein level
High inflammatory cytokines (IL-1, IL-6, IL-18, TNF-α)
High chemokines (MIP-1α, IL-8, MCP-1)
Reduced level of IGF-1 and IL-10
Inflammatory and anti-inflammatory cytokine gene polymorphisms
IL-6, TNF-α, IL-10

Abbreviations: CMV, cytomegalovirus; EBV, Epstein–Barr virus; IGF-1, insulin-like growth factor; IL, interleukin; MCP; MIP macrophage inflammatory protein; NK, natural killer; TNF, tumor necrosis factor; TREC, T-cell receptor rearrangement excision circles.

TABLE 18 Major Diseases Associated with Aging in Immune Function

Increased tumor incidence and cancer
Increased incidence of infectious diseases caused by the following:
Escherichia coli
Streptococcus pneumonia
Mycobacterium tuberculosis
Pseudomonas aeruginosa
Herpes virus
Gastroenteritis, bronchitis, and influenza
Reappearance of latent viral infection
Autoimmune diseases and inflammatory reactions as follows:
Arthritis
Diabetes
Osteoporosis
Dementia

therapeutic measures to assure successful aging and survival and to counteract the rapid progression of the aging process.

Potential strategies aimed at reducing chronic immune activation and oxidative stress (systematic reduction of antigenic burden, elimination of chronic infections, and preventive treatment with anti-inflammatory and antioxidant drugs) might prove effective in delaying the onset of immunosenescence and minimizing aging of the lymphocyte population. A wide range of possible approaches to partially reverse aging of the immune system is being uncovered. For instance, physical activity has been shown in men to influence immunological response with aging (Chapter 24). Both humoral and T-cell–mediated responses were more vigorous in an active older group of individuals upon exposure to a particular antigen compared to sedentary controls (109). Caloric restriction (which can extend life span) is also frequently applied, although it has not been definitively demonstrated to be effective (Chapter 23). A recent approach is that of applying hormesis (Chapter 9) in aging therapy, which is based on the principle of stimulation of maintenance and repair pathways by repeated exposure to mild stress. In this light, caloric restriction and moderate physical exercise could be interpreted as mild stressors (110–112).

Infectious diseases such as bacterial infections (lung, gastrointestinal, urinary tract, skin, and soft tissue) and viral infections (reactivation of herpes zoster and significantly increased morbidity and mortality due to influenza virus) are a great clinical problem in the elderly (Tables 2 and 18). In addition, changes in immunity create difficulty in detecting active and inactive tuberculosis. Moreover, atypical presentation of diseases in geriatric patients may result in an infection being recognized later than usual and cause a delay in starting treatment. The points raised above make prevention of disease even more necessary in the elderly. Response to vaccination, which requires intact cell-mediated immunity to drive the humoral response, is clearly diminished (108). There are worldwide efforts to improve vaccination in the elderly, for example, by doubling the vaccine dose or using new adjuvants (113). One candidate for a new adjuvant is CpG-DNA (which consists of DNA of over 50% GC content) to restore immunological function. In old mice, administration of this adjuvant has been found to enable an immunological response more like that of young (114).

It may also be advantageous to reduce exposure throughout life to antigens (e.g., reduce exposure to adverse environments, proper hygiene, and appropriate diet), thus lessening the antigenic load. This may help to possibly preserve acquired immunity and reduce the negative consequences of a proinflammatory state.

The deleterious effects of malnutrition or the lack of even a small quantity of nutrients on the cellular and humoral immune response have been largely demonstrated (111,112). The experimental supplementation of diet (Chapter 23) with one or several nutrients, for example, zinc, selenium, vitamin E, etc., has demonstrated an improvement in many immunological parameters in the elderly. Manipulations of dietary lipid intake (N-3 fatty acid fish oil enrichment) can have a significant effect on immune function, perhaps via altered eicosanoid production or influenced membrane fluidity and access to receptors. Another type of manipulation that seems to be successful in improving the immune function of aged rodents is hormone replacement or supplementation, which has also yielded unconvincing results in humans. Melatonin supplementation (Chapter 12) has been utilized by the general public in an uncontrolled fashion, with dubious and unproven benefits. The antioxidant effects of some products (vitamins, flavonoids, and melatonin) (Chapters 5 and 12), which help to restore cell redox balance, might be responsible for or contribute to the benefits observed in some experimental models and trials (82,108). The administration of ILs, such as IL-2, which seems to be defective in the elderly, has been found to have some benefits. IL-12 cytokine immunotherapy, in association with influenza vaccination, could enhance CTL responses and reduce influenza morbidity and mortality among high-risk elderly persons (113).

Another challenge for the future is to determine selective manipulation of cell death (AICD and DICD) in specific cell subsets that is able to preserve lymphocyte function and boost immunity in the elderly.

Also encouraging is a study that suggests that T-cell priming, which is defective in old mice, may be restored upon stimulation of a TNFR (CD137) by an antibody agonist. It is postulated that this may be also related to the restoration of T-cell proliferative capacity and survival, which is reduced in the elderly (114).

Future research on strategies to modulate the process of T-cell replicative senescence, by using genetic techniques to enforce telomerase expression or by manipulating CD28 expression, may also lead to novel approaches to improving the immune function in the elderly as well (16,23).

Reconstitution of the aged immune system with hematopoietic stem cells from young donors could reestablish a normal

young-like peripheral B-cell compartment consisting primarily of naïve B-cells. Other strategies for improving B-cell output from the bone marrow of aged individuals could include the gene therapy approach. For example, since decreased B-cell production may result from impaired signaling through IL-7 receptors, it might be possible to bypass this defect using a gene therapy approach. Interventions aimed at restoring thymic function and complementary modes of T-cell reconstitution are also possible therapeutic strategies to delay aging. Such approaches, while not providing a "fountain of youth," may someday enhance the quality of life of the aged by increasing their resistance to infectious agents (53).

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15

Cardiovascular Alterations with Aging: Atherosclerosis and Coronary Heart Disease

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INTRODUCTION

Cardiovascular disease continues to be one of the major causes of death in the United States and other industrialized, socioeconomically advanced countries. People now live longer and are more susceptible to the occurrence of degenerative diseases. Hence, despite the current, extraordinarily rapid decline in mortality (Chapter 2), cardiovascular disease associated with advancing age remains the most important single cause of death worldwide, in old age and in both sexes. However, during the last 50 years, there has been a marked decrease in total death rates due to heart disease and stroke (56% and 70%, respectively). Indeed, it is estimated that 73% of the decline in total death rates over this time period is due to this reduction in cardiovascular disease mortality (Chapters 2 and 3). Several concomitant factors—better control of hypertension, changes in lifestyle (diet and physical exercise), scientific breakthroughs in understanding the disease, improvement in medical care, and decline in cigarette smoking may have contributed to the decrease in cardiovascular morbidity and mortality.

This chapter will review some of the age-associated changes in structure and function of the arteries (see section entitled Definitions); it will focus on atherosclerosis, the major and universal manifestation of cardiovascular pathology (1–6) (see sections entitled Pathology, Timetable of Changes in Atherosclerotic Lesions, and Theories of Atherosclerosis) and it will select coronary heart disease (CHD) (see section entitled Coronary Heart Disease) as an example of a medical complication of atherosclerosis (7,8). Aging of the heart, not directly related to atherosclerosis of the coronary arteries, is discussed in Chapter 20.

The question of how long we might live were it possible to prevent atherosclerosis remains unanswered; it would seem that complete prevention of atherosclerosis would add several years to life (1,4,9,10). Scientists throughout the world and representing many disciplines are now striving to further elucidate the nature of the disease and to find more efficient means of preventing or curing it. The 1990s witnessed the beginning of a "new era" of atherosclerosis research in which it is possible not only to prevent but also to arrest or to induce regression of the lesions and their clinical manifestations (4,11).

Some Structural and Functional Characteristics of the Cardiovascular System

Blood vessels are part of a complex circulatory system, which includes the heart, representing the mechanical pump that propels the blood into the blood vessels, the nervous system that regulates contraction and relaxation of the heart and the vessels, and the lymphatic vessels that are also part of the immune system. A synoptic list of the major components and functions of the cardiovascular system (also called circulatory system) includes:

- The blood and blood vessels that carry gases, metabolic products, and hormones, from all tissues to the heart and back
- The lymph and lymphatic vessels that carry lymphocytes, clotting factors, and proteins to lymphatic organs and drain into veins (Chapter 14)
- The heart that pumps the blood through the blood vessels to all organs, tissues, and cells (Chapter 20)
- The brain and the central and peripheral nervous centers that coordinate cardiovascular activity

Types and Structure of Arteries

In its journey from the heart to the tissues, the blood passes through channels of six principal types:

- Elastic arteries
- Muscular arteries
- Arterioles
- Capillaries
- Venules
- Veins

In this system, the arteries show a progressive diminution in diameter as they recede from the heart, from about 25 mm in the aorta to 0.3 mm in some arterioles. The reverse is true for the veins; the diameter is small in the venules and progressively increases as the veins approach the heart. All arteries are comprised of three distinct layers, intima, media, and adventitia, but the proportion and structure of each varies with the size and function of the particular artery.

The morphology of the arteries is summarized in Box 1, illustrated in Figure 1, and represented diagrammatically in Figure 2.

DEFINITIONS

Arteriosclerosis and Atherosclerosis

Arteriosclerosis is a generic term for any vascular damage that leads to progressive thickening and loss of resiliency of the arterial wall. One type of arteriosclerosis is atherosclerosis, which refers to specific alterations occurring in the vascular (arterial) wall, such as atheromas or plaques characterized by a combination of lipid accumulation in the intima and an increase in connective tissue in the subintimal layers. It is this form of arteriosclerosis that is the most widespread and, at the same time, the most threatening, inasmuch as it affects those arteries such as the aorta, the coronary, and the cerebral arteries that are crucial in providing the necessary blood supply for the heart, brain, and other vital organs.

BOX 1 *Some Morphologic Characteristics of the Arterial Wall*

A large artery, such as the *aorta,* consists of the following layers, going from the lumen to the most external layers:

- *The intima,* or innermost layer, consists of a layer of endothelial cells separated from the elastin layer underneath by a narrow layer of connective tissue, which anchors the cells to the arterial wall.
- A large layer of elastic fibers forms the *elastica interna* layer.
- Below this layer are concentric layers of smooth-muscle cells intermixed with elastic fibers forming the *media*. Elastic lamellae, smooth-muscle cells, and occasional fibroblasts are imbedded in a ground substance rich in proteoglycans (starch-protein complexes) that serve as binding or "cement" material in the interstitial spaces. The outer layer of the media is penetrated by branches of the vasa vasorum.
- Between the smooth-muscle layer and the *adventitia,* there is again another layer of elastic fibers, the *elastica externa*. Layers 2, 3, and 4 form the media.
- The outer layer or *adventitia* is formed of irregularly arranged collagen bundles, scattered fibroblasts, a few elastic fibers, and blood vessels that, because of their location, are called *vasa vasorum* (vessels of the vessels); they provide blood to the adventitia and the outer media layers.
- In addition to the *endothelial, elastic, smooth-muscle* and *collagen cells,* a few cells of the immune system, *monocytes,* are occasionally present in the arterial wall. During the early events of lesion development, monocytes enter the intima in regions of endothelial damage. Cytokines, immune growth factors, trigger the differentiation of monocytes into macrophages that scavenge oxidized low-density lipoproteins (Chapter 16). With the progression of the lesion, macrophages accumulate in the arterial wall, degenerate and die and are transformed in the so-called foam cells.

The structure of the aorta and large arteries carry out their function as blood reservoir and for stretching or recoiling with the pumping of the heart. The wall of the arterioles contains less elastic fibers but more smooth-muscle cells than that of the aorta. The *arterioles* represent the major site of resistance to blood flow and small changes in their caliber cause large changes in total peripheral resistance. *Muscle cells* are innervated by noradrenergic nerve fibers, constrictor in function, and in some cases, by cholinergic nerve fibers, which dilate the vessels (Table 1).

The structure of *capillaries* shows a diameter just large enough to permit the red blood cells to squeeze through in single file. In the same manner as the intima of the arteries, the capillary wall is formed of a layer of endothelial cells resting on a basement membrane. The major function of the capillaries is to promote exchange of nutrients and metabolites between the blood and the interstitial tissues. Such exchanges are facilitated by the presence of specialized junctions, gaps, or fenestrations.

Atherosclerosis, then, is the vascular disorder that plays a major role in CHD (due to atherosclerosis of the coronary arteries) as well as in stroke (due to atherosclerosis of cerebral arteries). Arterial diseases may also arise from:

- Congenital structural defects
- Infectious diseases (e.g., syphilitic aortitis)
- Hypersensitivity or autoimmune diseases that principally affect the smaller vessels and lead to their occlusion (e.g., inflammation of the intima with clot formation as in thromboangiitis obliterans)
- Specific capillary lesions as in diabetic microangiopathy (Chapter 13)

The arteries show a progressive diminution in diameter as they recede from the heart (see above). The reverse is true for the veins; the diameter is small in the venules and progressively increases as the veins approach the heart. All arteries are comprised of three distinct layers, intima, media, and adventitia, but the proportion and structure of each varies with the size and function of the particular artery.

■ Progressiveness and Universality of Atherosclerosis

Atherosclerosis, as a prototype of cardiovascular changes with age (12), is characterized by the following:

- Onset at young age
- Progression through adulthood
- Culmination in middle and old age with overt disease manifestations
- Widespread distribution throughout the arterial tree
- Consequences leading to severe disability or death

Today, atherosclerosis must be viewed as a disease that, sooner or later, affects everyone. Working silently over the years from early childhood, it gradually destroys the arteries, ultimately preventing the exchanges of gases and nutrients necessary to keep organs, tissues, and cells alive and functioning normally.

Although a scourge of modern civilization and often discussed in relation to the pressures of an urban technocratic society, atherosclerosis has been with us from ancient times; the disease has been detected in Egyptian mummies and described in early Greek writings. There are several ways in which atherosclerosis impairs the normal function of the arteries:

- It may corrode the arterial walls to such a degree that they suddenly yield to the pressure of the blood inside and explode in a massive hemorrhage (e.g., rupture of an aneurysm).
- It may set off, in reaction to its destructive processes, a secondary proliferation of its tissues, thereby gradually blocking the arterial lumen (e.g., formation of a thrombus).

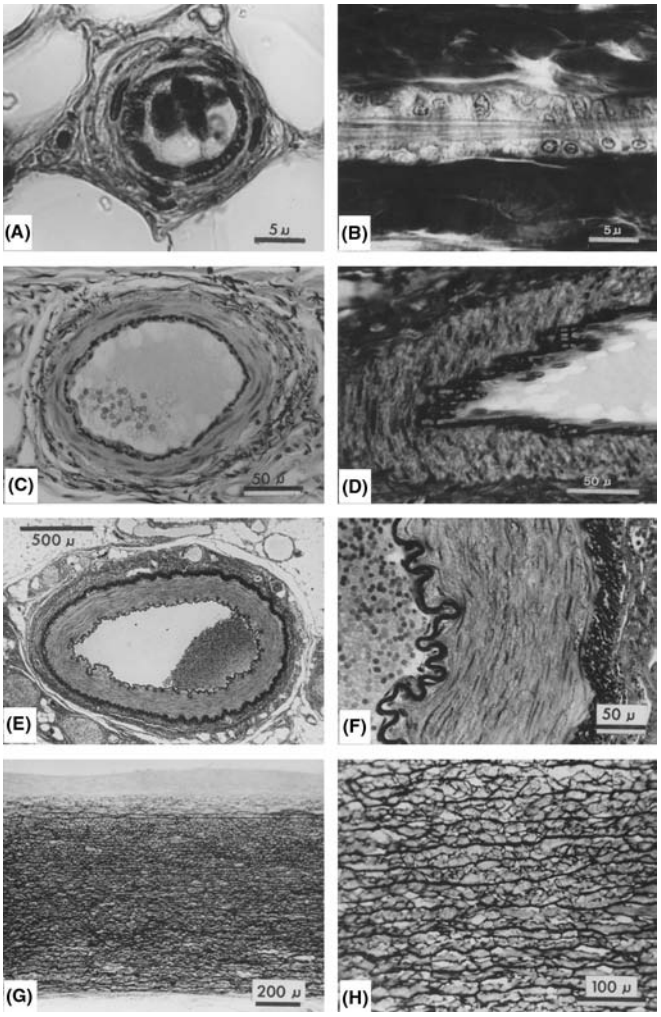

FIGURE 1 Morphology of normal arterial vessels. (**A**) Arteriole cross-section (human mesentery). Stain: iron hematoxylin-aniline blue. Note red blood cells in lumen, endothelial nucleus, internal elastic membrane, and smooth-muscle cells with elongate nuclei. (**B**) Arteriole, longitudinal section (cat ileum submucosa). Stain: Mallory-Azan. Note smooth-muscle cells coiling around endothelial tube that contains the nucleus of an endothelial cell. (**C**) Small artery, cross-section (human external ear, subcutaneous tissue). Stain: Verhoeff and Van Gieson. Note distinct internal elastic membrane and smooth-muscle of media; elastic fibers are beginning to accumulate from an external layer. (**D**) Small artery, tangential section showing fenestrated internal elastic membrane (human external ear, subcutaneous tissue). Stain: as in (**A**). Note all elastic membranes in the arterial tree bear fenestrae (window-like openings). (**E**) Medium artery, cross-section, low power (human mesenteric artery). Stain: as in (**A**). Note all layers of the wall, intima (with internal elastica), media, and adventitia (with externa elastica) are distinct. (**F**) Medium artery, cross-section, high power. Stain: as in (**A**). Note internal elastic membrane, well-developed muscular media, adventitia with external elastic tissue disposed as coarse fibers in helices, hence, cut tangentially. (**G**) Large artery, cross-section, low power (human aorta). Stain: as in (**C**). Note thick intima and high content of elastic tissue (appearing as black lines). (**H**) Large artery, cross-section, high power (human aorta). Stain as in (**C**). Note multiple thick membranes forming concentric tubes interconnected by finer cross membranes. The interstices are filled mainly with collagenous connective tissue and sparse smooth muscle. *Source*: Courtesy of Dr. O. N. Rambo.

- It may induce clotting of the blood within the diseased artery and, in this way, obstruct blood flow (also formation of a thrombus).
- It is a progressive disease that develops slowly over years or decades; however, the final "accidents" for which it is responsible (hemorrhage, thrombosis, gangrene, and infarct) may be initiated within only a few seconds.

One of the characteristics of atherosclerosis is its universality; it is present in almost all animal species, where it has been investigated, and throughout all populations within a species. So insistent and progressive is its onslaught with advancing age that it is generally considered to be an inevitable manifestation of aging—a "wearing out" of the arteries, as in the common saying "a man is as old as his arteries" [undoubtedly applicable also to women (13)]. Indeed, death by some of its consequences (e.g., heart attack or stroke) is now so common that we have come to regard it as a natural end of life. One of the burden of atherosclerosis is the accepted conviction that it kills us prematurely, in the sense that alterations in the arteries are capable of irreparably damaging such vital organs as the heart and brain, at a time when the functional competence of these structures is otherwise sound (4,11): from the analogy of the heart or brain as a motor and the arteries as the pipes that convey the fuel to the motor, if the motor is deprived of fuel because of a breakdown in the pipe system, it will stop working, even though the motor is fully operative (14).

Etiology (cause) and pathogenesis (pathology) of atherosclerosis are similar in all organisms, although there are some individual differences, probably related to the preponderance of one risk factor over the others (8,15). Thus, the first effects (early lesions) on the arterial lining may differ in time of onset, rapidity of progression, and severity, whether the risk factor is hyperlipidemia, diabetes, hypertension, smoking, increased lipid deposition (Chapter 16), concomitant endocrine alterations (Chapter 13), or accumulation of free radicals (Chapter 5). Consequences of risk factors may also vary according the genetic makeup of the affected individual (15–19). According to studies in twins, the greater role of genetic factors in increasing susceptibility to death from CHD at younger than at older ages implies that the genetic influence decreases as the individual ages (15,16).

■ Protocols of Atherosclerosis Studies

Currently, the study of atherosclerosis is being approached from two main directions: studies in humans or in experimental animals and tissue culture. Variables in the study of atherosclerotic lesions in humans may involve

- the timetable of their appearance,
- their location,
- their eventual regression,
- their clinical consequences,
- their adaptability to respond to internal metabolic needs as well as to changes in the external environment of the body,
- the multifactorial nature of morphologic and biochemical components of the lesions, and
- the identification of genetic factors, directly or indirectly implicated in vascular diseases.

In humans, progress in the study of atherosclerosis has been greatly facilitated by the use of imaging techniques for the arterial wall in vivo, such as angiography (e.g., X-ray visualization of blood vessels after the injection of radio-opaque, contrast material, associated with computer analysis) (9,20–25). The coronary and carotid are the arteries most frequently examined by these methods to quantify the lesions and to follow their progression (or regression) under baseline conditions or in response to various treatments. Additional noninvasive diagnostic techniques include vascular and intravascular magnetic resonance imaging, computerized

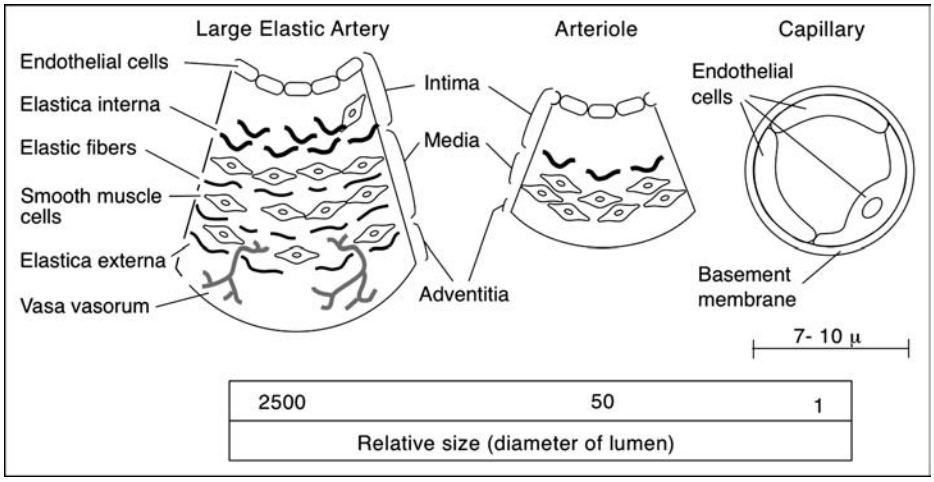

FIGURE 2 Diagrammatic representation of the arterial wall illustrating a large (elastic) artery, an arteriole, and a capillary.

tomography, positron electron tomography, ultrasound, and applications of nanotechnology both for diagnosis of pathology and for treatment with targeted delivery systems (26).

The study of atherosclerotic lesions in animals aims to discover the mechanisms that induce or, conversely, may be capable of preventing or curing atherosclerosis. The purpose is to attempt to reproduce the human disease in animals by

- manipulation of the diet,
- administration of "stress" hormones (e.g., glucocorticoids),
- exposure to trauma (e.g., mechanical, bacterial, or viral injury of arterial wall),
- alterations of clotting system and platelets,
- administration of oxidants (to induce accumulation of free radicals and oxidative damage), and
- use of genetic manipulations (transgenic, knockout animals with vascular pathology resembling atherosclerosis) (27–29).

However, it is recognized that observations in animals with respect to changes in cardiovascular function—as is the case for all other body functions—are not always referable to humans and, atherosclerotic lesions, in particular, differ depending on the species.

■ PATHOLOGY

■ Course of Atherosclerosis

The consequences of atherosclerotic lesions usually become manifest clinically in the fourth decade of life and thereafter. However, atherosclerosis is not exclusive to advanced age, but rather, it represents the culmination of progressive changes in the arterial wall beginning at a very early age (30,31). For example, microscopically identifiable vascular changes may start prenatally under conditions of impaired fetal development: this is the case of low-birth-weight (small-for-date) newborns who are at higher risk for cardiovascular disease later in life than individuals of the same age but born with normal body weight (30). Microscopic alterations consist, initially, of intimal thickening, later followed by cell proliferation, and accumulation of proteoglycans (i.e., high molecular weight complexes of proteins and starch). Some of these alterations, but not all, contain lipids and, in this case, can be readily observed as fatty streaks.

Despite species and individual variability, the approximate time sequence involved in the development of early atherosclerotic lesions with respect to specific pathologic changes has been generally established (at least in North America) to proceed in the following order (Fig. 3):

- The fatty streaks appear in the arteries during the first decade of life (as early as the first years or even months or days of life) and continue into the second or third decade.
- The fibrous or "pearly" plaques appear from the second decade on; clinical consequences (e.g., cardiac infarct, stroke, gangrene, and aneurysm) occur from the fourth decade on.

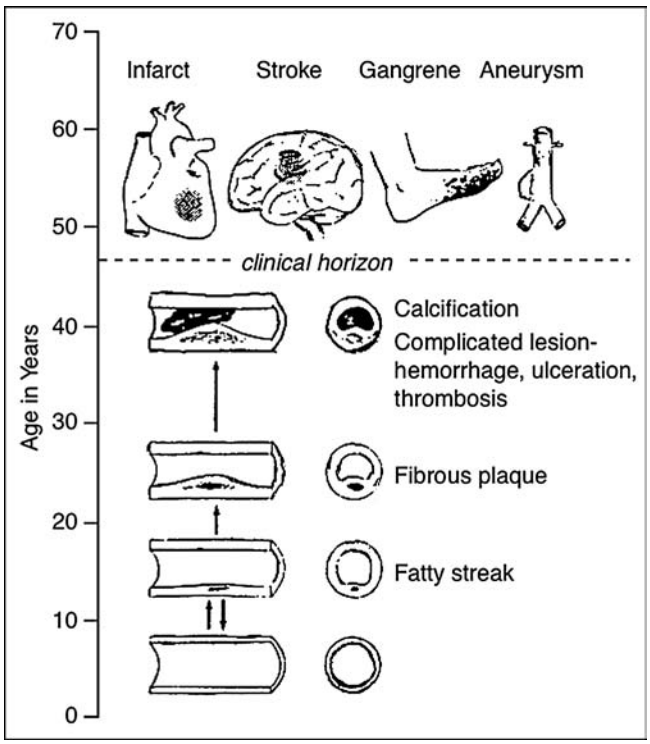

FIGURE 3 Natural history of atherosclerosis shown in this diagrammatic concept of the pathogenesis of human atherosclerotic lesions and their clinical manifestations. *Source*: From Ref. 12.

Atherosclerosis seems to develop in several "waves" throughout the life span, inasmuch as early and late stages of the lesions can be found side by side in the same vessel or in different vessels of the same person (Figs. 4 and 5).

However, this view assumes that the early stage is represented by the fatty streaks and advanced stages of the same lesion by the transformation of the streaks into plaques; this assumption is challenged by the observation that the two types of lesions are often found in different locations and that arterial areas with a fair amount of fatty streaks do not subsequently develop a commensurate number of plaques. The constituents of the lesions are similar and include, primarily, lipid and free radical accumulation, smooth-muscle cell and connective-cell proliferation, and migration and proliferation of immune cells reminiscent of inflammation. Not all lesions progress to the same degree in all individuals of the same age, and it is possible for the two types of lesions, early and late, to coexist in neighboring regions along the same artery.

■ Some Characteristics of Blood Flow and Arterial Function

The arterial system provides not only for the circulation of the blood, as a whole, but also, when necessary, for the special needs or functions of a particular organ. Certain organs—brain, heart, and kidney—receive a larger proportion of blood than others. Additionally, within the same organ, blood flow varies considerably depending on the degree of activity, as dramatically evidenced in the 30-fold increase in blood flow to the exercising muscle.

The velocity of blood flow declines gradually with the size of the artery, from approximately 8 cm/sec in the medium-sized arteries to 0.3 cm/sec in the arterioles; pressure, on the other hand, remains high in the large- and medium-sized arteries but falls rapidly in the small arteries to low levels of 30 to 40 mmHg; the magnitude of the drop varies depending on degree of arteriolar constriction.

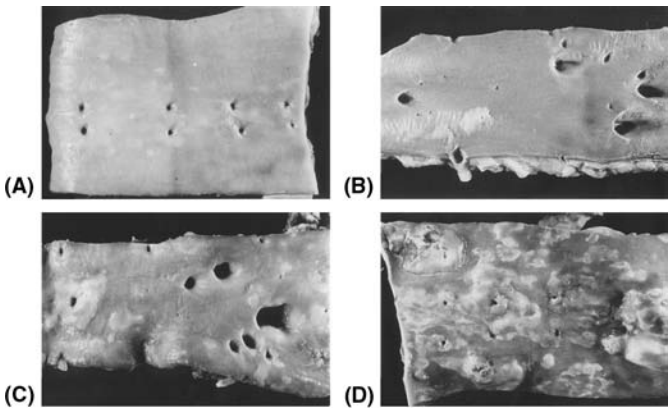

FIGURE 4 Progression of morphologic changes in human aorta from early to advanced atherosclerosis. Aortas were split open and the intima exposed for photography. (**A**) Aorta (thoracic) of a 32-year-old male showing early fatty plaques (represented by lighter coloration) localized mainly around the orifices of the intercostal arteries. As discussed in the text, because of the contribution of hemodynamic factors in the genesis of the atherosclerotic lesion, orifices of collateral branches are frequently the site at which lesions first appear. (**B**) Aorta of a 24-year-old female showing early fatty plaques, also around the orifices of collateral branches. (**C**) Aorta of a 55-year-old male showing advanced plaques characterized not only by a greater amount of fatty material but also by fibrotic thickening of the wall. (**D**) Aorta of a 65-year-old male showing large, complicated, and calcified plaques. *Source*: Courtesy of Dr. O. N. Rambo.

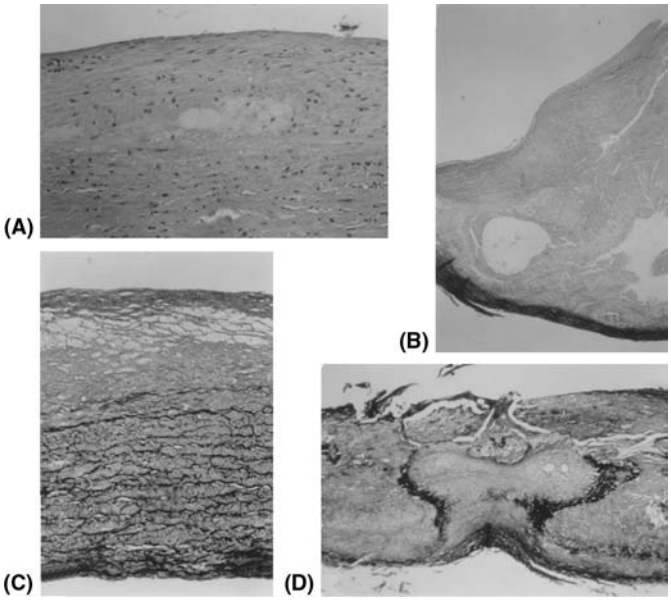

FIGURE 5 Progression of microscopic changes in human arteries from early to advanced atherosclerosis. (**A**) Earliest fatty plaque in fibrous intima (aorta from 24-year-old male: hematoxylin–eosin stain; (**B**) Large, atheromatous cystic plaque (carotid artery from a 65-year-old male; Verhoeff and Van Gieson stain; (**C**) Atheromatous plaque showing fatty infiltration and alterations of elastic tissue [carotidendarterectomy in 68-year-old male; stain as in (**B**); (**D**) Large ulcerated calcified plaque with metaplastic bone [carotid artery with occlusion in 79-year-old male; stain as in (**B**). *Source*: Courtesy of Dr. O. N. Rambo.

The mechanisms that regulate arteriolar diameter and, hence, blood flow through the arteries involve local (e.g., release of chemical substances into intercellular space) and systemic, primarily nervous, control (Table 1).

Nervous control is exerted by groups of neurons in the brain medulla (the vasomotor center), and by peripheral stretch receptors, the baroreceptors (32), located on the arch of the aorta, at the bifurcation of the common carotid artery, and in the walls of the cardiac atria and of the large veins. The arterial system, as a whole, is never static but continuously undergoes structural changes and adaptations that permit the organism to respond to changing requirements for blood supply.

Blood vessels are continuously subjected to the mechanical forces generated by blood flow in the form of stretch, including cyclic mechanical strain due to the pulsatile nature to blood flow (in synchrony with the cardiac cycle) and shear stress. Significant variations in mechanical forces induce structural modifications of the arterial wall cells. The numerous receptors on these vascular cells allow them to detect and respond to the mechanical forces generated by pressure and shear stress. The cytoskeleton and other structural cell components have an established role in mechanotransduction; they respond to the mechanical forces by initiating complex signals that augment damage to the vascular cell and contribute to the pathology of the arterial wall (33,34).

Even at birth, arteries vary in structure and in distribution depending upon the hemodynamic conditions under which

TABLE 1 Summary of Factors Regulating Arteriolar Diameter

Vasodilator	Vasoconstrictor
↓ Oxygen tension	↑ Norepinephrine
↓ pH	↑ Epinephrine
↑ CO_2	↑ Angiotensin II
↑ Temperature	↑ Vasopressin
↑ Lactic acid	
Histamine release	
Potassium ions	
Adenosine and nucleotide	
Kinins	

Note: With aging, capacity for arteriolar dilation or constriction is reduced due to: (*i*) reduced elasticity, (*ii*) collagen cross linkage, (*iii*) calcification, (*iv*) changes in adrenergic receptor sensitivity, and (*v*) atherosclerosis.

TABLE 2 Major Substances Secreted by the Vascular Endothelium

Substance	Synthesis, metabolism, receptors	Biological actions
Prostacyclin (PGI_2)	Synthesized from arachidonic acid via cyclooxygenase pathway, with ↑intracellular Ca^{2+}	Contributes to vasodilation Inhibits platelet aggregation
Endothelium-derived relaxing factor, identified also as NO, present in three forms	Synthesized from *L*-arginine by NO synthase (three isoforms: in brain, in macrophages, in endothelial cells)	Vasodilation has antithrombotic activity Others: influences brain function, stimulates cytotoxicity by macrophages, relaxes GI smooth muscle
ET-1, ET-2. ET-3, also in the intestine: vasoactive intestinal constricting peptide	Derived from a larger preproendothelin peptide by the endothelin-converting enzyme: ↑Intracellular Ca^{2+}; ↑ Ca^{2+} channel activation Two receptors have been cloned: ET_A receptor, specific for ET-1, and ET_B responding to all three ETs	Induce vasoconstriction Others: cardiac (coronary constriction) neuroendocrine (e.g., ↑ANP, renin, aldosterone, catecholamines), ↑renal vascular resistance, bronchospasm, others
Vascular endothelial growth factor (VEGF)	Family of four peptides with endothelial cells as specific target with receptors on both endothelial and tumor cells	Induces mitogenesis and promotes angiogenesis Promotes embryonic development, wound healing, reproductive functions
Cytokines (also secreted by lymphocytes, neutrophils, monocytes, and expressed on the surfaces of various cells)	Growth factors with specific receptors lacking tyrosine kinase domains, e.g., IL-1 to IL-8; platelet-derived growth factor; FGFa, FGFb, others	Participate in formation and repair of the vascular wall Promote cell adhesion, diapedesis, and chemotaxis Stimulate thrombotic activity

Abbreviations: ANP, atrial natriuretic peptide; ET, endothelin; FGF, fibroblast growth factors; IL, interleukin; NO, nitric oxide.

they operate and continue to change with maturation and advancing age. In the process of adapting to extrinsic and intrinsic stimuli, structural changes occur at particular sites, and the gross pattern of vascular distribution to an organ or body part may undergo considerable change (as in the development of collateral circulation).

Current evidence from human and experimental observations argues against a rigid classification of the various components of the arterial wall into definite "species" of cells. Instead, they favor a more versatile view of the cellular configuration of the arteries, in which cells are not irrevocably specialized but, rather, can assume more than one function when the need arises. For example, a muscle cell will not only contract or elongate upon stimulation, but, under certain conditions, it will also phagocytize lipids—i.e., becoming a cell full of fatty droplet, or foam cell, and even produce collagen or elastic fibers—a potential that might have a bearing on the formation and stability of atherosclerotic lesions (33,34).

Smooth-muscle cell mutations may be triggered by injury to the vascular wall [e.g., mechanical trauma, high blood lipids or oxidized low-density lipoprotein (LDL), altered carbohydrate metabolism or immune function, accumulation of free radicals, pollutant toxicity, bacteria, and viruses]. Mutations, associated with the local release of growth factors, would be responsible for the abnormal proliferation of smooth-muscle cells, with their subsequent migration to the intimal and elastic layers of the vascular wall. Invasion of the intima by smooth-muscle cells would represent the first event in the pathogenesis of atherosclerosis; it forms the basis of the myoclonal theory of atherosclerosis (34). This theory is consistent with the role of smooth-muscle cells in the formation and stability of the plaque, their potential transformation from a contractile to a different phenotype in response to injury, and their expression of genes that facilitate plaque formation (35).

Role of Endothelial Cells in Autoregulation of Vascular Motility

Cardiovascular adjustments to continuously changing demands of the organism are made possible, in part, by the capacity of blood vessels to constrict or relax (i.e., dilate) and, thereby, decrease or increase blood flow to a given tissue. Such regulation is under central and peripheral nervous control as well as under control from locally acting agents (Table 2).

Some of the signals generated locally derive from the endothelial cells of the vascular intima. Endothelial cells are of great interest (36–42) because

- they provide the vascular lining,
- they have a life span of 10 years and longer,
- they undergo aging-associated changes, and

■ they are involved in the pathogenesis of atherosclerosis.

Major products of endothelial cells include vasodilators [prostacyclin and nitric oxide (NO), formerly named endothelium-derived relaxing factor], and vasoconstrictors (endothelins) (Table 2). Among these vasodilator and vasoconstrictor compounds, NO plays a key role in mediating vasodilation through its actions on vascular tone, structure, and function. NO is synthesized, in the endothelium, from the amino acid *L*-arginine, through the action of the enzyme NO synthetase. Decreased bioavailability of NO results in arterial stiffness, hypertension, atherosclerosis, and, eventually contributes to cardiovascular pathology (see below) (39,40). With aging, overall changes in vasomotility are characterized by (*i*) decreased relaxation (*ii*) increased constriction, and the resulting imbalance of vascular tone leading to increased vasoconstriction with disruption of blood flow. Simultaneously, vasomotor responses to extraendothelial stimuli are altered: for example, the vasodilator actions of acetylcholine and histamine may be diminished, whereas the vasoconstrictor action of adrenergic stimulation may be increased. *These changes in vasomotility may contribute significantly to cardiovascular pathology.* By manipulating the various isoforms of the enzyme NO synthetase, it is possible to stimulate NO production with consequent enhanced vasodilation and relaxation of the vascular wall; thus, pharmacological advances promoting NO production suggest new beneficial approaches in prevention and treatment of atherosclerosis and its consequences (39,43).

Changes with aging in the function of endothelial cells also involve alterations in blood coagulation that would favor conversion from anticoagulatory conditions to a procoagulant, prothrombogenic state. Finally, with aging, endothelial-cell proliferation and responsiveness to local growth factors [e.g., epidermal growth factor and fibroblast growth factor (FGF)] are diminished, and equally diminished would be cell migration, perhaps due to increased cell adhesion; such changes would underlie the greater susceptibility of the endothelial lining to injury and the longer repair time that occurs in older individuals. A summary of these actions is presented in Table 3.

It may be noted that vascular endothelial cells secrete factors [e.g., vascular endothelial growth factor (VEGF)] that promote angiogenesis (i.e., formation of new blood vessels) (44) and that tumor growth depends on an appropriately sustaining blood circulation of the rapidly dividing carcinogenic cells (45). Under these conditions, formation of new blood vessels (angiogenesis) as well as the secretion of the vasodilator NO are stimulated. These observations have led to numerous attempts to reduce tumor growth by blocking blood supply to the fast-proliferating cells.

■ Blood Supply to the Arterial Wall and Metabolic Exchanges

Blood supply of a nonfunctioning organ or part of an organ can be diminished and, conversely, blood supply of an actively functioning part can be increased by three basic mechanisms:

1. Arteriovenous anastomoses, i.e., short channels that connect arterioles to venules, bypassing the capillaries
2. Specialized muscular arrangements in the walls of arteries, as in the sphincters of hepatic and splenic arteries
3. Arrangement of the capillaries in the capillary bed in such a manner that there is a preferential capillary channel from arteriole to venule (a controversial assumption that presupposes contractile structures in the capillaries)

One of the best examples of blood circulation adjusted to hemodynamic requirements is the establishment of a collateral circulation (i.e., circulation that is carried through secondary channels) when the main arterial supply to a specific organ or tissue is reduced or cut-off. Adequate blood supply to the heart is assured at all times by the two (right and left) coronary arteries (the first branches of the aorta) and by the presence of a rich system of anastomoses, not only between the two main coronary arteries but also between the coronary arteries and the arteries from the pericardium, lungs, thorax, and diaphragm. Thus, in the case of narrowing (stenosis) or occlusion (thrombosis) of one of the coronaries, if the process of occlusion is sufficiently slow, there can be time for competent collateral circulation to be established. This is the rationale for the administration of substances that promote angiogenesis (e.g., VEGF) and, thus, stimulate collateral circulation in the myocardium (44).

The extent of vascularization of the arterial wall itself varies from species to species: for example, vascularity is less well developed in man, rabbit, and chicken (species highly susceptible to atherosclerosis) than in the horse, goat, and cow (species less susceptible to the disease) (46). Lack of blood supply to the arterial wall may represent a major cause of arterial disease (Table 4).

The intima and inner media obtain their nutrition by diffusion from the lumen; thus, any process that causes a thickening of the intima or that damages the vasa vasorum of the adventitia might be expected to cause an ischemic-type injury (i.e., due to inadequate blood flow) to the arterial tissue (47).

Conversely, when the atherosclerotic lesion has reached the stage of plaque, regardless of the causes of plaque formation (e.g. lipid infiltration and thrombosis), the lesion becomes vascularized. Thus, although an inadequacy of blood supply to the arterial walls has been implicated in the initial stages of atherosclerosis, the increased vascularization associated with further development of the lesion has been viewed as an aggravating factor in advanced stages of the disease. Increased vascularization may also be responsible, at least in part, for the sudden accidents such as hemorrhage, with or without thrombosis, which are characteristic of these advanced stages.

Efficiency of exchanges of metabolic products between vascular wall and lumen and vice versa is determined by the special structure of the arterial wall. Diffusion, which normally regulates nutritional exchanges between blood and tissues, varies with the layer of the arterial wall. In the intima, for example, because of its proximity to the bloodstream, nutrients diffuse from the blood and products of arterial tissue

TABLE 3 Summary of Changes in Vascular Endothelium with Aging

Endothelial cells undergo significant changes indicative of abnormal function
The imbalance of vascular tone is manifested by increased vasoconstriction
Vascular integrity (cell proliferation and migration, wall remodeling) and injury repair through local growth factors are impaired
Maintenance of blood fluidity is disrupted with increased cell adherence, blood coagulation, and thrombogenic properties
These alterations by themselves may induce pathology or may predispose with other factors to atherosclerosis

TABLE 4 Localized Factors Contributing to Atherosclerotic Lesions

Marginal vascularization (i.e. blood supply) of arterial wall
Relative ischemia
Limited metabolic exchange
Blood turbulence and mechanical stress

metabolism are discharged into the lumen in the reverse direction. The other layers are too thick to be nourished by diffusion; in the adventia and outer media, metabolic needs are met by the vasa vasorum, leaving the inner portion of the media metabolically undersupplied and, therefore, at risk. Hence, any increase in thickness of this layer (due to increased proliferation of smooth-muscle cells, macrophages, and fibroblasts) or compromise of the circulation of the vasa vasorum will lead to alterations in metabolic exchanges and accumulation of metabolic by-products. Once the lesion has been established, metabolic injury will aggravate it and impair eventual recovery processes (46,47).

■ Localization of Lesions

It must be kept in mind that atherosclerotic lesions are focal (i.e., circumscribed to a specific location); they have preferred sites of occurrence and others where they are seldom found. Thus, other factors to be considered in evaluating the etiology and progression of the atherosclerotic lesion are its location along the arterial wall and the influence of blood turbulence. Preferred sites are around the orifices of arteries branching from a major artery (Fig. 4), such as the intercostal arteries from the descending aorta; another is at the bifurcation of a large artery into two smaller ones, such as the abdominal aorta bifurcating into the two iliac arteries. Blood flow at such orifices and bifurcations increases in velocity, exceeds critical velocity, and becomes erratic and turbulent. This turbulence creating a mechanical stress favors onset and progression of the lesion at these sites (Fig. 4 and Table 5) (47).

■ TIMETABLE OF CHANGES IN ATHEROSCLEROTIC LESIONS

■ Progression of Lesions

As mentioned above, atherosclerotic lesions begin at an early age and progress continuously throughout life. The localization, rate of progression, or type of lesion varies widely depending on several factors (Table 5) that are related to the following:

- The structure and function of the vessel considered
- The specific hemodynamic physiologic requirements
- The number of associated pathologic conditions, either local (e.g., hemorrhages, thrombi) or systemic (e.g., hypertension, diabetes)

Atherosclerosis occurs fundamentally as a localized lesion of the arterial wall. Although expansion of the atherosclerotic site—in the vessel, in the area of the vessel, and throughout the whole body—may be considerable, the steadily progressing pathologic process is always confined to one focal point, the form and size of which depends on local and generalized conditions. The atherosclerotic plaque or atheroma represents the characteristic site at which the histogenesis of the disease can best be analyzed. Although it has not yet been possible to detect the precise beginning of an atherosclerotic lesion, there are a sufficient number of characterizing features in those lesions to assign them the descriptive terms "early" and "advanced" (Figs. 4 and 5). Currently, animal models have proven to be particularly useful in studying atherosclerosis progression by identifying advantages and limitations and in identifying new methodologies and developments (29).

TABLE 5 General Characteristics of Atherosclerotic Lesions

Early onset—progressive
Focal lesions
Early lesions
Advanced lesions
Damage
Repair
Regression
Progression of localized-type lesions influenced by
Local factors—vessel structure and metabolism, blood turbulence
Systemic factors—diabetes, hypertension, stress, genetic predisposition

■ Early Lesions

For large elastic arteries such as the aorta, lesions usually begin as scattered foci in which the innermost layers of the vascular wall show signs of damage accompanied by the growth of repair tissue (Table 5). In the smaller arteries, the progression of events is less easily identifiable, but damage and repair processes still represent the most important characteristics of the early lesion in these structures.

Often, the membranes of intimal endothelial cells become "sticky." A simultaneously developing stickiness of circulating monocytes [from the immune system (Chapter 14)] facilitates the mutual attraction of these two cell types and *the invasion of the arterial wall by the monocytes.* In the wall, *the monocytes are transformed into lipid- and free radicals–engorged macrophages and become foam cells* (i.e., cells engorged with lipid droplets that are dissolved in the course of histologic staining and appear as numerous vacuoles resembling foam).

The intima thickens as a result of an increase in tissue fluid in the intimal ground substance. Disruption and disintegration of the innermost elastic lamellae, are followed first by moderate influx and then swelling and flooding of the area with amorphous materials, primarily proteins and sulfated proteoglycans. The proteins are probably derived from the blood, as a consequence of the increased permeability of the damaged endothelium (as in all inflammatory edemas); they are often coupled with lipids, which become visible when uncoupled. Proteoglycans may derive from the blood or may be formed in situ, but, in any case, they are similar to the materials that accumulate in most young repair tissues of the body, where they help to build collagen fibers, the principal component of scars (Chapter 21). The early lesions, at this stage, are essentially proliferative, due to the release of growth factors by endothelial and smooth-muscle cells and macrophages; T-lymphocytes are present. Inflammatory processes in the arterial wall occur early and persist with the plaque progression.

Signs of inflammation consist of accumulation, in the fatty streak, first, and in the plaque, later, of monocyte-derived macrophages (with or without lipid) and a varying number of T-lymphocytes producing proteolytic enzymes (47,48). Inflammatory reactions associated with early and late atherosclerotic lesions appear to be directly related to the level of C-reactive protein, a marker for systemic inflammation (49–51). Inflammatory processes would increase the risk of a first thrombolytic event and of myocardial infarction and stroke (48). These observations have led to the formulation of an inflammatory theory of atherosclerosis; they have suggested the use of anti-inflammatory agents such as aspirin for the prevention of cardiovascular diseases (52). Whether these still-disputed beneficial effects are mediated by aspirin's anti-inflammatory action or by other mechanisms (e.g., thrombolytic action) remains to be established.

This phase of the atherosclerotic lesion involves the repair and protective processes that characterize any inflammation (Table 5). One of the consequences of these processes is the further aggravation of intimal hyperplasia or thickening. Sooner or later, lipid appears in many of these lesions, mostly in their basal portions and not only within the cells (local smooth-muscle cells and invading and proliferating macrophages) but also in the matrix and on the disintegrating elastic lamellae. The lipid material, first in the form of small droplets, gradually fills the cells, imparting a "foamy" appearance in histologic section: its ubiquitous presence is the basis for the lipid accumulation theory of atherosclerosis. With the increase in the number of *foam cells and extracellular lipid,* the fat accumulation becomes visible to the naked eye as tiny yellow spots or streaks in the inner lining of the arteries, the so-called *"fatty streaks"* (Figs. 4 and 5). Taken as evidence of lesion, these fatty streaks are most commonly found in the aorta of children and younger individuals, although similar foci have been described in octogenarians and centenarians who earlier showed a so-called "juvenile" atherogenic index.

Changes in Vascular Ground Substance with Aging

Given the importance of wall thickness in the exchanges between blood and vascular wall, the ground substance or extracellular matrix, and its major constituents, the *proteoglycans* have been studied extensively. *Proteoglycans are high-molecular-weight complexes of proteins and polysaccharides (polymers of cellulose and starch) that form ground substances in the extracellular matrix of connective tissue.* The proteoglycans show progressive quantitative and qualitative changes as the atherosclerotic lesion progresses. Alone or in combination with other components of the ground substance, such as *hyaluronic acid,* and embedded substances such as *collagen* and *elastin*, the proteoglycans regulate some *important viscoelastic and water-binding properties of tissues,* including those of the arterial wall, where they serve as lubricants and support elements (Table 6).

Impairment of these properties with aging result in

- weakening of the arterial wall,
- reduced ability to provide mechanical support and hydration,
- decreased ability of the arterial wall to support compressive load of normal blood pressure, and
- chemical alterations of transport and binding of water-soluble substances.

■ Advanced Complicated Lesions

These lesions, found mainly in adults and elderly persons in whom autopsy examinations are conducted more frequently, have been studied more exhaustively than the early lesions typical of the first decade of life. Detailed morphologic descriptions of advanced human atherosclerosis can be found in most textbooks of pathology and in specific texts dealing with atherosclerosis. Only a brief summary will be presented here.

With the passing of years, more and more lipids—especially *cholesterol esters—accumulate in the fatty streaks of the established lesion* (Table 7); the foam cells increase in number to the extent that those in the center of the arterial wall die—probably due to lack of oxygen and the enormous amounts of fat in their cytoplasm displace or alter the organelles concerned with normal cellular function (Fig. 5).

The lipids released from the disintegrating foam cells, with those already present extracellularly, assemble in large pools, partly as cholesterol crystals and partly as an amorphous mixture of triglycerides, phospholipids, and sterols: these lipid pools have the consistency of a soft paste or gruel (hence, the term "atheroma" from the Greek "ather," indicating a gruel-like substance). An aorta that is riddled with atheromatous plaques may contain as many as several times its normal lipid content (Table 7).

The mass of extracellular lipid acts as an irritant to the arterial wall and provokes a proliferative reaction in the surrounding vascular tissue, similar to the inflammatory reaction that occurs in response to any foreign body encountered by the organism. The major lipid component is the LDL, and, particularly, its oxidized form (Chapter 16). While newly formed LDL is relatively benign, oxidized LDL acquires a new configuration, binds to specific receptors, and becomes less susceptible to removal by the high-density lipoprotein (54). Monocytes attracted from the circulating blood to the arterial wall and transformed into macrophages become trapped in the wall due to oxidized LDL, which inhibits their motility.

The resulting atheroma develops like a sac, encapsulating the gruel, but remaining much thicker on the lumen side of the arterial wall, where it forms a thick barrier between the bloodstream and the gruel (Fig. 5). At this stage, the atherosclerotic lesion has progressed considerably from its earlier manifestation as a fatty streak; not only is it larger and thicker, but it also seems to rise above the inner surface of the artery like a cushion, resembling an encapsulated abscess. Because of its appearance, the lesion has been given the name of "atheromatous abscess," but it is also called a "raised plaque" or a "fibrous plaque," and its characteristic pearly white color resulting from the high content

TABLE 6 Probable Role of Ground Substance in Early Atherosclerotic Lesions

Major components	Properties
Glycosaminoglycans (proteoglycans)	Viscoelastic impaired with aging, (reduced mechanical support)
Hyaluronic acid	
Collagen	Water-binding (with aging, reduced hydration, altered transport)
Elastin	

TABLE 7 Percentage of Total Lipids in Human Aortic Intima at Different Ages and in Different Types of Lesions

	Normal intima		Fatty streak[a]	Fibrous plaque[a]	Calcified fibrous plaque[a]
	Age 15	Age 65			
Total lipid (mg/100 mg dry tissue)	4.4	10.9	28.2	47.3	50.0
% of total:					
Cholesterol ester	12.5	47.0	59.7	54.1	56.3
Free cholesterol	20.8	12.2	12.7	18.4	22.4
Triglycerides	24.8	16.6	10.0	11.1	6.5
Phospholipids	41.9	24.2	17.6	16.6	14.8

[a] Irrespective of age (see text).
Source: Adapted from Ref. 53.

of collagen fibers in the capsule has also given it the name of "pearly plaque" (Figs. 4 and 5).

One of the main characteristics of the advanced atheroma is its progressiveness. As the plaque grows with fat, it consumes more of the arterial wall underneath it, transforming the cells into foam cells, and disintegrating one elastic lamella after another. In this process, the entire media layer is destroyed, and the atheroma invades the adventitia, which then reacts by setting up a series of inflammatory-like responses, such as *hyperemia* (due to vascular invasion) and *lymphocytic infiltration*. Simultaneously, the capsule of the advanced atheroma, perhaps in a compensatory effort, thickens considerably, building a new arterial wall. However, as it does not contain muscle or elastic fibers but contains almost exclusively scar (connective) tissue, this wall becomes functionally less efficient. As the pearly plaque becomes established, calcium deposits precipitate on the gruel, the capsule, or both, in the form of fine granules, thin strips, or huge masses (55). In the coronaries, for example, with the *accumulation of large amounts of calcium* over the years, the arteries become exceedingly hard and brittle, hence, the term *"hardening" or "sclerosis" of the arteries*. Associated with these progressive changes are alterations of the mitochondria: not only do mitochondria appear susceptible to damage mediated by oxidative stress but they also play significant roles in the regulation of cardiovascular function and the predisposing action of risk factors (56).

Until this stage, repair processes have counterbalanced the changes in the atherosclerotic lesion and no loss of tissue has occurred; indeed, in the sense that the lesion continues to form scar tissue, it can be viewed as "productive." In this respect, the function of the vessel, although impaired, is not drastically altered inasmuch as there is still a lumen, though smaller, and an intact, though thickened, wall with a relatively smooth lining permitting blood supply to the tissues. Some lesions remain in this stage for an indefinite period of time, whereas others eventually undergo changes that cause the breakdown of the vessel, inviting the perils that have made atherosclerosis a deadly disease. *The sequence of events summarized here underlines the important role of lipid accumulation, oxidative damage, and inflammatory responses in the formation of the atheroma and forms the basis of the "lipid accumulation" and "inflammation" theories of atherosclerosis (see above).*

■ Complications of Advanced Lesions

When the capsule of the atheroma breaks away,

- the plaque is transformed into an ulcer,
- blood clots cover the uneven surface (forming thrombi),
- part of the exposed gruel is carried away by the bloodstream (forming emboli),
- hemorrhage may occur in the gruel or under the lips of the ulcer,
- the ulceration breaks through the remnant of the wall, causing rupture of the artery and massive hemorrhage into the space outside (as in an aneurysm), and
- the end result may be thrombosis and embolism, or aneurysm and hemorrhage.

Although little is yet known of the precise factors that promote ulceration and hemorrhage in plaques, they are, perhaps, favored by certain local changes in the lesions (e.g., extensive cell necrosis in the capsule) as well as hemodynamic events (e.g., sudden rise in blood pressure) (57,58). Other complications of advanced atherosclerosis include narrowing or widening of the arterial lumen, thrombosis (59,60), and dilation, with possible rupture, of the arterial wall, aneurysm (61). The view that atherosclerosis narrows and tends to shut down the arterial lumen is applicable mainly to some arteries such as the coronaries, which are embedded into an unyielding environment. Arterial occlusion by stenosis (narrowing) of the lumen is usually a slow process, and collateral circulation, mentioned above, frequently has time to establish itself so that a sufficient blood supply reaches the area normally serviced by the stenotic vessel.

Large arteries, such as the aorta, are widened by the atherosclerotic process as a result of the progressive weakening of the wall by the formation of scar tissue, causing it to give way to the mounting pressure within. In these cases, the arteries not only widen but also tend to lengthen, bending and twisting in the process. *Aneurysms,* balloon-like bulges that press upon neighboring structures and often burst (with subsequent hemorrhage), frequently occur in a given spot in the wall that is much weaker than the rest (61). Rupture of the arterial wall that has been weakened by atherosclerosis can also be triggered by hypertension. When the rupture occurs in the relatively small cerebral arteries, the result is a "stroke." When it occurs in the aorta, especially the arch or the descending portion of the aorta, the usual result is massive bleeding into the thoracic or abdominal cavities.

■ Thrombosis, Embolism, Platelets and "Clot Busters," and Growth Factors

According to the "Virchow's Triad" formulated by the German pathologist R. Virchow, more than one century ago, *three factors—vascular injury, altered blood flow, and changes in blood coagulability*—are *responsible for vascular thrombosis. Thrombosis represents the process by which a plug of blood clot, or thrombus, is formed in a blood vessel (or in one of the heart cavities) by coagulation of the blood (62,63).* The thrombus

- contains few platelets, abundant fibrin, and many trapped red blood cells,
- is produced by activation of the plasma coagulation system, and
- forms in areas of slow blood flow (stasis).

The thrombus, distinguished from the embolus, is also a clot of coagulated blood and often originates from a thrombus detached from the arterial wall where it was formed. *The embolus is carried in the blood current, and an "embolism" represents the plugging of an artery by a clot (embolus) that has been brought to its place by the blood current.* Thrombi develop more frequently in atherosclerotic than in normal arteries, appearing particularly on the ulcerated plaques (62,63), on the arterial wall, or wherever there is a crack or fissure in the plaque. "Mural" thrombi are small and flat, develop over the surface of the wall of the large arteries, and are relatively harmless as long as they do not seriously impede the flow of blood through the vessel. When, however, the thrombus is large or develops in a small artery (such as a coronary artery or a cerebral vessel), it can fill its entire lumen and block all flow of blood, with disastrous results for the tissues that are to be supplied by the plugged vessel. Such occlusions generally occur suddenly, within minutes or hours, leaving little or no time for a collateral circulation to become established. The occurrence of thrombosis (forming the basis for the thrombogenic theory of atherosclerosis) has been related also to blood chemistry (e.g., high blood lipids) and hemodynamic changes (e.g., hypertension) (33,57,58,62,63).

Platelets are small, circulating, granulated cell fragments of bone marrow megakaryocytes; platelet number is about 300,000, their life duration about 10 days, and their production is regulated by colony-stimulating factors (Chapter 17). Platelets contain two types of

granules, one type is made up of nonprotein substances involved in platelet activation, the other, protein substances including blood clotting factors and platelet-derived growth factor (PDGF), also produced by macrophages and vascular endothelial cells. *The major function of platelets is to participate in the repair of breaks in the vascular wall to prevent blood loss, a complex process called hemostasis (64–66).* The first step in this process is the formation of a platelet plug, over the arterial wall break, by adhesion of platelets to the wall; this is followed by platelet activation and aggregation to form a clot. When the clot is in place, tissue repair begins, triggered by PDGF. Platelets work in concert with the endothelial cells (see above), also damaged by the injury, and initiate a coagulation cascade involving several factors [e.g., platelet-activating factor (PAF), a cytokine]. The resulting formation of the proteins, fibrinogen, and fibrin stabilizes the platelet plug. With healing of the injury, the clot is removed by enzymatic action (fibrinolysis) and by phagocytosis from immune cells (64–66).

Given the important role of platelets and endothelial factors in clot and, hence, thrombus formation, several fibrinolytic drugs are used to dissolve the clots, especially life threatening, in the coronary arteries (see below). Among these, some of the most frequently used are the enzymes urokinase (extracted from the kidneys) and streptokinase (extracted from bacteria) and the genetically engineered tissue plasminogen activator (t-PA) (67), a natural-occurring promoter of the fibrinolytic enzyme, plasmin. A number of inhibitors of platelet aggregation include aspirin, a weak inhibitor, probably more efficient in decreasing the incidence of myocardial infarctions than in treating acute coronary thrombosis, and inhibitors of the platelet glycoprotein IIb/IIIa complex that promotes platelet aggregation and is a member of the family of integrins (involved in the adhesion of cells to the extracellular matrix) (68). Current evidence has linked an inherited platelet trait involving one allele on glycoprotein IIIa to coronary artery disease (68,69).

To be noted here is the increasing list of growth factors participating in atherosclerotic processes and in repair of lesions. Among them, FGF, PDGF, and PAF have important roles in plaque formation. Factors stimulating or inhibiting proliferation of vascular cells are being investigated actively to determine if this aspect of plaque formation is amenable to pharmacologic interventions.

■ THEORIES OF ATHEROSCLEROSIS

It is evident that the pathogenesis of the atherosclerotic lesion is an extremely complex, multifactorial process not yet fully understood. At the present state of our knowledge, it may be advantageous to clarify certain specific steps of the process rather than to attempt to formulate a single, all-inclusive theory of atherosclerotic pathology. Such an aim is consistent with the major, currently proposed theories, dealing each with different processes in the arterial wall. Such theories have been referenced throughout this chapter and are summarized in Table 8.

■ CORONARY HEART DISEASE

One of the major life-threatening consequences of atherosclerosis involves the coronary arteries that supply blood to the heart muscle. Atherosclerosis of these vessels leads to CHD, also called ischemic heart disease, which implies reduced blood flow to the heart and consequent angina pectoris and myocardial infarction. Other possible consequences include arrhythmias due to defects of impulse conduction and electrocardiographic (ECG) changes. All of these conditions lead to heart failure, severe disability, and death (8,69–71).

CHD continues to be the major cause of disability and death in the United States (Chapters 2 and 3). Its prevalence increases with age and shows a significant sex difference, women having a lower incidence than men; this sex difference disappears after 70 years of age. Stress is another significant contributing factor to the high CHD incidence in the elderly (Fig. 6) (Chapter 9).

However, as indicated at the beginning of this chapter and more extensively discussed in Chapter 2, after a rise in the first half of the last century, mortality from this cause has been decreasing since the late 1960s. This decline includes all sectors of the adult population. In the 35- to 74-year age group, the rate of mortality due to CHD has fallen considerably, which is all the more remarkable because this decrease occurred after a sustained period of increase in this disease beginning about 1940. Apparently, we have been doing the "right things" (e.g., improvement of lifestyle and medical advances in control of hypertension and atherosclerosis) during the past decades to decrease mortality from CHD and the overall adult cardiovascular diseases (70,72).

■ Coronary Circulation

The right and left coronary arteries arising from the aorta, as it emerges from the left ventricle, are the major vessels supplying blood to the myocardium (the muscle layer of the heart). Venous drainage is through a superficial system ending primarily in the coronary

TABLE 8 Theories of Atherosclerosis

Lipid Accumulation	Myoclonal	Thrombogenic	Inflammation	Free radicals
Alterations of lipoproteins with accumulation of oxidized LDL in arterial wall (Chapter 16)	Chronic smooth-muscle proliferation in response to damaging agents	Lesions may be initiated through alterations of endothelial cells with consequent hemorrhage and thrombus formation	Early inflammatory processes in arterial wall followed by macrophage, T-lymphocyte migration	Increased accumulation of free radicals and induction of oxidative stress (Chapter 5)
Lipid infiltration involves muscle cells, monocytes, macrophages, and T-lymphocytes	Smooth-muscle cell mutations responsible for abnormal proliferation of smooth-muscle cells with migration to intima and elastic layers	Importance of platelets, growth factors, and blood clotting	Associated with increased C-reactive protein and responsive to anti-inflammatory agents	

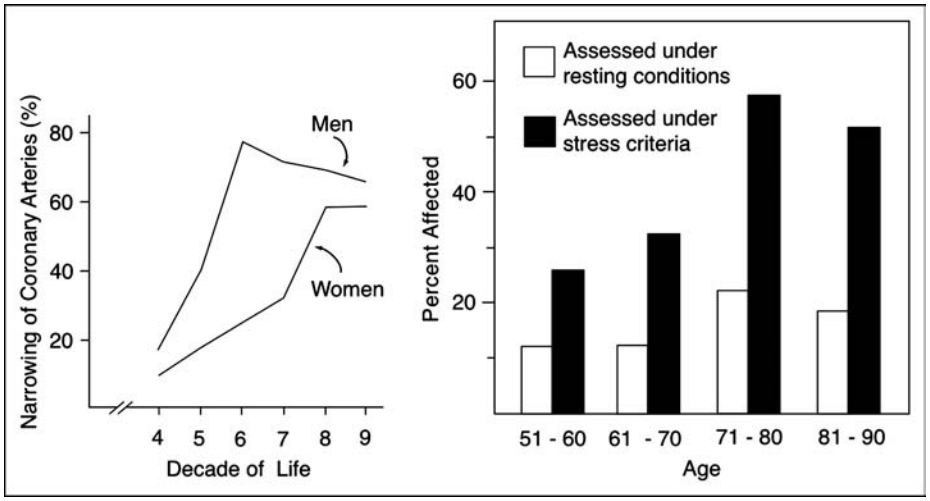

FIGURE 6 Percentage narrowing of coronary artery lumen with aging (*left*). Note sex differences with narrowing coronary artery occurring earlier and being more severe in men than in women. Incidence of coronary artery disease with aging (*right*). Note the increase in incidence with age, particularly under conditions of stress.

sinus and through a deep system that drains the remainder of the heart and empties directly into the heart chambers.

Coronary flow at rest (250 mL/min) is 5% of the cardiac output. The heart extracts 70% to 80% of the oxygen from this blood. Therefore, oxygen consumption can be significantly increased on demand only by increasing blood flow. Coronary flow is influenced by several local factors that regulate flow through vasodilation or vasoconstriction (Table 9).

Major vasodilators include reduced oxygen and increased carbon dioxide concentration, increased products of metabolism (hydrogen ions, potassium, and lactic acid), local agents (prostaglandins, adenine nucleotides, and adenosine), and neural stimulation (stimulation of parasympathetic innervation). Major vasoconstrictors include angiotensin II and stimulation of the sympathetic innervation. Cardiac circulation is preferentially preserved, as is cerebral circulation, when circulation of other organs is compromised.

■ CHD Consequences

When progressive narrowing of the coronary lumen reduces flow through the coronary artery to the point that the myocardium becomes ischemic (i.e., reduced blood flow that is insufficient to sustain function), angina pectoris develops. If the myocardial ischemia is severe and prolonged, irreversible changes occur in the cardiac muscle, and the result is *myocardial infarction*. The heart stops functioning, and death ensues within a few minutes. If ischemia is less severe, death may not occur, but it may generate permanent functional impairment.

TABLE 9 Local Regulation of Coronary Blood Flow

Vasodilation	Vasoconstriction
Low oxygen	Angiotensin II
High CO_2	Sympathetic stimulation (direct)
High H^+	
High K^+	
High lactic acid	
High prostaglandins	
High adenine nucleotides and adenosine	
Vagal stimulation	

■ Signs and Symptoms

The major sign of angina pectoris is squeezing or pressure-like pain, retrosternally, radiating to the left shoulder, arm, hand, neck, and jaw. The pain often appears with exertion, emotion, or after a large meal. Anginal pain in the elderly is often less marked, possibly due to reduced activity or altered pain perception; it may be effort induced or may occur at rest or in bed and may present as headache or epigastric (around the stomach) pain relieved by antacids (Table 10).

Major signs and symptoms of myocardial infarction are variable in the elderly, although chest pain remains the commanding feature in patients admitted to coronary care units (Table 10). Other symptoms include breathlessness, confusion, behavior change, fainting, palpitations, vomiting, sweating, abdominal pain, and hypotension. The pain may last over 30 minutes and is not relieved by multiple doses of nitroglycerine. Diagnosis is confirmed by ECG changes. With the establishment of the infarct, irreversible changes occur in the myocardium: muscle cells first become leaky, and the rise in serum enzymes and isoenzymes is a biochemical diagnostic sign of the infarct. The first enzyme to be elevated is serum glutamic oxaloacetic transaminase followed by creatinine phosphokinase and lactic dehydrogenase.

■ Risk Factors for CHD

The decreased mortality from CHD reported in recent decades (Chapter 2) may be ascribed primarily to the identification of risk factors and to their prevention (Chapter 16). Studies in various populations identified the following risk factors that predispose one to the development of CHD:

TABLE 10 Symptoms of Angina Pectoris and Acute Myocardial Infarction in the Elderly

Angina pectoris
Pain, less marked than in adult; may present as headache or epigastric distress
Myocardial infarction
Variable presentation with chest pain, including breathlessness, confusion, fainting, GI symptoms, sweating, hypotension, etc.

Abbreviation: GI, gastrointestinal.

- Age
- Genetic predisposition
- Hypertension
- Diabetes mellitus
- Hypercholesterolemia
- Cigarette smoking

Other risk factors include

- obesity,
- poor physical fitness,
- lack of exercise, and
- personality type (Table 11).

These lists dictate specific preventive measures such as treatment of hypertension and diabetes, elimination of cigarette smoking, amelioration of dietary habits toward an optimal body weight, and encouragement to follow regimens to improve nutrition and physical fitness (Chapters 23 and 24). An important risk factor, on which prevention and treatment are currently being focused, is the presence of elevated blood LDL cholesterol as a risk factor, and of reduction of LDL cholesterol as the prevention and treatment of choice for CHD (Chapter 16). However, not all patients with CHD have high blood LDL cholesterol levels (Chapter 16). In fact, the normal cholesterol levels preserved in some patients clash with the current emphasis on high blood cholesterol as a major risk factor for CHD.

Homocysteine is another substance that has gained attention because of its potential beneficial effects on patients at high cardiovascular risk (73). *Homocysteine is a nonprotein amino acid and intermediate in methionine metabolism; it is a donor of methyl groups to choline and creatinine and stabilizer of cell membrane fluidity.* In early studies, homocysteine blood levels were markedly higher in men who later had CHD than in age-matched controls who remained free of cardiac infarction. Later studies reported that the relationship of homocysteine to cardiovascular pathology persisted even when homocysteine levels were only moderately elevated. Accumulation of mutations in enzymes involved in the elevation of homocysteine blood levels correlates with increased CHD risk and occurrence of thrombogenesis. Hyperhomocysteinemia may damage the blood vessels by (*i*) impairing the production of NO from endothelial cells, (*ii*) stimulating smooth-muscle proliferation, (*iii*) acting as a thrombogenic agent, and (*iv*) increasing oxidative stress (73,74).

Blood levels of homocysteine are inversely related to those of folic acid, a vitamin (vitamin B9) part of the hematopoietic vitamins (Chapter 23). Folic acid is necessary for normal erythropoiesis (Chapter 17). The inverse relationship of homocysteine to folic acid and the low folic acid intake in a large percentage (40%) of the population have prompted the recommendation for the widespread screening of homocysteine blood levels and for the use of dietary folic acid supplements. However, the actual clinical benefits of normalization of elevated levels of homocysteine on CHD occurrence are still unproven (75). In addition, inasmuch as B12, B6, and folic acid are cofactors in the metabolism of homocysteine, folate supplements may mask the danger of anemia due to low levels of B12 (Chapter 17). Currently, the role of homocysteine in CHO and its preventive and therapeutic potential are still controversial (76).

■ New Approaches to Management

The reduction of mortality due to CHD reported in the 1980s had arisen from the awareness of contributing factors and their alleviation or amelioration (Table 12).

New studies demonstrate that appropriate dietary and drug interventions can induce regression and reversal or at least arrest the progression of atherosclerotic lesions. Reduction of blood levels of LDL (the atherogenic lipoprotein), by administration of drugs or manipulation of the diet, resulted in a significant regression of atherosclerotic lesions in cases of hyperlipoproteinemia or hypercholesterolemia (familial or not) in men and women and in younger as well as older (65+ years) individuals (Chapter 16). Current studies show that the administration of antioxidants, by reducing the level of oxidized LDL, may also be beneficial (Chapter 5). So far, promising results with antioxidant therapy have been reported in nonhuman primates and swine. Facilitation of collateral circulation (time permitting) and stimulation of angiogenesis are other interventive procedures under study. The effectiveness of these treatments supports the view that the atherosclerotic process is not, as previously thought, an inexorably progressive condition. Current advances in molecular biology offer new approaches to therapy and prevention by targeting molecules that are as diverse as adhesion molecules and transcriptions factors. While the biological rationale and progress of these therapies is still being chartered, they undoubtedly will contribute substantially to "remodeling" and repairing the arterial wall.

Several epidemiological studies have compared the risk factors associated with the early-onset versus late-onset CHD to determine whether the risk factors associated with middle-aged populations—the source of much of our current epidemiological information—are similar to those for CHD in younger or older populations. The main objective of these studies is to determine the importance, at different ages, of such conventional cardiovascular disease risk factors as smoking, hypertension, inactivity diet on the treatment, and prevention of the disease at progressive ages (77). Current medical treatment includes:

1. Treatment of the underlying disease, if any (hypertension, diabetes mellitus, and hyperlipidemias)

TABLE 11 Major Risk Factors in Coronary Heart Disease

Age
Genetic predisposition
Hypertension
Diabetes mellitus
Hypercholesterolemia
Cigarette smoking
Also
Obesity
Poor physical fitness and lack of exercise
Personality type

TABLE 12 Major Types of Coronary Heart Disease Treatment

Medical treatment
Diet
Exercise
No smoking
Pharmacologic agents
Surgical treatment
Aortocoronary bypass graft
Percutaneous coronary angioplasty with
Streptokinase/tissue plasminogen activator
Anticoagulant therapy

2. Behavioral therapy: low-cholesterol, low-fat diets, cessation of smoking, reduced stress, and increased physical exercise, especially for cardiac rehabilitation
3. Administration of pharmacologic agents with the intention of
   a. reducing LDL and cholesterol blood levels,
   b. decreasing free radical levels (antioxidants),
   c. increasing cardiac blood flow, reducing cardiac work,
   d. promoting collateral circulation and angiogenesis, and
   e. preventing clotting.

Surgery has also been successful with aortocoronary bypass grafts and transluminal coronary angioplasty (mechanical dilation of the area of constriction) with anticoagulant therapy (78) and by the intracoronary injection of the enzyme streptokinase or, even better, of t-PA, a recombinant protease (see above) (67,78–80). Attempts are being made with some success using gene therapy (81) as well as to transplant stem cells in specific cardiac areas to replace the muscle cells injured by the ischemia responsible of the cardiac infarct (82). Finally, heart transplant may represent, theoretically, another therapeutic alternative; however, this alternative is, realistically, out of reach for the elderly because of the still unknown complications that might arise after such an intervention in this age group, with a complex comorbidity and the low priority given to the elderly for organ transplantation (Chapter 3).

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16

Lipids, Lipoproteins, and Atherosclerosis

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INTRODUCTION

Atherosclerotic cardiovascular disease is the major cause of morbidity and mortality in industrialized nations. The disease is complex, and many factors singly or in combination contribute to its etiology, including genetic predisposition, hypercholesteremia (elevated cholesterol), hypertension, diabetes mellitus, smoking, obesity, and stress. Atherosclerosis, the condition whereby lipids accumulate in the artery wall, starts in the first decade of life and progresses throughout life; its severity is linked to the aforementioned risk factors. As the list of risk factors indicates, cholesterol, and by extension, plasma lipoproteins, have a major role in the process of atherogenesis. The structure, synthesis, and metabolism of lipoproteins are reviewed in this chapter along with their role as positive or negative risk factors in atherosclerosis, particularly in early or premature forms of the disease (Chapter 15).

LIPOPROTEIN NOMENCLATURE, STRUCTURE, AND COMPOSITION

Lipids in the plasma including cholesterol, phospholipids, and triglycerides are transported in the form of lipid complexes stabilized by specific proteins called apolipoproteins (apo). These complexes form lipoproteins that transport lipids in plasma and interstitial fluid. The generalized features of a lipoprotein are illustrated in Figure 1. The lipoprotein particle is essentially an oil droplet (hence, its globular shape) stabilized by a surface coat of hydrophilic molecules, including proteins, phospholipids, and unesterified cholesterol. The hydrophobic core of the particle consists of the highly water-insoluble lipids, cholesteryl ester and triglyceride. As the protein (apolipoprotein) content of the particles increases relative to the lipid content, the particles become smaller and denser. The differences in densities of the particles are the fundamental basis for the nomenclature used for defining lipoproteins, e.g., very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). Chylomicrons (CM) secreted by intestinal absorptive cells are the largest and the most buoyant of the lipoprotein particles. The major classes of lipoproteins and their compositions are summarized in Table 1.

Major Classes of Lipoproteins

As the compositions in Table 1 indicate, lipoproteins can be grouped into three major categories based on their composition:

CM and VLDL. Triglyceride-rich lipoproteins. These are relatively poor in protein but high in triglyceride (55–95% of total particle weight).

IDL and LDL. Cholesterol-rich lipoproteins. These particles are characterized by high levels of cholesterol that is mainly in the form of the highly insoluble cholesteryl ester. Because up to 50% of the LDL mass is cholesterol, it is not surprising that LDL has a significant role in the development of atherosclerotic disease.

HDL. Protein-rich lipoprotein. This particle has high protein content (~50%) and relatively high phospholipid content (~30%). HDL is generally divided into two subclasses, HDL_2 and HDL_3; of the two, HDL_2 is the larger and more buoyant particle.

Major Apolipoproteins

In addition to their core lipids that help define the lipoprotein particles, the particles are also defined by the apolipoproteins on their surfaces. The apolipoproteins act as markers that determine the metabolic fate of the particles. The major apolipoproteins and the lipoprotein class in which they are found are summarized in Table 2. Some apolipoproteins, e.g., apoBs, are found uniquely associated with specific classes of lipoproteins, whereas others such as apoCs can associate with almost all classes of particles.

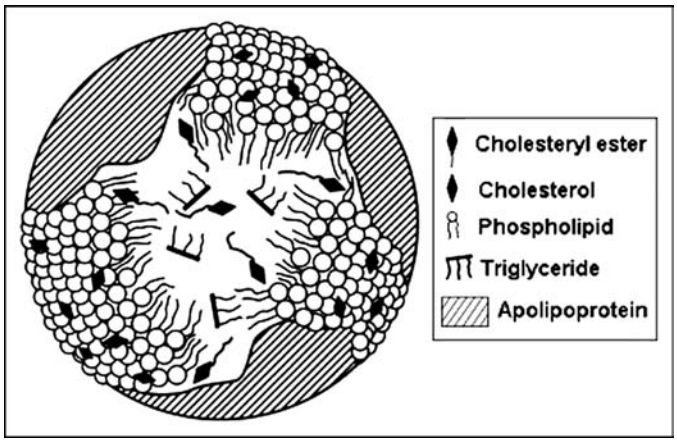

FIGURE 1 Generalized organization of mature plasma lipoproteins. Particles are overall globular in morphology with polar (water-soluble) components on the surface to stabilize the particle in the aqueous plasma environment. The polar constituents are primarily protein (apolipoprotein) and phospholipid. Some cholesterol is also on the surface. The "core" of the particle consists of nonpolar (highly insoluble in the aqueous environment) components, cholesteryl esters, and triglyceride.

TABLE 1 Composition (Weight Percentage) of the Major Classes of Lipoproteins

Lipoprotein	Protein	Phospholipid	Cholesterol	Triglyceride
CM	1–2	3–6	2–7	80–95
VLDL	5–10	15–20	10–15	55–65
IDL	19	19	38	23
LDL	20–25	22	45	10
HDL	45–50	30	20	5

Abbreviations: CM, chylomicron; VLDL, very low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

■ PLASMA CONCENTRATIONS OF LIPOPROTEINS

Generally, one thinks of lipoproteins in terms of pathologic conditions such as premature atherosclerosis, but in fact, they are macromolecules necessary for the maintenance of cell and tissue function and integrity.

Triglycerides transported by lipoproteins are a major source of energy for cells.

Cholesterol transported by lipoproteins is utilized by cells for cell division, cell growth, and membrane repair; cholesterol is also essential for the production of steroid hormones (adrenocortical hormones and sex hormones).

Another important function of lipoproteins is the transport of fat-soluble vitamins.

An overabundance of lipoproteins, however, particularly those carrying cholesterol, can be deleterious, by predisposing to premature cardiovascular disease (1).

The most commonly used clinical indicator for measuring potential risk of premature cardiovascular disease is the level of plasma lipids. Fasting levels of triglyceride, cholesterol, and HDL cholesterol can often be used to identify possible abnormalities. The expected normal adult plasma lipid levels are shown in Table 3. Females characteristically have lower triglyceride concentrations than males and have higher HDL cholesterol (55 mg/dL vs. 43 mg/dL); it is well known that there is an inverse relationship between HDL cholesterol levels and risk for heart disease; thus, the female has the more protective profile. The ratio of total cholesterol to HDL cholesterol is an important value, because values of 4.0 and above are associated with increased risk for coronary heart disease. Table 3 provides, for comparison, the plasma lipid levels from cord blood of normal, full-term newborns. The newborn infant has triglyceride and total cholesterol levels one-half to one-third those of the adult. The HDL cholesterol concentrations are relatively high (35 mg/dL) in the newborn, where the ratio of total cholesterol to HDL cholesterol is 2 compared with the adult values of 3.5 for females and 4.6 for males. HDL is considered beneficial; that is, it is protective against atherosclerosis, whereas LDL is a positive risk factor (Table 4). The lipid levels in infants are perhaps the most "ideal," as LDL levels are low and HDL relatively high. Except for genetic abnormalities (such as homozygous familial hypercholesterolemia), the vascular walls of neonates are free of fatty streaks. Fat accumulation appears, however, in the first years of life, indicating that dietary input and environmental factors probably influence initiation and progression of atherosclerosis. At birth, no distinction can be seen between male and female infants, because sex hormone concentrations are low and apparently have little metabolic influence at this stage of development.

TABLE 3 Normal Plasma Lipid Levels (mg/dL)

	Triglyceride	Total cholesterol	HDL cholesterol
Adult female	80	190	55
Adult male	120	200	43
Neonate	38	70	35

Abbreviation: HDL, high-density lipoprotein.

■ RISK FACTORS IN ATHEROSCLEROSIS

Three major indicators of increased risk of atherosclerosis are triglyceride concentrations greater than 150 mg/dL, LDL cholesterol concentrations greater than 130 mg/dL, and HDL cholesterol concentrations less than 40 mg/dL.

Aging is associated with a progressive increase in plasma triglyceride and cholesterol concentrations; thus, the process of aging is an important risk factor in atherosclerosis. As Table 4 indicates, besides age, there are other positive and negative factors that affect atherogenesis. In the adult, gender differences have a definite effect on plasma lipid levels, as shown by the higher levels of triglyceride and total cholesterol and lower levels of HDL cholesterol in males as compared to females (Table 3). In females before menopause, the most functionally significant difference is the higher HDL cholesterol concentration, 55 mg/dL, as compared to males, 43 mg/dL. This increase in HDL cholesterol is in a specific subclass of HDL, the HDL_2.

TABLE 2 Major Apolipoproteins

Apolipoprotein	Molecular weight (Da)	Major lipoprotein class
ApoA-I	28,000	HDL
ApoA-II	17,000	HDL
ApoB-100	540,000	VLDL, IDL, LDL
ApoB-48	260,000	CM
Apo(a)	300,000–800,000	LDL
ApoC-I	6,000	CM, VLDL, HDL
ApoC-II	9,000	CM, VLDL, HDL
ApoC-III	8,800	CM, VLDL, HDL
ApoE	34,000	CM, VLDL, IDL, HDL

Abbreviations: CM, chylomicron; VLDL, very low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

TABLE 4 Positive and Negative Risk Factors in Atherosclerosis

Positive	Negative
Age: Males >45 yrs; Females >55 yrs	Elevated HDL cholesterol
Family history of early CHD	Low LDL cholesterol
Elevated LDL cholesterol (>130 mg/dL)	Good genes
Low HDL cholesterol (<40 mg/dL)	Female gender (estrogen)
Diabetes mellitus	Exercise
Hypertension	
Obesity	
Smoking	

Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein; CHD, coronary heart disease.

■ High-Density Lipoprotein

Epidemiologic studies suggest that elevated HDL_2 concentrations play a protective role in cardiovascular disease (2). In women, the hormone estrogen is important, because it elevates HDL_2 and, thus, tends to protect premenopausal women from an early onset of atherosclerosis. Indeed, the incidence of cardiovascular disease increases after menopause.

HDL_2 levels can also be increased by exercise. It is known that male marathon runners have HDL patterns similar to those of females. An obvious conclusion is that exercise is beneficial in maintaining healthy HDL levels. Cigarette smoking, on the other hand, decreases HDL levels, whereas cessation of smoking reverses the effect. Other factors that contribute to atherosclerosis include genetic disorders that increase VLDL and LDL and decrease HDL levels, diabetes, obesity, and hypertension (3). Regulation of plasma lipid levels is clearly complex and involves genetic and environmental components.

■ Small, Dense LDL

Elevated LDL cholesterol is directly linked with an increased risk of cardiovascular disease. However, within the LDL class, one can distinguish specific subclasses, LDL subclass pattern A and LDL subclass pattern B, which correlate differently with regard to their contribution to cardiovascular disease (4). Pattern A LDLs are large (>25.5 nm diameter), buoyant particles, whereas pattern B LDLs are less buoyant and smaller in size (small, dense LDL, <25.5 nm diameter). The latter LDL pattern (but not pattern A) is associated with an increased risk of atherosclerosis; pattern B is also associated with a constellation of risk factors that predispose to atherosclerosis, including low HDL, particularly HDL_2, elevated apoB concentrations, and elevated triglyceride (5). LDL subclasses are genetically influenced; pattern B appears to be associated with an autosomal-dominant allele(s) that has a rather high population frequency of 25% to 30%. Interestingly, expression of the pattern B phenotype is age dependent. This phenotype is not expressed in males until approximately 20 years of age and in females until menopause.

■ SYNTHESIS OF LIPOPROTEINS

There are two major sites of synthesis of lipoproteins: the small intestine and the liver (Fig. 2).

■ Small Intestine

Lipoprotein secretion by the intestine is regulated to a great extent by what we eat. Pancreatic lipase in the intestinal lumen hydrolyzes dietary triglycerides to fatty acids and monoglycerides. These moieties, together with dietary cholesterol, form micelles by interaction with bile. The lipid micelles are taken up by absorptive cells in the small intestine, and triglycerides are resynthesized from fatty acids and assembled into triglyceride-rich lipoproteins, the CM. The composition and size of the CM depends on dietary lipids, where more saturated fats yield smaller particles and unsaturated fats yield larger CM. Formation of CM requires the stabilization of the particle surface with apoB-48, a truncated form of apoB-100 necessary for the release of CM from the cell. ApoC-II and C-III are also added to the surface; the secreted CM acquires apoE upon release into the circulation. ApoE and apoC-II are crucial for the rapid removal of these large particles from the circulation.

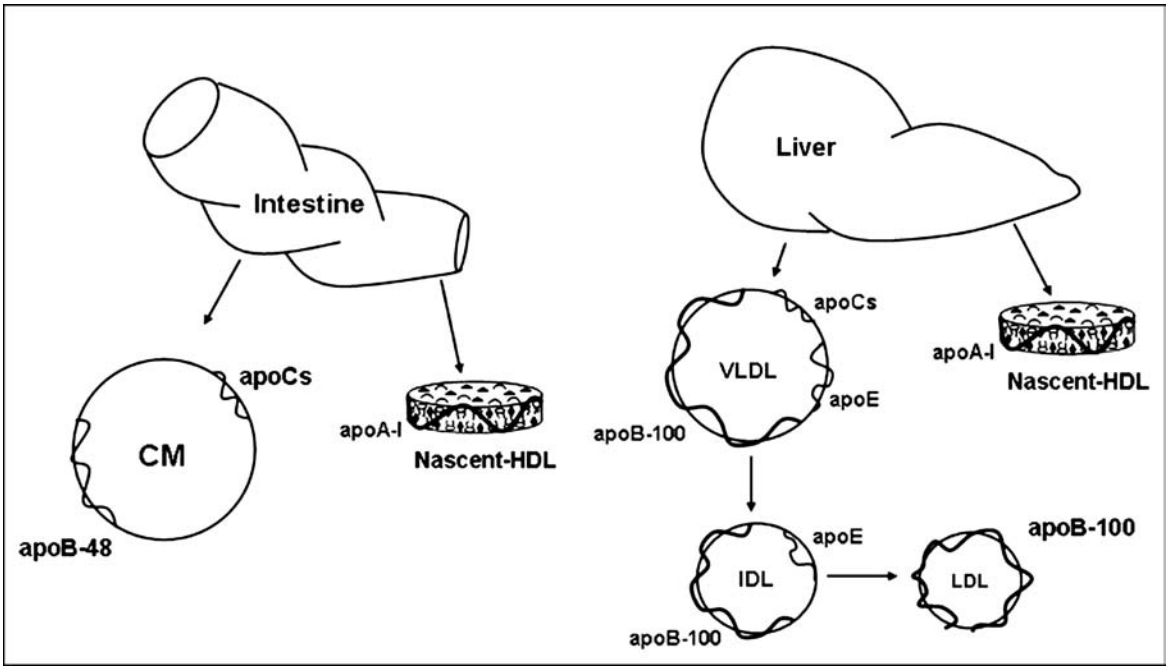

FIGURE 2 Sites of synthesis of the major plasma lipoproteins. The small intestine secretes mainly triglyceride-rich particles in the form of large and small CMs. The intestinal cells also secrete nascent HDLs that are discoidal in shape. The liver secretes VLDLs, which, following lipolysis, generate smaller, denser particles including IDLs and LDLs. HDLs are also secreted by the liver; like intestinal HDL, the nascent or precursor particle is discoidal in shape rather than spherical. Nascent HDL particles are subsequently transformed in the plasma into the mature, spherical forms by the enzyme LCAT (Fig. 4). *Abbreviations*: CM, chylomicron; HDL, high-density lipoprotein; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; LCAT, lecithin:cholesterol acyltransferase.

The small intestine is also the site of synthesis for HDL-containing apoA-I. Newly secreted HDLs, however, are chemically and structurally different from circulating HDL. The newly secreted particles possess mainly phospholipid and unesterified cholesterol and are organized as a bilayer with a disk-like structure stabilized by apoA-I on its rim. Such discoidal HDLs are "immature" forms of HDL, whereas the spherical ones are mature HDL. Discoidal HDLs are also termed "nascent HDL," and normally, they are rapidly converted to mature HDL by the plasma enzyme, lecithin:cholesterol acyltransferase (LCAT) discussed below.

■ Liver

The liver is the major organ regulating cholesterol homeostasis. Parenchymal liver cells synthesize and secrete VLDLs, which are large particles rich in triglycerides; the major protein on their surface is apoB-100. Additional proteins found on the surface of the VLDL include apoE and the apoCs. In the circulation, VLDLs rapidly lose core lipids, mainly triglyceride, through lipolysis and give rise to an intermediate-sized particle, the IDL (also referred to as the VLDL remnant). IDL is relatively rich in cholesteryl ester and possesses apoB-100 and apoE on its surface. Lipolysis of the IDL yields the cholesteryl ester–rich LDL particle that possesses only apoB-100; clearly, the cascade of events demonstrates that VLDLs are precursors of LDL (Fig. 2).

The liver also synthesizes and secretes nascent or precursor HDL possessing apoA-I. Like those from the small intestine, these nascent HDLs are discoidal particles that require LCAT for maturation.

■ APOLIPOPROTEINS AS DETERMINATORS OF LIPOPROTEIN METABOLISM

Although the lipid moieties of lipoproteins are involved in processes of growth and survival as well as the development of atherosclerosis, it is the protein associated with the lipids that directs the metabolic fate of the lipoproteins. The origin and function of major apolipoproteins (apo) are summarized in Table 5.

■ Apolipoprotein A-I

As previously mentioned, apoA-I is the major protein of HDL, constituting approximately 75% of the total protein in mature HDL; the protein is synthesized in the liver and small intestine. Epidemiological studies have abundantly shown an inverse relationship between plasma concentrations of apoA-I HDL and risk for atherosclerosis (6). Transgenic mouse models have also been developed that experimentally demonstrate that apoA-I plays an important role in reducing atherosclerosis (7). These studies were carried out with the atherosclerosis-susceptible C57BL/6 mouse strain that develops aortic atherosclerotic lesions when the mice are maintained on a high-fat, high-cholesterol, atherogenic diet for three to four months. Transgenic mice expressing elevated levels of human apoA-I had little or no lesions compared to the nontransgenic mice on the same diet. Such studies are good evidence that apoA-I may have a direct role in the prevention of atherosclerosis.

TABLE 5 Function and Origin of Major Apolipoproteins

Apolipoprotein	Function	Origin
ApoA-I	Activator of LCAT; cholesterol efflux via ABCA1	Intestine, Liver
ApoA-II	Modulates LCAT activity	Liver
ApoB-100	Recognition of LDL receptor; triglyceride transport from liver cell	Liver
ApoB-48	Triglyceride transport from intestinal cell	Intestine
Apo(a)	Inhibits fibrinolysis	Liver
ApoE	Recognition of LDL receptor	Liver, macrophage
ApoC-I	Activator of LCAT	Liver
ApoC-II	Activator of lipoprotein lipase	Liver
ApoC-III	Modulate apoE uptake; lipoprotein lipase inhibitor	Liver

Abbreviations: ABCA1, ATP-binding cassette transporter A1; LCAT, Lecithin: cholesterol acyltransferase; LDL, low-density lipoprotein.

Apolipoprotein A-I in Reverse Cholesterol Transport

One of the most widely recognized functions of apoA-I is its role in reverse cholesterol transport (RCT), schematically illustrated in Figure 3. RCT is the process whereby lipid-free apoA-I and specific subclasses of HDL mediate the removal of excess cholesterol from peripheral cells, including those of the artery wall and transport this cholesterol to the liver for catabolism or to the adrenals for reutilization for steroid hormone synthesis (8). The first step of RCT involves the removal of unesterified cholesterol and phospholipid from cells. Cholesterol assimilated into nascent HDL, or mature HDL, is then esterified to cholesteryl ester by the enzyme LCAT; apoA-I is a cofactor that activates the enzyme.

RCT can be initiated by at least two mechanisms, aqueous diffusion and apo A-I–mediated efflux via the ATP-binding cassette transporter A1 (ABCA1) transporter.

Aqueous Diffusion

This mechanism involves the diffusion of membrane cholesterol from the cell surface to preformed HDL by a nonspecific, energy-independent process called "aqueous diffusion." This reaction is largely dependent on the capacity of LCAT to esterify cholesterol on the surface of HDL, thus creating a concentration gradient that favors net movement of cholesterol out of the cell membrane and onto the surface of the phospholipid-rich HDL.

Apolipoprotein A-I–Mediated Efflux and the ABCA1 Transporter

This mechanism involves a specific metabolic process whereby lipid-free apoA-I promotes phospholipid and cholesterol efflux from cells to form nascent HDL particles. This process is termed "apolipoprotein-mediated efflux" to distinguish it from the process of aqueous diffusion that requires mature HDL. Unlike the aqueous diffusion mechanism, apolipoprotein-mediated efflux requires metabolic energy and the ABCA1. The ABCA1 transporter was discovered following the analysis of cholesterol efflux from fibroblasts from Tangier patients. These patients have severe HDL and apoA-I deficiency. The patient's fibroblasts were shown to lack specific apolipoprotein-mediated efflux, but the capacity to release cellular cholesterol to mature HDL was normal (9). This observation prompted an intense search for the gene responsible for apolipoprotein A-I–mediated efflux.

The defective gene product responsible for Tangier's disease was shown to be the ABCA1 transporter, which in affected patients, exhibits numerous deletions, insertions, and substitutions (10). The normal transporter consists of a single polypeptide chain containing two domains, each possessing six helical segments that form "pores" in membranes. Adjacent to each of the "pore"-forming domains are two nucleotide-binding

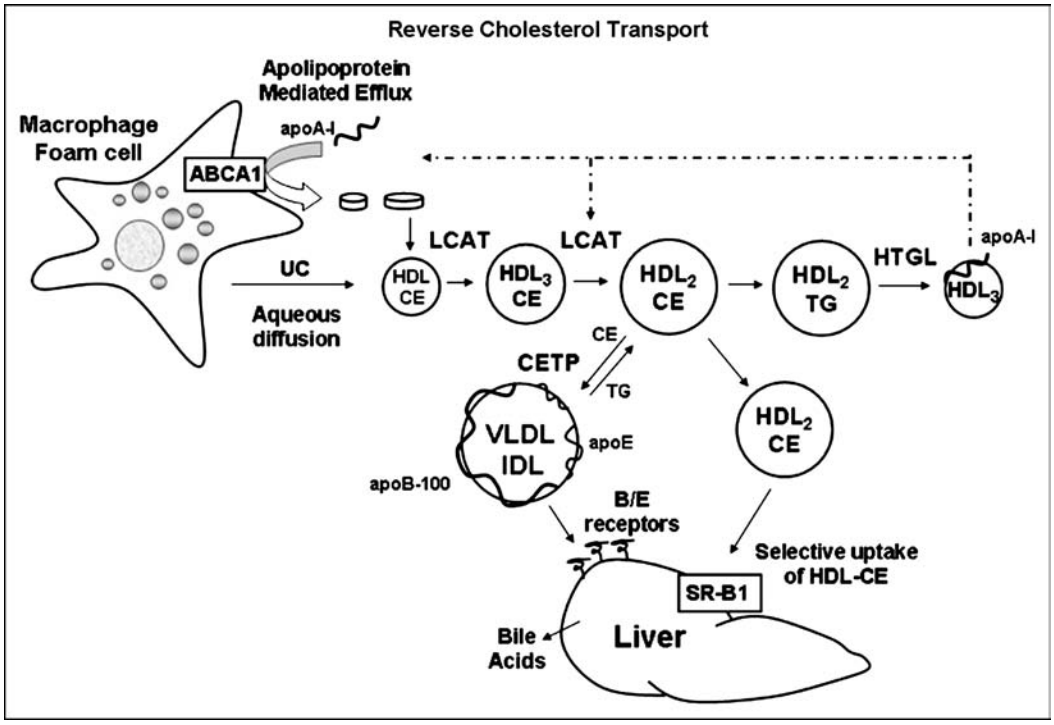

FIGURE 3 Schematic of RCT. The initial stages of RCT involve cholesterol efflux from extrahepatic cells such as macrophage foam cells in the artery wall. The mechanism of cholesterol efflux can be mediated by two processes, one involving aqueous diffusion of cholesterol to mature HDL_3 and the other involving lipid-free apoA-I that interacts with the ABCA1 transporter and directly recruits cholesterol and phospholipid from cells. In the latter, efflux of lipid to apoA-I results in the formation of discoidal HDLs, which are substrates for LCAT, subsequently giving rise to mature spherical particles. As CEs accumulate in HDL, the particles become larger and more buoyant HDL_2. CEs of large HDL_2 can be transferred to apoB-containing VLDL in exchange for TG; this exchange is mediated by CETP. TGs accumulating in HDL are hydrolyzed by HTGL, thus reducing the size of HDL and regenerating HDL_3. ApoA-I is also released from the surface, thus recycling apoA-I for ABCA1-mediated efflux. Alternatively, HDL CEs can be directly transferred to the liver and the adrenal gland by a process called "selective uptake" of CE, which does not require whole particle internalization by cells. Selective uptake is mediated by the cell surface SR-BI. CEs transferred to VLDL/IDL by CETP return to the liver via LDL receptor uptake for conversion to bile acids and excretion. *Abbreviations*: RCT, reverse cholesterol transport; HDLs, high-density lipoproteins; CE, cholesteryl esters; VLDLs, very low-density lipoproteins; TG, triglyceride; CETP, cholesteryl ester transfer protein; HTGL, hepatic triglyceride lipase; ABCA1, ATP-binding cassette transporter A1; LCAT, lecithin:cholesterol acyltransferase; IDLs, intermediate-density lipoproteins; SR, scavenger receptor.

domains responsible for the hydrolysis of ATP. The energy released from the hydrolysis of ATP facilitates the transport of phospholipid and cholesterol across the plasma membrane. Mutations in the ABCA1 transporter are associated with an inability of lipid-free apoA-I to remove excess cholesterol from cells with the resulting accumulation of cholesterol in the cells. In Tangier patients, lipid-free apoA-I cannot be adequately lipidated, and the protein is then removed by the kidneys, leading to a severe deficiency of apoA-I and HDL in these patients. Current studies with the ABCA1 transporter indicate that familial hypoalphalipoproteinemia (FHA), a condition associated with low HDL and elevated triglyceride concentrations (both risk factors in atherosclerosis), is also associated with mutations in the ABCA1 transporter (11). Age is a modifier of the phenotype, where younger FHA individuals (< 30 years) have higher concentrations of HDL cholesterol and lower concentrations of triglyceride than FHA individuals above 30 years do.

ApoA-I and LCAT

In addition to its role in removal of excess cholesterol from cells, a major function of apoA-I is the activation of the enzyme, LCAT. This enzyme, which is synthesized and secreted by the liver, is essential for the normal maturation of nascent HDL into the mature plasma form. The action of LCAT on nascent discoidal HDL is shown in Figure 4. LCAT, which associates with the surface of the discoidal HDL particle, is activated by apoA-I. The enzyme removes the acyl chain from the sn-2 position of phospholipid and transfers it to the 3'-hydroxy group on unesterified cholesterol, thus forming the hydrophobic cholesteryl ester molecule. Lysophospholipid is also generated and is rapidly removed by association with albumin. Cholesteryl ester, because of its hydrophobicity, moves into the core of the HDL particle, where it coalesces into a lipid droplet, thus transforming the disk to a sphere. Cholesteryl esters are considered a storage and transport form of cholesterol. LCAT is a necessary enzyme for normal cholesterol homeostasis because a deficiency of the enzyme, familial LCAT deficiency (FLD), is known to result in the abnormal accumulation of unesterified cholesterol in cell membranes. The latter alters vital function of cells and is also associated with an increase in premature atherosclerosis in some, but not all, patients (12). FLD subjects are characterized by exceedingly low levels of HDL and apoA-I and by the presence of nascent HDL in their plasma.

■ Apolipoprotein A-II

ApoA-II is the second most abundant apolipoprotein on HDL and is produced primarily by the liver. Its functional significance is not completely understood, although it is thought to inhibit LCAT activity and thereby modulate HDL metabolism by influencing the conversion of free cholesterol to cholesteryl

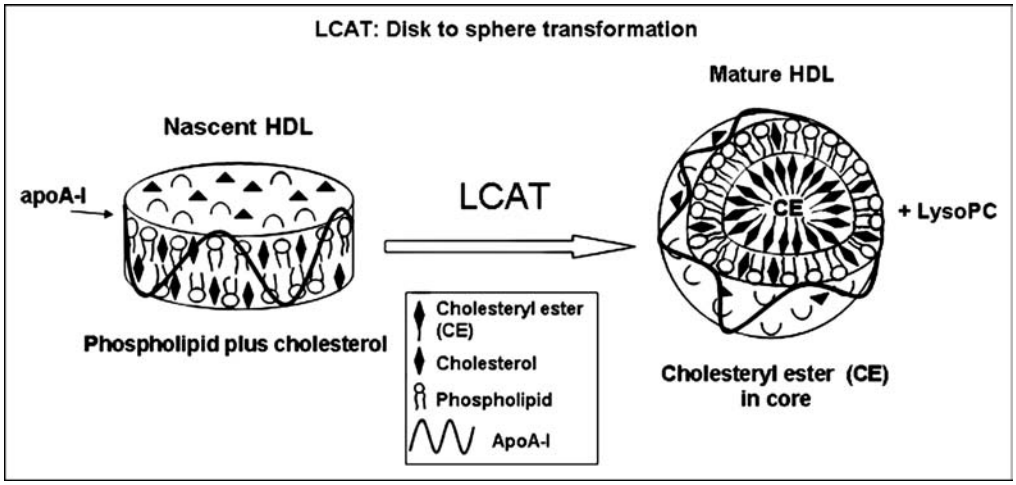

FIGURE 4 The LCAT reaction and transformation of nascent discoidal HDL to mature plasma HDL. Nascent HDL particles are discoidal in shape because of the lack of a CE core. CEs are generated as a result of the LCAT reaction. This enzyme removes a fatty acyl chain from the sn2 position of phosphatidylcholine and transfers the fatty acid to the 3'- hydroxyl group of cholesterol. The CEs thus formed are highly insoluble and form a lipid droplet between the phospholipid bilayer of discoidal HDL. As the CE droplet expands, HDL assumes its spherical shape. Lysophospholipid, the other product of the reaction, binds to albumin. *Abbreviations*: HDLs, high-density lipoproteins; CE, cholesteryl esters; LCAT, lecithin:cholesterol acyltransferase.

ester. Transgenic mice overexpressing mouse apoA-II become more atherogenic than nontransgenic littermates, leading investigators to suspect that apoA-II may be proatherogenic (13). Recent studies with transgenic mice overexpressing apoA-II suggest that elevated apoA-II increases leptin (Chapter 23), thus contributing to obesity and insulin resistance (Chapter 13), both known to be risk factors in atherosclerosis (14).

■ Apolipoprotein B-100

ApoB-100 is an important protein synthesized by the liver and associated with VLDL, IDL, and LDL (15). It is the sole protein on LDL that possesses one molecule of apoB-100 per particle. This apolipoprotein has two important functions:

1. It is necessary for the assembly and secretion of triglyceride-rich particles by the liver. In a rare genetic disease, abetalipoproteinemia, apoB-100 is not synthesized by the liver, and, as a consequence, this organ becomes fatty because of the intracellular accumulation of triglycerides. The lack of apoB-100 has serious metabolic implications because lipolysis of liver-derived VLDL normally produces LDL. The latter is an important transporter of cholesterol required for normal growth and development as well as for steroid hormone production.
2. It is a ligand for the LDL receptor. This receptor binds and internalizes LDL, thus delivering cholesterol to cells for various cellular functions. An overabundance of LDL and apoB-100 can lead to saturation of the receptors, principally in the liver, with a consequent accumulation of excess cholesterol in the plasma and initiation of the atherosclerotic process. The concentration of apoB-100 in plasma is a good indicator of atherosclerotic risk; elevated apoB levels correlate with elevated levels of circulating cholesterol.

■ Apolipoprotein B-48

ApoB-48 is a truncated form of apoB-100 synthesized in the human intestine but not in the liver (16). It is required for the assembly and secretion of CM that transport dietary lipids into the bloodstream. ApoB-48 is a product of the *apoB-100* gene, but editing of the mRNA in intestinal cells leads to a stop codon that signals premature termination of apoB translation, with the end result that the molecular weight of the apoB is only 48% that of the apoB-100 protein. Because apoB-48 is required for the transport of CM from the intestinal cell, in abetalipoproteinemia, where apoB is not secreted, the intestinal cells become lipid laden. Such individuals have steatorrhea (presence of excess lipids in stools) and diarrhea along with malnutrition.

ApoB-48 is not recognized by the LDL receptor. Following lipolysis, CM remnants are removed by the liver through the action of apoE (also found on CM), which recognizes the LDL receptor.

■ Apolipoprotein (a)

Lipoprotein (a) is a unique subset of LDL and its lipid composition is like that of LDL. In the 1960s, Berg discovered a novel protein associated with LDL in some individuals (17). This lipoprotein antigen was named apolipoprotein (a), and the lipoprotein is known as lipoprotein (a) or Lp (a). Apo(a) is a glycoprotein of variable size with molecular weights ranging from 300,000 to 800,000 Da and is synthesized in the liver. Apo(a) is covalently bound to apoB-100 through a disulfide bridge located in the carboxyl terminal region of apoB-100. The apo(a) protein has high homology with plasminogen, which hydrolyzes fibrin and aids in the dissolution of clots. Plasminogen possesses five pretzel-shaped protein units called "kringels." Apo(a) contains kringel 5 of plasminogen and variable numbers of the kringel 4 unit. The number of kringel 4 units in the apo(a) structure is responsible for the variation in molecular weight of the protein; it is now accepted that there is an inverse relationship between apo(a) size and Lp (a) concentration. Lp (a) is an independent risk factor for atherosclerosis, and its plasma concentrations are genetically controlled. Individuals with Lp (a) levels greater than 30 mg/dL are at increased risk for heart disease. Lp (a), however, is not correlated with other known risk factors such as concentrations of LDL cholesterol, HDL cholesterol, apoA-I, and apoB. The physiological role of Lp (a)

in coronary artery disease is not completely understood but appears to be involved in atherogenesis (18). The mechanisms whereby Lp (a) contributes to atherosclerosis may involve the following: (*i*) inhibition of fibrinolysis because the molecule can interfere with plasminogen function and (*ii*) binding of Lp (a) particles to the extracellular matrix in the subendothelial space of the artery wall, where they are subsequently oxidized. The oxidized Lp (a) particles can be taken up by the scavenger receptor (SR) on macrophages (see section Receptors in Cholesterol Metabolism), thus contributing to foam cell formation in fatty streaks.

■ Apolipoprotein E

The liver is the major site of synthesis of apoE, although macrophages, adrenal gland, and brain also synthesize this protein. ApoE is another apolipoprotein recognized by the LDL receptor (also known as the apoB-E receptor). As a ligand for the LDL receptor, apoE has an important role in targeting CM and VLDL cholesteryl ester–rich remnants to the liver for catabolism, thus preventing the abnormal accumulation of cholesterol in the plasma. ApoE deficiency in mice is associated with strikingly elevated plasma cholesterol levels and the onset of severe atherosclerosis even when such apoE knockout mice are maintained on a chow diet (19). In humans, apoE deficiency is also associated with premature coronary artery disease and elevated plasma cholesterol levels, thus suggesting that apoE plays a significant role in cholesterol metabolism.

Some apoE is synthesized by macrophages. During secretion of apoE by macrophages, excess cell cholesterol is transported from these cells, thus reducing the accumulation of intracellular cholesterol and the potential development of foam cells. ApoE, therefore, has an important function in RCT.

There are several genetic variants of apoE. The normal apoE molecule possesses a single cysteine at amino acid residue 112 and an arginine at residue 158, identifying it as the E3 isoform. A point mutation at residue 158 can occur wherein the arginine is replaced by cysteine (E2 isoform). In the homozygous state, this mutation is associated with an inability of the apoE-containing lipoproteins to recognize the LDL receptor. This results in reduced clearance of cholesteryl ester–rich remnant lipoproteins and the elevation of plasma cholesterol concentrations and is a risk factor for atherosclerosis. Another mutation in apoE exists wherein the cysteine at residue 112 is replaced by an arginine. This isoform, known as apoE4, is associated with risk for familial late-onset Alzheimer's disease (AD) (Chapter 7). As the number of apoE4 alleles increases, there is an increase in risk for AD and also a decrease in average age of onset (20).

■ Apolipoprotein Cs

The apoC proteins are synthesized mainly in the liver and function principally as cofactors in enzyme reactions that have a critical role in lipid metabolism and cholesterol homeostasis (Table 5). ApoC-I functions as an activator of LCAT and, in this respect, is functionally similar to apoA-I. It is likely that this protein may have other, as yet unidentified, function(s).

ApoC-II is a cofactor in lipoprotein lipase (LPL) activation; the protein is essential for the hydrolysis of the triglyceride core in CM and VLDL with the resultant formation of remnant particles (Fig. 6). A deficiency of plasma apoC-II leads to chylomicronemia and a severe elevation of plasma triglyceride concentrations.

ApoC-III has a functional role in two areas of lipoprotein metabolism:

1. It acts as an inhibitor of LPL activity and, hence, modulates triglyceride hydrolysis.
2. It modulates the cellular uptake of apoE-containing lipoprotein particles. An increase in apoC-III can result in the elevation of plasma lipoprotein remnants, thus elevating plasma cholesterol.

■ LIPOLYTIC ENZYMES

■ Lipoprotein Lipase

This enzyme is important for the metabolism of triglyceride-rich lipoproteins. LPL is synthesized by adipocytes, heart, and kidney and migrates from the sites of synthesis to the capillary endothelium, where it is bound to the cell surface. LPL is responsible for catabolism of the large, triglyceride-containing lipoproteins, principally CM and VLDL. It catalyzes the hydrolysis of triglyceride to free fatty acids and glycerol and requires the presence of apoC-II, the obligatory enzyme activator. The action of LPL in the degradation of CM and VLDL is illustrated in Figure 5. VLDL and CM interact with LPL at the endothelial cell surface where lypolysis occurs. The triglyceride core of these large particles is hydrolyzed, thus generating fatty acids, excess surface material, and lipoprotein remnants. The fatty acids are utilized by cells for energy; excess fatty acids are taken up by adipocytes and stored as triglycerides. Chylomicron and VLDL remnants generated during lipolysis are rich in cholesteryl ester and contain apoE on their surfaces. Remnants are rapidly cleared from the plasma through the LDL receptors of the liver; this receptor also recognizes apoE. The removal of apoE-containing remnants is vital for normal cholesterol metabolism.

Excess surface components are produced when the triglyceride core in CM and VLDL is removed. These components, especially phospholipids and free cholesterol, are incorporated into HDL as shown in Figure 5.

■ Hepatic Triglyceride Lipase

This enzyme, as suggested by its name, is secreted by the liver and is associated with the surface of hepatocytes. The major substrate for hepatic triglyceride lipase (HTGL) is the VLDL remnant that carries a small amount of triglyceride. Lipolysis of triglyceride in remnants results in the formation of LDL. HTGL also has the ability to hydrolyze triglycerides that accumulate in HDL through the action of cholesteryl ester transfer protein (CETP). CETP transfers excess cholesteryl ester from cholesteryl ester-rich HDL_2 to apoB-containing lipoproteins in exchange for triglyceride. This exchange is important in RCT as shown in Figure 3. HTGL hydrolyzes the triglyceride in the triglyceride-enriched HDL_2, and in so doing, regenerates smaller HDL_3 particles. The latter HDL have a crucial role in RCT, as indicated in Figure 3, by acting as acceptors of cholesterol from cells.

■ RECEPTORS IMPORTANT IN CHOLESTEROL METABOLISM

■ LDL Receptor

LDL is the major transporter of cholesterol in the circulation; cholesterol in the form of cholesteryl ester is delivered to cells by internalization of LDL by a specific receptor mechanism, the

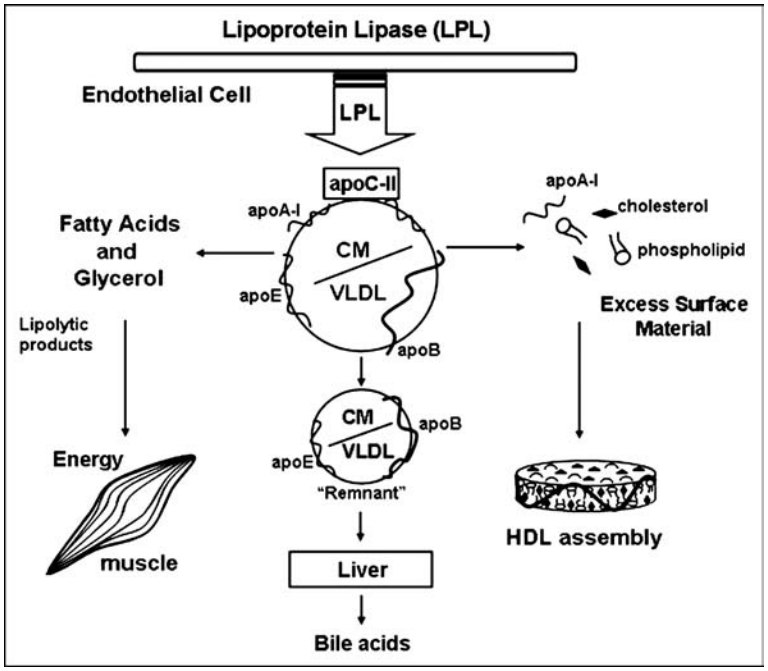

FIGURE 5 Schematic diagram of the physiological function of LPL. This enzyme is the key in clearing TG-rich lipoproteins from plasma. LPL is found on the lumenal surfaces of the capillary endothelium and requires apoC-II for activation. TG-rich particles including CM and VLDL carry apoC-II and activate the enzyme; in so doing, the TG core is hydrolyzed, and the constitutive parts of TG, free fatty acids and glycerol, are liberated and used to produce energy. Removal of core TG creates remnant particles that are enriched in cholesteryl ester. Most remnants are removed by the liver while excess surface components (apolipoproteins, phospholipid, and cholesterol) generated by shrinking of the core are used to form HDL. *Abbreviations*: LPL, lipoprotein lipase; TG, triglyceride; CM, chylomicrons; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein; apoC-II, apolipoprotein C-II.

LDL receptor. This receptor is also referred to as the apoB-E receptor because the receptor recognizes apoE in addition to apoB-100. Although almost all cells possess LDL receptors, the liver possesses the largest number of receptors. Functionally, this receptor is extremely important in the regulation of intracellular cholesterol synthesis and flux, and in addition, it regulates further synthesis of the receptor. Based on the work of Brown and Goldstein, the LDL receptor function is outlined in Figure 6 (21). The liver possesses high-affinity receptors that recognize apoB-100–containing LDL (or apoE-containing

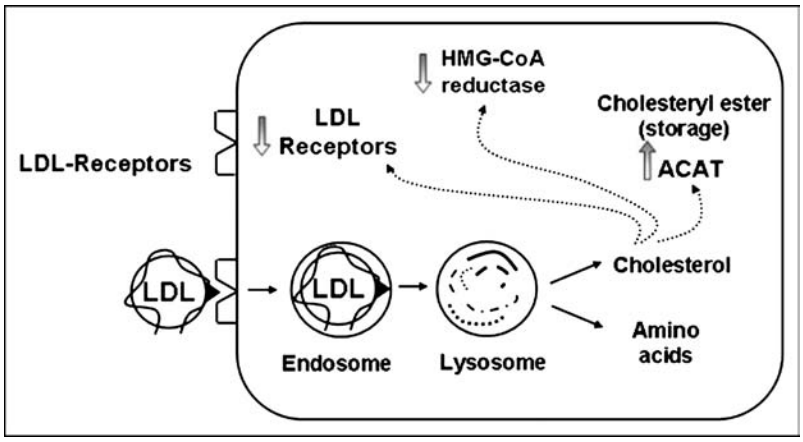

FIGURE 6 Schematic outline of the function of the LDL receptor (also called the apoB/E receptor). This receptor plays an important role in the catabolism of cholesterol-containing lipoproteins. Lipoproteins carrying apolipoproteins (apo) B-100 or E are recognized by the receptor and are bound. The bound particles are internalized in a membrane-bound sac, the endosome; acidification of the endosome releases the receptor, which recycles to the cell surface. The LDL is then delivered to the lysosome where the protein and lipids are broken down to amino acids and unesterified cholesterol. In the cytosol, cholesterol is esterified to cholesteryl esters by the cellular enzyme, ACAT. Cholesterol coming into the cell downregulates, or decreases, the activity of the cell's own cholesterol-making machinery. The rate-limiting step in the de novo synthesis of cholesterol is HMG CoA reductase; therefore, this enzyme is the one regulated by receptor-mediated cholesterol accumulation. In addition to a decrease in HMG CoA reductase, accumulation of cellular cholesterol also decreases the number of LDL receptors on the cell surface. Overall, the receptor-mediated process is a finely tuned system in the regulation of cholesterol metabolism. *Abbreviations*: ACAT, acyl-coenzyme A: cholesterol acyltransferase; LDL, low-density lipoprotein; HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A.

remnants). The LDL (or remnant) binds to the receptor and is internalized into a structure referred to as the endosome. Acidification of the endosome releases the receptor, which recycles to the cell surface while the LDL is delivered to the lysosome, wherein hydrolysis and degradation of the internalized lipoprotein takes place. The LDL is degraded into its molecular constituents, amino acids and cholesterol.

In the cell, catabolism of LDL results in the activation of several processes. Accumulation of unesterified cholesterol upregulates the enzyme, acyl-coenzyme A:cholesterol acyltransferase (ACAT), which in turn reesterifies cholesterol. The accumulation of cellular cholesterol then downregulates the enzyme, 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, which is the rate-limiting step in cellular cholesterol synthesis. In other words, the cell slows its machinery for synthesizing cholesterol when adequate amounts are being delivered to it by the lipoproteins. In addition to downregulating HMG CoA-reductase, degraded LDL regulates synthesis of the LDL receptor and, thus, the cell reduces its uptake of cholesterol. The liver is finely tuned for the maintenance of cholesterol homeostasis.

Receptor-mediated uptake and degradation of apoB-100–containing and apoE-containing lipoproteins are important in maintaining normal plasma cholesterol levels. Elevated levels of cholesterol-containing particles such as VLDL remnants and LDL that result from the overproduction of cholesterol by the liver could result in saturation of the LDL receptor and accumulation of excess cholesterol in the plasma and the increased risk to cardiovascular disease. The risk for cardiovascular disease is also enormously increased when the LDL receptor is defective or deficient. This happens in certain genetic defects, where either the receptor protein is not synthesized or the synthesized protein is defective. Patients with such receptor defects are hypercholesterolemic, and their condition is called familial hypercholesterolemia. In the homozygous state, patients can have staggeringly high plasma cholesterol levels, as high as 1000 mg/dL as compared to the normal 150 to 200 mg/dL. The patients have precocious atherosclerosis and, unless managed extremely carefully, will not survive the second decade of life. The disease state is a clear case of underutilization of LDL. Heterozygotes for the disease have decreased numbers of functional receptors, and therefore, have elevated plasma cholesterol and premature coronary artery disease.

■ Macrophage Scavenger Receptor (SR)

This receptor is found on the surface of macrophages in the subendothelial space and plays a key role in the process of atherogenesis in the artery wall. The scavenger receptor (SR) recognizes modified LDLs. Modification of LDL is an important contributor to the atherogenic process; modification can happen through several mechanisms. In the case of hypercholesteremia, such as that resulting from defective LDL receptors, the residence time of LDL in plasma is increased, thus increasing the likelihood of protein alterations by oxidation events. Glycation of apoB-100 protein in diabetes also leads to modification of the LDL particles; it is well known that patients with diabetes mellitus have an increased risk of atherosclerosis. LDL entering the subendothelial space can be retained and bound to the extracellular matrix of the artery wall, where they then undergo oxidation. The oxidatively modified LDLs are recognized and internalized by SRs on macrophages. As indicated in Figure 7, the current theory on the development of the atherosclerotic lesion implicates oxidized/modified LDL as a key player in the process (22). During the early events of lesion development, monocytes enter the arterial intima in regions of endothelial damage. Cytokines, specialized cell signals, trigger the differentiation of monocytes into macrophages and also stimulate smooth-muscle cell proliferation. Macrophages scavenge the oxidatively modified LDL in the artery wall via the SR. Uptake of the modified LDL is probably a protective function; however, if the insult continues, more macrophages amass at the injury site, and modified LDL accumulate intracellularly in great abundance. The macrophage, however, cannot degrade the extra burden of cholesterol, which then accumulates within the cell in the form of lipid droplets, thus transforming the macrophage into a foam cell. These cells eventually die, and the lipids, cell debris, and cholesterol crystals are released into the extracellular space and form the nucleus for more complicated lesions.

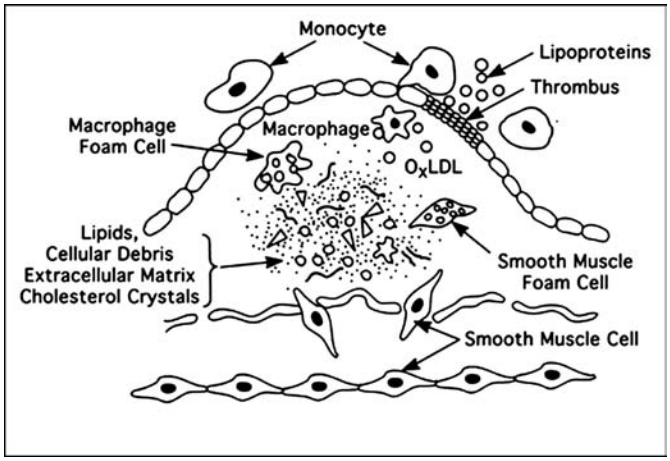

FIGURE 7 Schematic of the role of macrophages and lipoproteins in foam cell and atheroma formation. Early steps in atherosclerotic-lesion formation involve the appearance of foam cells in the subendothelial space. Current research suggests that OxLDL plays an important role in the production of foam cells. OxLDLs are taken up by the scavenger receptor of macrophages in the artery wall; however, the internalized OxLDL cholesterol is not re-utilized, and it accumulates within the cell in the form of lipid droplets. Macrophages with large quantities of accumulated lipid droplets are foam cells. The macrophage foam cells in the artery wall ultimately die and release their residue of cholesterol, which accumulates in the lesion. The macrophages also release factors that stimulate smooth-muscle cells to proliferate and accumulate lipids, thus further augmenting the atherosclerotic process. *Abbreviation*: OxLDL, oxidatively modified low-density lipoprotein.

As noted in a previous section, HDL and apoA-I are important in reversing the early steps of cholesterol accumulation in macrophages. When RCT works efficiently, foam cell development is minimized.

■ SR-B1 Receptor

Cholesteryl esters accumulating in HDL_2 can be delivered to the liver (and also adrenal cortical cells) by a process of "selective uptake." Selective uptake involves the selective transfer of HDL cholesteryl esters to hepatocytes (or adrenal cells) without the uptake of the whole HDL particle. This process is distinct from that of receptor-mediated endocytosis and involves a membrane "docking" protein for HDL particles. This "docking" protein is the SR-B1 receptor that belongs to a family of Class B SRs. Cholesteryl ester entering the liver via the SR-B1 receptor is hydrolyzed to form unesterified cholesterol that is converted into bile acids for excretion from the body. Cholesteryl esters

delivered to the adrenal gland via the "selective-uptake" mechanism are stored or converted into steroid hormones.

■ METABOLIC SYNDROME AND HYPERLIPOPROTEINEMIA

■ Metabolic Syndrome

This syndrome, also referred to as Syndrome X, represents a constellation of several health risks that increase one's chance of developing coronary artery disease, peripheral vascular disease, stroke, and type 2 diabetes (Chapter 13) (23). The appearance of three or more of the following features in an individual defines metabolic syndrome:

- Abdominal obesity (waistline 40 inches or more in males and 35 inches or more in females)
- Triglyceride concentration above 150 mg/dL
- HDL cholesterol concentration below 40 mg/dL in males and below 50 mg/dL in females
- Elevated blood pressure (130/85 mm Hg or higher)
- Fasting blood glucose greater than 100 mg/dL

Conditions contributing to metabolic syndrome include aging, physical inactivity, obesity, hormonal imbalance, and genetic predisposition. It is estimated that the prevalence of metabolic syndrome in the United States is high, with greater than one in five individuals demonstrating this syndrome. The age-adjusted prevalence for metabolic syndrome demonstrates its relationship with aging where prevalence in 20- to 29-year-olds is 6.7% but that of 60- to 69-year-olds is 43.5%. Prevalence appears to be similar for both sexes.

■ Hyperlipoproteinemia

Lipid metabolism is normally tightly regulated with trigylceride and cholesterol fluxing through the enterohepatic circulation as illustrated in Figure 8. Clinical concern arises when concentrations of lipoproteins such as VLDL, IDL, and LDL are abnormally elevated (a condition termed hyperlipoproteinemia), because such elevations can accelerate the development of atherosclerosis. Not only may atheromas form, but deposition of cholesterol may also occur in tendons and skin, producing raised nodules called xanthomas; these are overt manifestations of severe hypercholesterolemia.

Hyperlipoproteinemias are designated primary or secondary (Table 6). Primary hyperlipoproteinemias are due to a single gene defect or to a combination of genetic factors; in addition, genetic factors may be exacerbated by environmental or dietary factors. The incidence of the different primary hyperlipoproteinemias ranges from 1 in 250 in the population to 1 in 1 million. Secondary hyperlipoproteinemias are complications of more generalized metabolic disturbances such as diabetes mellitus, hypothyroidism, excessive intake of alcohol, or chronic kidney failure. Knowledge of the plasma concentrations of cholesterol and triglycerides usually reveals the class of lipoproteins that is high, and this is useful in making a diagnosis and in designing proper drug and diet therapy.

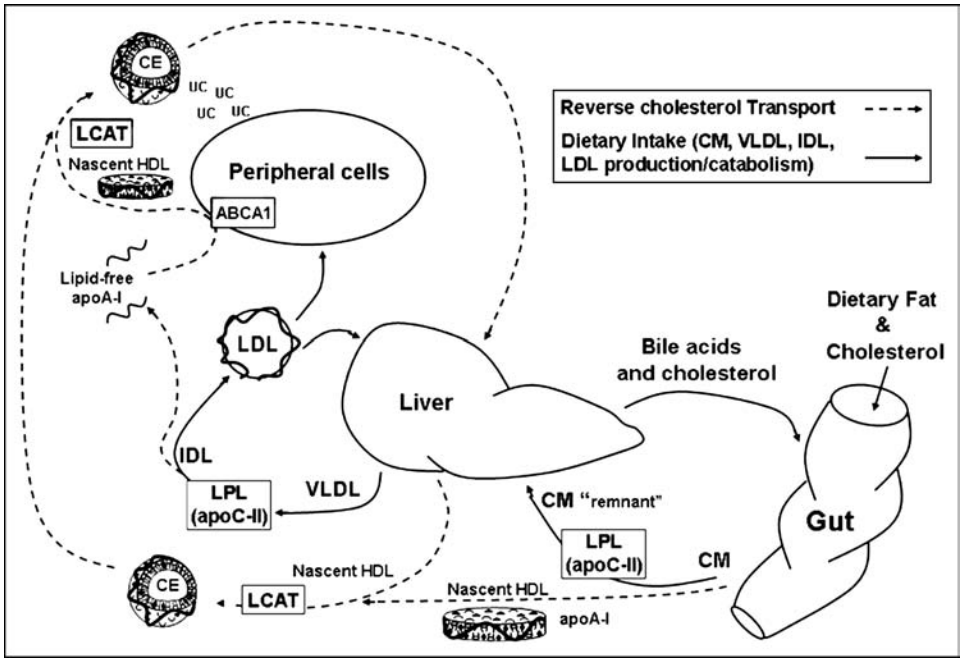

FIGURE 8 Schematic diagram summarizing whole-body lipoprotein metabolism and cholesterol transport. Intestinal epithelial cells absorb dietary fat and cholesterol and package the lipid in the form of CMs, which are secreted into the blood. Lipoprotein lipase activated by apoC-II hydrolyzes the core triglycerides within the CM, generating remnants that are taken up by the liver. The liver repackages the lipids into VLDL particles that are secreted into the bloodstream. Lipoprotein lipase mediates the hydrolysis of VLDL triglycerides to generate IDLs and LDLs particles as part of a lipolytic cascade. LDLs transport cholesterol to peripheral cells and the liver. The cholesterol content in peripheral cells is tightly regulated at least, in part, as a result of cellular cholesterol efflux mediated by lipid-free apoA-I and plasma HDL particles. Nascent HDL generated by the intestine and liver is converted to mature HDL by the action of LCAT. Nascent HDL generated upon the interaction of apoA-I with the ABCA1 transporter is also a substrate for LCAT, giving rise to mature HDL. Mature HDL, with the help of LCAT, can promote the efflux of cholesterol from peripheral cells via the aqueous diffusion mechanism. HDL transports cholesterol in the form of cholesteryl esters back to the liver for production of bile acids, which are secreted into the intestine for excretion. *Abbreviations*: HDL, high-density lipoprotein; VLDL, very low-density lipoprotein; ABCA1, ATP binding cassette transporter A1; LCAT, lecithin:cholesterol acyltransferase; CM, chylomicrons; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein.

TABLE 6 Diseases Related to Lipoprotein Abnormalities

Disorder	Clinical findings
Primary hyperlipoproteinemias	
Single gene	Atheromas Pancreatitis Xanthomas
Multiple genes	Atheromas
Secondary hyperlipoproteinemias	
Diabetes mellitus	Atheromas Pancreatitis Xanthomas
Hypothyroidism	Atheromas
Estrogen excess (oral contraceptive)	Pancreatitis Xanthomas

Treatment protocols for hyperlipoproteinemia are summarized in Table 7. Basic to the treatment of all hyperlipidemias is a diet that maintains normal body weight and that minimizes the plasma cholesterol concentration. If a patient is overweight, weight loss should be attempted and then maintained by a diet low in cholesterol and saturated animal fats and relatively high in mono- and polyunsaturated vegetable oils.

Patients with secondary hyperlipidemia require treatment of the underlying disorder (diabetes, hypothyroidism, excess alcohol consumption, etc.) and should reduce all other risk factors such as smoking and hypertension, while maintaining physical fitness.

Primary hyperlipoproteinemia requires more aggressive treatment. In addition to a proper diet, drugs that lower plasma cholesterol and/or triglyceride concentrations are used (24). These drugs function by diminishing the production of lipoproteins or by increasing the efficiency of their removal.

TABLE 7 Treatment of Hyperlipoproteinemias

Diet therapy	
Low cholesterol, low animal fat, relatively high polyunsaturated fats	
Drug therapy	
Class	**Metabolic effect**
HMG CoA-reductase inhibitors (lovastatin, pravastatin, simvastatin, fluvastatin, atorvastatin)	Inhibits cholesterol biosynthesis; enhances LDL clearance; lowers plasma cholesterol
Bile acid sequestrants (cholestyramine, colestipol)	Binds and removes bile acids in intestine; increases cholesterol conversion to bile; increases LDL clearance
Fibric acid derivatives (gemfibrozil, fenofibrate)	Reduces synthesis and increases catabolism of VLDL; lowers triglyceride; increases HDL
Cholesterol absorption inhibitor (ezetimibe)	Blocks dietary cholesterol absorption by intestinal cells; lowers LDL cholesterol
Nicotinic acid	Reduces synthesis of VLDL; lowers LDL; elevates HDL by reducing its clearance
Antioxidant (Probucol)	Modestly lowers LDL; may prevent oxidation of LDL

Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein; HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A; VLDL, very low-density lipoprotein.

The most widely used drugs in treating hypercholesteremia are the statins. This class of drugs, including lovastatin, parvastatin, simvastatin, atorvastatin, and fluvastatin, inhibits HMG CoA-reductase and with it cholesterol synthesis. To compensate for a reduction in cholesterol synthesis in the liver, the number of hepatic receptors for LDL increases, and this brings about a reduction in plasma LDL by increasing clearance of circulating LDL.

A recent addition to the arsenal of drugs to decrease plasma cholesterol levels is ezetimibe. This drug blocks dietary cholesterol uptake by intestinal absorptive cells and, in doing so, decreases cholesterol transported from the gut to the plasma compartment.

Nicotinic acid (niacin), a member of the Vitamin B group (Chapter 23), has long been used to reduce the production of VLDL and, in so doing, it also lowers LDL. It also increases HDL concentrations by decreasing HDL clearance from the plasma. Nicotinic acid can produce cutaneous flushing and pruritus (itching) involving the face and upper body, but this appears to subside with continued use. It may, however, interfere with compliance, that is, the patient's willingness to continue with the drug.

Fibric acid derivatives such as fenofibrate and gemfibrozil are effective in decreasing the synthesis of VLDL and triglyceride. This type of drug also increases VLDL catabolism by increasing the activity of LPL. The decrease in triglyceride is paralleled by an increase in HDL.

Normally, cholesterol returned to the liver is converted to bile acids that are delivered to the small intestine lumen where some of the cholesterol in the form of bile acid is ultimately excreted and some is reabsorbed. Formation of bile acid has a negative-feedback effect on further production of bile acids. Removing the bile acids so that they no longer exert negative feedback speeds up the conversion of cholesterol to bile acids and reduces body pools of cholesterol, including cholesterol sequestered in xanthomas. Resins, such as cholestyramine and colestipol, have such an effect. They readily bind bile acids in the intestinal lumen and increase the flux of cholesterol from the liver to bile.

Probucol is an antioxidant that has been found to have a modest effect in lowering LDL cholesterol although the mechanism is not fully understood. It is possible that by its antioxidative properties, probucol can protect LDL from oxidation, thereby reducing its accumulation in macrophages (Chapters 5 and 15).

Both nicotinic acid and HMG CoA-reductase inhibitors may be used in combination with one of the bile acid–binding resins. These combinations are usually synergistic, allowing the doses of both substances to be lowered. It is thus apparent that drugs utilized in treating dysfunctions of lipid metabolism must be selected and tailored according to the individual condition.

■ ACKNOWLEDGMENTS

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17

The Pulmonary Respiration, Hematopoiesis, and Erythrocytes

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THE PULMONARY RESPIRATORY SYSTEM

The respiratory function includes two major processes:

1. An external process consisting of the absorption of oxygen (O_2) from atmospheric air into the lungs; O_2 is carried by the blood to cells, tissues, and organs throughout the body; and the removal of carbon dioxide (CO_2) from cells, tissues, and organs; CO_2 is carried by the blood to the lungs for elimination into the atmospheric air.
2. An internal process consisting of gaseous exchanges at the cellular level to support cell metabolism; the resulting CO_2 is transported by the blood to the lungs for elimination into the atmospheric air.

This chapter is concerned with the external pulmonary respiration dealing with the input of atmospheric air (rich in O_2) and output of bodily air (rich in CO_2) (Fig. 1), as well as the transport of gases by the red blood cells (RBCs) (erythrocytes) throughout the body and their gas exchanges at the tissue level. Changes that may occur with old age in the structure and the function of the lungs are described in the first section. These changes (*i*) may result from environmental insults, (*ii*) may contribute to alterations of pulmonary O_2 uptake and CO_2 excretion, and (*iii*) may represent the cause of some of the respiratory diseases prevalent in the elderly.

The section entitled Hematopoiesis and Erythrocytes, which describes the aging of the hematopoietic system, focuses on the study of the erythrocytes (RBCs) and their function in carrying O_2 and CO_2 to and from the lungs and tissues under steady-state and stress conditions. Focus is on the hematopoietic potential for erythrocyte remodeling in old age within the bone marrow (BM) environment and the proliferative capacity of stem cells. *Human erythrocytes have a relatively short life span of about 120 days and have been extensively used as a model for the study of cellular aging.*

Respiration was assessed traditionally, by tests of physiologic competence in the whole organism. In the last decade, advances in organ and tissue imaging [e.g., magnetic resonance imaging (MRI), positron emission tomography (PET), computed tomography (CT)] and other noninvasive measures as well as tests of cellular and molecular biology have facilitated the diagnostic evaluation of function at cellular and molecular levels, have provided a better understanding of disease processes, and have suggested new and more effective approaches to therapy (1,2).

AGING-ASSOCIATED CHANGES IN THE LUNG

Air passes from the atmosphere into the lungs in the following manner:

1. It passes first through the nasal passages, where it is filtered of the larger contaminants.
2. It enters the pharynx, where it is warmed and absorbs water vapor.
3. It flows down the trachea and through the bronchi and bronchioles.
4. It proceeds through the respiratory bronchioles and the alveolar ducts to the alveoli. The alveoli are the functional units of the lungs (Fig. 1).

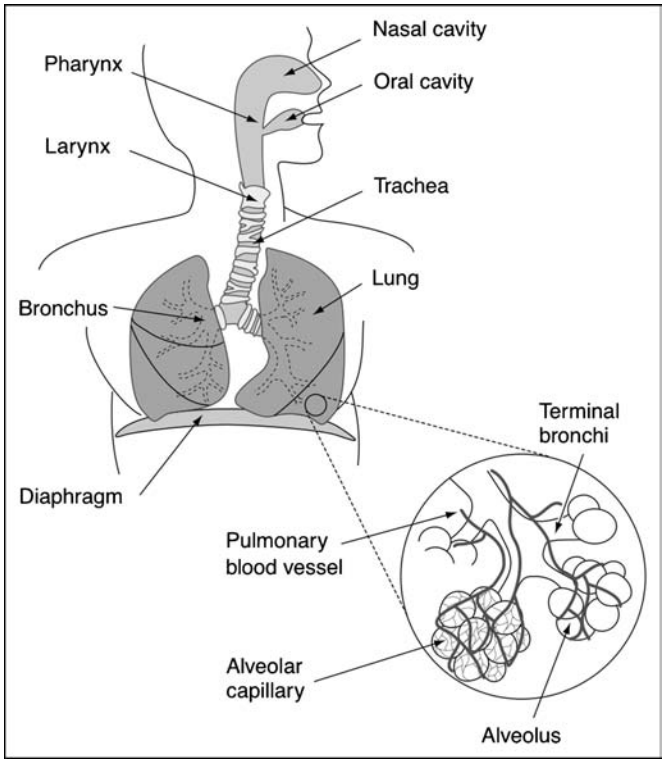

FIGURE 1 Diagram of the major structures of the pulmonary respiratory system including the airways, the gas-exchange organ (the lungs), the pump that ventilates the lungs (the chest wall and respiratory muscles), the functional units of the lungs (the alveoli and terminal bronchi), and the rich vascular network that surrounds them.

BOX 1 *Structure and Functions of the Pulmonary Respiratory System*

In the respiratory system:

- The gas-exchange organ is comprised of the two lungs.
- The pump that ventilates the lungs comprises
  1. the chest wall,
  2. the respiratory muscles (that by contracting or relaxing increase or decrease the size of the thoracic cavity), and
  3. the brain centers and nerve tracts (that control the muscles).

In addition to regulating gaseous exchange, the lungs participate in

- immunologic defenses of the body (by phagocytizing particles from the inspired air and from the blood),
- metabolic functions (by synthesizing, storing, or releasing into the blood such substances as surfactants and prostaglandins),
- endocrine functions [by transforming angiotensin I into angiotensin II, a vasoconstrictor and stimulus for aldosterone secretion (Chapter 9)],
- the actions of a few biologically active peptides, some with pressor activity (e.g., vasoactive intestinal peptide) and some with neuronal (e.g., opioid peptides) activity,
- an important defensive action capable of cleansing or clearing blood clots from the circulation. Small blood clots are usually formed during walking or running in the plantar region of the feet; they reach general circulation and are promptly cleared in the lungs by powerful endogenous thrombolytic mechanisms. Larger clot formation in the lower extremities may become a pathologic sign in elderly individuals affected by obesity, affected by immobilization (e.g., after strokes), receiving cancer chemotherapy, or taking long travels in cramped positions (at the end of the trip, when rapid ambulation restarts).

Aging has profound effects on both lung architecture and lung function. These effects include the following:

1. Enlargement of air spaces
2. Decrease in surface area for exchange of gases
3. Loss of supporting tissue for peripheral airways (as in "senile emphysema"), resulting in decreased recoil (stretchability) and expiratory flow rates and increased residual volume (see below)
4. Changes in the pulmonary surfactant system

The major function of the lungs is to ensure the efficient exchange of air (O_2) from the environment to the blood and from the blood to the cells of the body (Box 1). Yet, this very function is performed at the peril of contamination and damage from the many toxic substances transported in the air. The degree and the rate of age-related changes in the structure and the function of the lungs are variable and depend on (*i*) the habits of the individual (particularly nutrition, physical exercise, and smoking), (*ii*) the individual's environment (e.g., urban vs. rural), and (*iii*) the concomitant occurrence of diseases (e.g., infections, industrial diseases) (3–5).

The respiratory system in humans is mature, i.e., reaches optimal adult function, by the age of 20 years (6). Pulmonary function begins to slowly and gradually decline in healthy subjects after the age of 25 years. This decline is linked to progressive deleterious changes that occur with aging in respiratory structures including the lung, the thoracic cage, and respiratory muscles (Table 1), as well as in the respiratory centers in the central nervous system (CNS). These changes, however, are minor compared to the constant effects of the environment and other insults on the respiratory system—infections, pollution, cigarette smoking, abnormal immune responses, and unfavorable living and working conditions—to which the organism is exposed throughout the life span (1).

In the absence of disease, none of these functional decrements, singly or in combination, is sufficient to severely incapacitate the old individual. The majority of the elderly are capable of maintaining their lifestyle and a satisfactory respiratory function under resting (steady state) conditions. Some of the impairments become manifest when ambient conditions worsen and may lead to increased pathology and mortality (1).

Given the long-term exposure of the lungs to external and internal insults, respiratory diseases are more prevalent in older individuals than in the general population. Among these diseases, incidence and severity of infections, chronic obstructive disease, and cancer increase with aging.

■ Alveolar Structure and Function

The major functional asset of *the alveolus* is its structure, which provides for close proximity of the capillary blood to the alveolar air. Air in the alveolus and blood in the pulmonary capillaries are separated only by the capillary endothelium and the thin basement membrane supporting the alveolar cells. This arrangement facilitates gas exchanges between blood and air.

Two types of epithelial cells (pneumocytes) line the alveolus. *Type I cells are extremely thin, with few intracellular*

TABLE 1 Signs of Impaired Pulmonary Respiration with Aging

Reduced maximum breathing capacity
Progressive reduction in arterial P_{O_2} and in P_{O_2} alveolar to arterial differences due to premature airway closure
Loss of elastic recoil (i.e., springing back of elastic fibers after stretching)
Weakening of respiratory muscles
Decreased elasticity of thorax cage and chest wall
Increased rigidity of internal lung structure
Less efficient emptying of the lungs
Earlier and easier fatigability

Abbreviation: P_{O_2}, partial pressure of oxygen.

organelles and are designated agranular pneumocytes; they serve to facilitate the exchange of gases between blood and atmospheric air. *Type II cells contain many organelles and lipid droplets and are designated granular pneumocytes. The type II cells produce surfactant, a proteolipid that coats the alveolar cells and lowers the surface tension at the air–fluid interface.* Lower surface tension (*i*) keeps the alveoli from collapsing and (*ii*) reduces muscular work required to ventilate the lungs. Changes in the surfactant system occur with aging (see below).

Another cell type in the lung is the *alveolar macrophage.* These cells are loaded with digestive enzymes and can digest foreign materials, as do white blood cells. They are part of the immune system (Chapter 14); they migrate from the bloodstream and patrol the tissues of the lung on the alveolar side, gliding on the surfactant.

■ Aging-Related Structural Changes

The architecture of the lung is altered in aging. Structural changes are associated with impairment of function. The lungs become more voluminous, and the alveolar ducts and respiratory bronchioles are enlarged, while the *alveoli become shallower and more flat with loss of septal tissue (i.e., forming dividing walls between alveoli).* These changes do not appreciably affect total lung capacity (the maximum volume of air in the lungs and airways) when this volume is corrected for the aging-related decrease in height (stature): the decrease of this volume between the ages of 20 and 60 years is less than 10% in both men and women. However, air distribution is altered with "an increase in alveolar duct air" but "a decrease in alveolar air." Alveolar surface area is 75 m^2 at 30 years, and decreases by 4% per decade thereafter. Given the fact that O_2 transfer into the blood is most efficient in the alveoli (and much less efficient in the alveolar ducts), a decrease in alveolar air space will impair optimal O_2 diffusion from alveolar air into pulmonary capillaries.

The amount of elastic tissue, abundant in the lung and partly responsible for the stretching ability of this organ, is decreased with age, while fibrous tissue is increased. The importance of lung elasticity is illustrated by the condition of emphysema (see below), in which loss of lung elasticity, due to disruption of elastic tissue, is associated with impaired ventilation. The nature of the exact changes in the elastic fibers during aging is unclear, but it appears that alterations in the distribution of elastic tissue are functionally as significant as are changes in amounts. With aging, abnormal location or structure of the elastic fibers may contribute to impairments of ventilation and perfusion of the lungs.

The dome-shaped diaphragm constitutes the floor of the thorax, thereby separating the thoracic from the abdominal cavity. The diaphragm is the major muscle involved in pulmonary respiration. Contraction of the diaphragm, by lowering its central portion (the diaphragm is anchored around the perimeter of the lower thorax), accounts for 75% of the increase in thoracic volume during quiet inspiration. Other muscles are the external and internal intercostal muscles, inserted on the border of the ribs and the costal cartilage below. *During inspiration, contraction of the external intercostal muscles increases thoracic volume by elevating and pulling outward the anterior ends of the ribs.* Increased thoracic volume and consequent decreased pressure allow for expansion of the lungs and O_2 to flow into the alveoli. Other auxiliary muscles from the abdomen and the shoulders are involved in inspiratory processes.

Normal expiration is mostly a passive process attributable to recoil of the elastic tissue of the stretched lungs and thorax. Also involved are the internal intercostal muscles, which, when they contract, lower the ribs and move them inwards, thereby contributing to the decrease of the thoracic volume. Some abdominal and shoulder muscles also participate in expiration. During exercise, the abdominal and rib-cage muscles assume a greater role in augmenting ventilation rates. Because of the increased stiffness of the rib cage with aging, the diaphragm takes over a higher proportion of the mechanical effort needed for increasing ventilation (7–12).

The structure, biochemical properties, and contractile function of respiratory muscles, like those of all skeletal muscles, change in response to variations (*i*) in the pattern of use (sedentary vs. physical exercise habits), (*ii*) in the nutritional state, and (*iii*) during growth and development, in response to the influence of hormones (growth hormone, insulin-like growth factor I, thyroid and gonadal hormones). The diaphragm is a muscle not easily fatigued; further investigation is needed to discover if, during increasing ventilatory activity, it becomes fatigued in old age to the same degree as the other (abdominal and intercostal) auxiliary respiratory muscles (10).

Exertional dyspnea (i.e., shortness of breath after exercise) is common among the elderly (8). Dyspnea may be due to a number of contributing factors, such as (*i*) decrements in muscle strength, (*ii*) increase in thoracic stiffness, and (*iii*) loss of stretchability (compliance) of the lungs and chest wall. Fatigue after muscular exercise has a strong CNS component that may contribute to the dyspnea (e.g., impaired coordination, insufficient motivation). Still other factors, such as arthritic involvement of the joints, may also play an important role in limiting the adaptive competence of the elderly in undertaking physical exercise (Chapters 20 and 24) (7–12).

With aging, it is well accepted that most muscles of the body undergo a certain degree of *sarcopenia* (loss of tissue mass), which may be due, in part, to progressive disuse with advancing old age (Chapters 20 and 24). *With respect to the respiratory muscles, it has been suggested that, inasmuch as they remain continuously active throughout life, they may be spared aging-associated sarcopenia. However, that does not seem to be the case* (7,13,14). The ability of the lungs to shift from resting to maximal function is impaired in some aged individuals, and this impairment depends on the decline in the strength and endurance of the respiratory muscles (Chapter 24). Observed changes in respiratory muscles in older humans and animal models are summarized in Table 2. The configuration and mechanical properties of the chest wall also change with aging; they follow the changes in bone structure and function with old age as discussed in Chapter 20. Major aging-associated signs are (*i*) increased curvature of the spine, and (*ii*) calcification of the intercostal cartilage.

■ Aging-Related Changes in Lung Volumes

Lung volumes and pressures change dramatically from birth to death, with major and rapid changes during childhood and adolescence and

TABLE 2 Changes with Aging in Respiratory Muscles

Muscle strength is decreased
Muscles of older individuals are more prone than those of adults to fatigue when the work of breathing is increased (as during physical exercise)
Atrophy of some respiratory muscles (primarily, type I muscle fibers of slow, red muscles, as in long muscles of back and shoulders)
Ratio of glycolytic (anaerobic) to oxidative (aerobic) metabolism is increased
Blood supply to muscle is decreased

slower but progressive changes with increasing age. These changes involve the lungs and chest wall and are often divergent (Table 3) (7). The chest becomes stiffer because of the calcification of the rib cartilage, while the lungs become more distended due to a slightly increased compliance (i.e., stretchability) and decreased recoil (i.e., ability of elastic fibers to spring back after stretching). Thus, lung volumes and ventilation rate are decreased at rest and, especially, during exercise. Lung volumes and measurements are summarized in Box 2.

Vital capacity (the largest amount of air that can be expired after a maximal inspiration) is often measured as an index of pulmonary function. Vital capacity, in some elderly in the seventh decade of life, may decrease to approximately 75% of its value at the age of 17 years (taking an average vital capacity of 4.8 L for men and women). During this time, residual volume (the amount of air left in the lungs after maximal expiratory effort) increases by nearly 50% (Fig. 2). Other measures of ventilatory mechanics decline as well, including forced expiratory volume/second (32 mL/yr decrease in males and 25 mL/yr decrease in females starting from the age of 25 years), and airway conductance, which also progressively slows with aging (14). All these changes underline the greater difficulty in old age to empty the lung adequately with each expiration, with the consequence that residual volume is increased and the ability of the lungs to promote adequate air diffusion to the alveoli is impaired and blood is less oxygenated. Expiratory flow rates begin to decline at an age when vital capacity is still intact (13). Perhaps this decline reflects a reduced elastic recoil in the elderly, and, during expiration, may cause a premature closure of some regions of the lungs (14). The trapping of air in sites distal to the closure may result in inadequate blood oxygenation (14). In contrast, P_{CO2} (i.e., partial pressure exerted by CO_2 in the pulmonary air) remains remarkably constant throughout life.

■ Aging-Related Changes in Surfactant

Surfactant is a liquid surface tension–lowering agent secreted by the type II pneumocytes that line the inner alveolar surface (see above). It is secreted as typical "lamellar bodies," i.e., membrane-bound organelles, containing phospholipids and forming complexes with proteins. If the surface tension is not kept low, during expiration, the alveoli will collapse. It has been calculated that, were it not for the presence of surfactant, the unopposed surface tension in the alveoli would produce a 22 mmHg of force favoring transport of fluid from the blood into the alveoli, with the consequent formation of lung edema. Surfactant production is impaired with aging in several mammals, from rats (15–17) to primates (18), with a decrease in the intracellular formation of lamellar bodies and the increase of cytoplasmic vacuoles. However, similar changes have not been reported in humans (19). Surfactant amount and composition is altered in a number of diseases [e.g., asthma (20), cystic fibrosis (21)] and under the effect of pollutants (22).

The role of surfactant is critical for the initiation and maintenance of optimal lung function. Surfactant deficiency leads to many functional perturbations, such as

- reduced lung compliance,
- increased alveolar permeability, and
- altered immune function.

The important role of surfactant continues in maturity and old age, and alterations of this role may induce respiratory dysfunction or aggravate preexisting pulmonary diseases. Inasmuch as the lung is susceptible to invasion by air-borne bacteria, fungi, and viruses, *another important role of the surfactant is to facilitate the protective function of the immune system by (i) reducing the work required for the airway cilia to beat, (ii) accelerating beat frequency, and (iii) improving cilia–mucus coupling*. In so doing, the particles are pushed to the upper respiratory tract and are removed by swallowing. In addition to this mechanical removal of foreign material, some of the proteins in the surfactant enhance the ability of lung macrophages to destroy bacteria (23,24). Although research on surfactant changes with age has focused, so far, on the vital role of surfactant, prenatally, at birth and at young ages, it is expected that a better understanding of the surfactant system in the elderly may provide future advances in the prevention and treatment of pulmonary dysfunction in old age (24).

TABLE 3 Morphologic Changes in the Thorax and Lung with Aging[a]

Morphological change	Functional significance
Thorax	
Calcification of bronchial and costal cartilage	↑ Resistance to deformation of chest wall
↑ Costovertebral stiffness	↑ Use of diaphragm in ventilation
↑ Rigidity of chest wall	↓ Tidal volume
↑ Anterior-posterior diameter	↓ Response to exercise hyperapnea
Wasting of respiratory muscles	↓ Maximal voluntary ventilation
Lung	
Enlarged alveolar ducts	↓ Surface area for gas exchange
↓ Supporting duct framework	↓ Decreased stretchability
Alveoli shallow, flatter	↑ Physiologic dead space (40%)
Thinning, separation of alveolar membrane	↓ Lung elastic recoil
↑ Mucous gland	Vital capacity ↓ 15–20%
↓ Number, thickness of elastic fibers	RV/TLC ↓ 35–40%[b]
↓ Tissue extensibility (alveolar wall)	↓ Ventilatory flow rate
↓ Pulmonary capillary network	↓ Ventilation distribution
↑ Fibrosis of pulmonary capillary intima	↑ Resistance to flow in small airways
	↓ Ventilation

[a] Modified with permission from Ref. 14.
[b] RV, residual volume; TLC, total lung capacity.

■ THE LUNG: A "BATTERED" ORGAN FROM WITHIN AND WITHOUT

The lungs and the skin (Chapter 21) are the only two organs of the body to be in direct contact with the atmospheric air. Not only are the lungs exposed to toxic substances (pollutants) in the atmospheric air, but also the very oxygen indispensable for terrestrial life may induce lung and brain damage. Indeed, animals may suffer irremediable damage within a few days of exposure to pure oxygen (25). Oxygen damage is due to a number of chemical reactions in the tissues (e.g., macromolecule reactions with sugars, aldehydes, and oxidants, alkylation by methylation agents, and spontaneous hydrolytic processes) that lead to accumulation of free radicals, with consequent toxic and even lethal reactions. However, animals, including humans, can survive longer oxygen exposure if they are gradually adapted to increasing oxygen concentrations (26).

■ Oxygen Toxicity in Lungs

The ability of adapting to elevated oxygen concentrations agrees with the view that aerobic organisms maintain, throughout life, antioxidant defenses capable of providing protection against toxic oxygen derivatives under optimal

BOX 2 *Lung Volumes and Measurements*

Total lung capacity of 6 L in men and 4.2 L in women comprises the following values (in men):

- *Tidal volume*: amount of air that moves into and out of the lungs with each quiet inspiration and expiration (0.5 L)
- *Inspiratory reserve volume* (3.3 L) and *expiratory reserve volume* (1 L): amount of air that moves into or out of the lungs following maximal inspiration or expiration
- *Residual volume*: air left in the lungs after maximal expiration (1.2 L)
- *Dead space*: air in the airways (150 mL)
- *Vital capacity*: the greatest amount of air that can be expired after a maximal inspiration. Vital capacity is frequently taken as an index of pulmonary function and ranges from 3 to 4 L in adult females to 4.5 to 5.5 L in adult males. A more precise index is gained by measuring vital capacity per unit of time; for example, in asthma, vital capacity appears normal but, when timed, shows significantly prolonged time because of bronchial constriction
- *Pulmonary ventilation rate* (or respiratory minute volume): normally 6 L/minute at rest by 12 breaths/minute
- *Maximal voluntary ventilation*: the largest volume of air that can be moved into and out of the lungs in one minute by voluntary effort; it may be as high as 125 to 180 L/min.

physiologic conditions. The eventual presence of oxidative stress occurs under special circumstances, when the balance between oxidants and antioxidants is disrupted. Consequent to this disruption, free radicals accumulate with advancing age, in agreement with the hypothesis that oxidative stress may be a possible cause of aging (Chapter 5). Indeed, the lungs are "battered" not only by external insults but also by the formation of endogenous oxygen radicals (27,28), so deleterious to all tissues, and, particularly to the pulmonary cells in immediate contact with the gaseous environment rich in O_2.

The chronic damage to lung tissue by oxygen results also, indirectly, from the inflammatory reaction that is induced by cell damage and death. The increased generation of free radicals released by the phagocytic cells that infiltrate the inflamed tissue damages nuclear and mitochondrial DNA, cell membrane, and cytoplasmic enzymes. Another example is the activation by the free radical of the enzyme elastase that breaks down the elastic tissue of the lungs. In this case, the stretching function of the lung necessary for optimal ventilation is markedly impaired (see below).

The acute O_2 toxicity is of particular significance for critically ill patients who require respirators (i.e., devices worn over the mouth or nose protecting the respiratory tract or providing for the administration of O_2). Although the lowest possible doses of oxygen are given, the potential damage to the lung must always be considered. Sometimes it may be necessary to choose between "allowing a patient to die immediately and giving pure oxygen which may kill in days" (28,29). Experiments in rats show that breathing oxygen increases the production of free radicals in pulmonary epithelial cells and macrophages and causes the consequent death of the animals within three days. If animals are protected by the administration of the antioxidant enzymes, superoxide dismutase, and catalase, death of the animal is prevented (Chapter 5).

■ CONTROL OF VENTILATION

Control of ventilation by brain centers in the medulla and pons and by the peripheral carotid and aortic body chemoreceptors is markedly

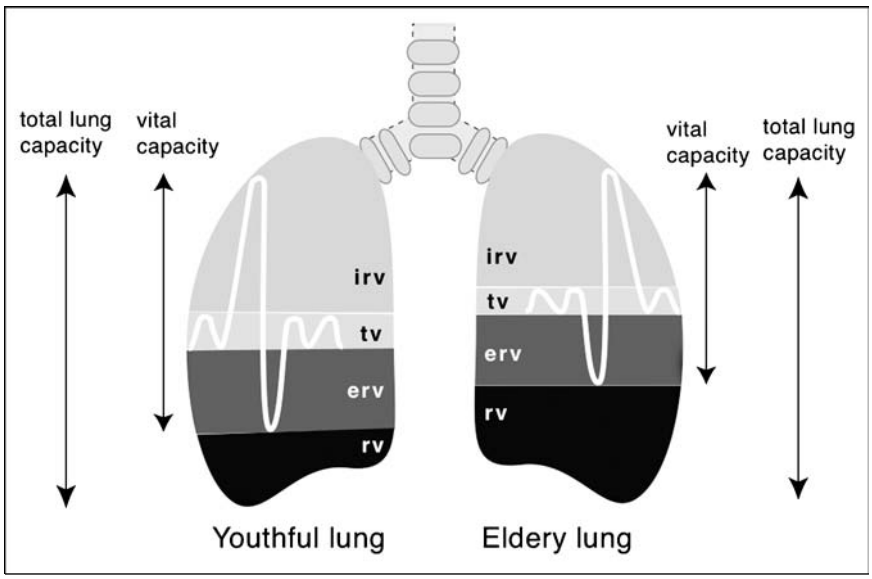

FIGURE 2 Changes in lung volumes with aging. Note particularly the decrease in VC and the increase in RV. *Abbreviations*: irv, inspiratory reserve volume; tv, tidal volume; erv, expiratory reserve volume; RV, residual volume; VC, vital capacity.

altered in the elderly (13,14). It is unclear whether altered ventilation may be due to one or several of the following, and more research is needed in this regard:

- Intrinsic alteration of neural control, such as decreased sensory perception of P_{CO2}, pH, and partial pressure of oxygen (P_{O2})
- Loss of synchrony among higher CNS inputs
- Alterations of mechanical factors such as stiffness of chest wall
- Reduced neuromuscular competence and responsiveness to neural inputs
- Responses to hypercapnia (increased P_{CO2}) and hypoxia (reduced P_{O2}), which are reduced by 50% in some aged individuals as compared to young individuals (30)

Despite an overall decline in performance during exercise, most elderly can and should undertake a regimen of physical exercise adequate to their capabilities and needs. Such regimens are briefly surveyed in Chapter 24. Other factors influencing performance of physical exercise are discussed in Chapter 20 in relation to skeletal and muscular changes with aging. Age-related respiratory changes during sleep are discussed in Chapter 7.

■ Responses to Exercise

Physical exercise stimulates the active tissue to utilize more O_2 and to eliminate more CO_2 through coordinated cardiovascular and respiratory adjustments. Circulatory changes increase blood flow to the exercising muscle (Chapter 24) (31,32). Respiratory adjustments include the following:

- Increased ventilation, to provide more O_2, eliminate more CO_2, and dissipate heat
- Increased extraction of O_2 from the blood by the exercising muscle
- Increased blood flow
- Shift to the left of the O_2–hemoglobin dissociation curve (that is, the percentage saturation of the O_2-carrying-power of hemoglobin to P_{O2}). The shift of this curve to the left means that a lower P_{O2} is required by hemoglobin to bind a given amount of O_2 and, therefore, blood oxygenation is improved; a shift to the right means that a higher amount of P_{O2} is required by hemoglobin to bind a given amount of O_2, and therefore blood oxygenation is impaired.

With aging, lung ventilation, if already impaired under quiet conditions, is further altered during exercise:

- Ventilation becomes inadequate for mustering the necessary adjustments to meet the increased demands of exercise.
- Loss of elastic recoil and decreased functional residual capacity (i.e., gas volume remaining in lungs at the end of quiet expiration) inhibit the effective range of tidal volume (i.e., the amount of air that moves into the lungs with each inspiration, or alternatively, the amount of air that moves out with each expiration).
- Early closure of the airways similarly inhibits the expiratory flow.
- Dyspnea (shortness of breath) ensues and necessitates the early cessation of exercise.

The reduction in vital capacity with aging restricts the potential tidal volume that may be reached during maximal exercise. At rest, only a minor fraction of the potential lung volume and flow changes is used, and even during maximum exercise, the maximal inspiratory and expiratory flow rates and volumes are not usually reached. With increasing age, the ability to reach maximal rates and volumes is severely curtailed during moderate-to-heavy exercise. Therefore, ventilation, in many elderly, cannot increase sufficiently to provide for the increased metabolic demands, keeping in mind significant variations in response to gender and ethnicity (8,12,30).

In addition to difficulty in adjusting ventilatory responses to exercise, the elderly have an earlier onset of the shift from aerobic (requiring O_2) to anaerobic (independent of O_2) metabolism (32). *Both the time required to reach a steady-state* level at the onset of exercise and the time required to return to preexercise resting levels are prolonged with age after moderate-to-heavy exercise. Alveolar–capillary gas exchange is reduced, and alveolar–arterial P_{O2} differences are increased with exercise in middle-aged men. Nevertheless, at 65 years and older, gas diffusion capacity (i.e., the movement of O_2 "downhill" from the air through the alveoli and blood into the tissues) is comparable to that at younger ages (33).

■ SOME RESPIRATORY DISEASES RELATED TO AGING OF THE LUNGS

Respiratory diseases are the cause of potentially preventable morbidity and mortality in elderly people. As mentioned earlier, no internal organ other than the lung is so directly exposed to external environmental influences. Thus, decline in physiological competence with aging and the environmental toll exacted by a number of conditions and agents (e.g., smoking-related airway obstruction, asthma, pulmonary tuberculosis) combine to induce multiple pathologies. One of the most frequent respiratory disorders in the elderly is chronic obstructive pulmonary disease (COPD) (34). COPD refers to a spectrum of chronic respiratory diseases. Other diseases include lung neoplasia and lung infections, and, particularly, pneumonia (Chapter 14). While some disorders, e.g., bronchitis and emphysema, are not necessarily life threatening, they represent a considerable "burden" for the well-being of the elderly in terms of days of hospitalization, physician consultation, days of sickness, and almost continuous discomfort (35). From the perspective of the entire life span, the proportion of deaths due to respiratory diseases (in Western countries) is highest (approximately 30%) in the first year of life, and falls (approximately 5%) in late adolescent and early adulthood. From the fifth decade on, the incidence of respiratory disease rises steadily, and in those over 85 years of age, it accounts for 25% of all deaths. In the last 20 years, the death rate from respiratory disease has fallen, except for bronchiogenic carcinoma (still rising, especially in young women) and pneumonia (remaining constantly high). Within the confines of the present chapter, COPD will be the representative disease briefly reviewed.

■ CHRONIC OBSTRUCTIVE PULMONARY DISEASE

This disease refers to a group of disorders characterized by airflow limitation and persistently impaired gas exchange, often associated with an inflammatory condition of the lungs (3–5,34). In the United States, COPD is ranked among the leading causes of death, with mortality rates on the rise, especially in women (Chapter 3). It is often the result of cigarette smoking. It is predicted that by the year 2020, worldwide, COPD will rise from being the sixth most common cause of death (currently) to the third (35). Reasons for this dramatic increase in COPD

include reduced mortality from other causes (e.g., cardiovascular diseases in industrialized countries and infectious diseases in developing countries), and the marked increase in cigarette smoking and air pollution in the developing countries.

COPD comprises at least three distinct pathologic processes that may occur separately or concurrently. They are as follows:

- Chronic bronchitis (inflammation of the bronchi), accompanied by hypersecretion of mucus and cough
- Emphysema, characterized by enlargement of air spaces, destruction of lung parenchyma, loss of lung elasticity, and closure of small airways
- Chronic asthma (constriction of the bronchi)

■ Genetics and Environmental Risk Factors in COPD

Although cigarette smoking is a major risk factor in the etiology of COPD, the disease occurs only in a small percentage (15–20%) of smokers. This suggests that genetic factors may determine which smokers will develop the disease (36). Other indications for genetic involvement include the following:

1. The familial clustering of early onset COPD (37).
2. Ethnic differences (38): for example, the higher than normal risk of COPD in a Taiwanese population is associated with increased production of tumor necrosis factor α (TNFα) (39), but not in a British population with the same increased TNFα (40).
3. Lower levels of the proteinase inhibitor α 1-antitrypsin, leading to early development of emphysema (41). [A hereditary association has been noted among individuals affected by an α 1-antitrypsin deficiency; in the homozygous individual, emphysema develops early in life (about 20 years of age) even in the absence of cigarette smoking.]
4. A polymorph variant of microsomal epoxide hydrolase, an enzyme involved in the metabolism of epoxides (chromosome breaking agents) that may be generated in tobacco smoking, has been associated with a significant increase in COPD incidence (42).

External factors that may contribute to disease pathogenesis may include, in addition to a genetic background, physical factors (e.g., temperature) socioeconomic status, and exposure to environmental stimuli (e.g., allergens, molds, air pollutants, tobacco smoke, sulfur dioxide, cadmium, particulates associated with cooking) (43,44). These factors are some among the many environmental conditions that can predispose to or aggravate COPD. Chronic infections of the respiratory tract and the inflammatory reactions they induce significantly contribute to the incidence and severity of COPD (Table 4) (42).

TABLE 4 Major Risk Factors for COPD

Cigarette smoking
Air pollution
Genetic factors
Bronchial inflammation
Chronic respiratory tract infections
Old age
Family history of COPD
Male sex

Abbreviation: COPD, chronic obstructive pulmonary disease.

■ COPD Pathology

Causes of COPD range from an inherent defect of elastic tissue to association with fibrotic pulmonary diseases such as silicosis, to consequence of chronic diffused bronchitis due to age and aggravation by cigarette smoking. Major structural and pathophysiologic signs of COPD are summarized in Table 5.

Chronic inflammation of the airways plays a critical role in producing symptoms of emphysema and asthma; hence, the use of anti-inflammatory treatments for COPD such as inhaled corticosteroid hormones (one of the commonly used treatments of asthma) (44–47). Smoking increases the number of pulmonary alveolar macrophages, which release a chemical substance that attracts leukocytes to the lungs. Leukocytes, in turn, release proteases such as elastase, which attacks the elastic tissue in the lungs. Normally, the plasma protein α1-antitrypsin inactivates elastase and other proteases. In emphysema, the activity of this enzyme is decreased. Inactivation of the enzyme may be promoted by oxygen radicals that are released by the leukocytes (Chapter 14). Thus, with smoking, there may be both an increased production of elastase and a decreased activity of the inactivating enzyme, with the resulting destruction of elastic fibers. The inflammatory changes and protease imbalance that occur in COPD as a consequence of either infections or irritants such as tobacco smoke would accelerate the functional decline in pulmonary respiratory function that occurs in old age (34). Latent viral infections have also been suggested as possible causative (48) or contributing factors, perhaps by amplifying the inflammatory responses (49,50).

Among the major metabolic disorders associated with emphysema is hypercapnia (excess carbon dioxide in the blood), which induces acidosis, which is at first compensated by urinary retention of bicarbonate. When this compensatory mechanism fails, especially in aged individuals in whom renal competence may be diminished (Chapter 18), the ensuing respiratory acidosis represents a medical emergency and must be treated accordingly. A consequence of hypoxia is stimulation of the production of RBCs that are increased in number (polycythemia). This contributes to hypertension, which causes the right side of the heart to enlarge and then leads to cardiac

TABLE 5 Major Signs of Chronic Obstructive Pulmonary Disease

Structural
Diffuse distention and overaeration of alveoli
Disruption of interalveolar septa (walls)
Loss of pulmonary elasticity
Restructuring of alveoli into large air sacs, resulting in poor, uneven alveolar ventilation and inadequate perfusion of underventilated alveoli
Increased lung volume
Barrel-shaped chest as the chest wall expands (increased lung volume and increased use of accessory shoulder and abdominal muscles)
Pathophysiologic
Disturbed ventilation
Altered air and blood flow
Frequently partial obstruction of bronchi (hence the often used name of obstructive disease)
Inspiration and expiration are labored (wheezing) and more work is required for breathing
Resulting hypoxia (low O_2 levels) and hypercapnia (high CO_2 levels)
Chronic productive cough with mucus
Minor respiratory infections of no consequence to young individuals with normal lungs are fatal or near fatal for the elderly

failure (the so-called "cor pulmonale" or congestive right heart failure, Chapter 20).

■ COPD Management and Treatment

Given the burden inflicted by the disease and its frequency, treatment and prevention are still inadequate. Although cigarette smoking is the major cause of COPD, quitting smoking does not appear to resolve the inflammatory response in the lung airways, and the first symptoms of the disease may become manifest several years after smoking was stopped (42). Treatment still remains for the most part symptomatic, despite significant advances (34,51–53). The major goals of management and available therapeutic strategies are listed in Table 6.

■ PNEUMONIA

Pneumonia is an inflammatory process of the lung parenchyma most commonly caused by infection. The infectious agent (often the Bacillus pneumococcus) is frequently present among the normal flora of the respiratory tract. The development of pneumonia must, therefore, be usually attributed to an impairment of natural resistance. Indeed, the decline of immune competence with aging (Chapter 14) may explain why pneumonia remains, despite the availability of antibiotics, a serious, life-threatening problem for the elderly.

In most cases of community-based epidemics of pneumonia, the elderly are more susceptible than the young with respect to severity and complications (e.g., lung abscess, bacteremia) as well as mortality (as high as 80% in those aged 60 and older) (54). The rise in pneumonia after the seventh decade registered in several Western countries, including the United States, may simply reflect the type of death certification, pneumonia being the terminal expression of other diseases. However, studies of hospitalized elderly suggest a true increase (55–57). *While the true cause may be failure of immunologic competence, the immediate cause has been ascribed to aspiration in the lungs of oropharyngeal flora during sleep or aspiration of food in the trachea during hand-feeding (as in severe dementia)* (57). These are common occurrences in the elderly, in whom the mucobronchial defense barrier is impaired due to reduced ciliary activity, mucus production, and blunting of mechanical reflexes.

TABLE 6 Treatment of Chronic Obstructive Pulmonary Disease

Goals of management:
To retard the progression of the disease
To educate patients
To improve air flow
To optimize functional capabilities
Therapeutic strategies:
Administration of pharmacological agents, such as
Broncodilators (to relieve bronchial spasm)
Mucus liquefiers (to thin the mucous secretions)
Anti-inflammatory agents such as steroids (see text)
Protease inhibitors, inhibitors of receptors or enzymes involved in immune responses
Antibiotics (to control potential infections)
Administration of O_2 to be used cautiously to prevent acidosis
Optimizing function by:
Physical exercise to strengthen abdominal muscles and diaphragm to aid in lung ventilation
Meeting social, emotional, and vocational needs
Use of respiratory aids in the form or aerosols, sprays, etc.

■ Presentation and Management

The diagnosis of pneumonia in the elderly is more difficult than in the young because of a frequent atypical presentation. The classic features of chest pain, cough, and purulent or blood-stained sputum (spit) are uncommon. There is a lack of cough, toxic confusion predominates, and dehydration occurs early. Progression of the disease may produce further lung damage (e.g., abscess) or aggravate extrapulmonary manifestations such as the confusional state or induce additional damage such as pericarditis (inflammation of the pericardium, the sac surrounding the heart), ischemic heart disease, and meningitis (infection of the membranes surrounding the brain). Treatment involves the use of antimicrobial agents, primarily antibiotics, the correction of dehydration, and ultimately the treatment of the underlying disease. *Prevention includes the use of anti-influenza vaccination and perhaps pneumococcal vaccination (still under investigation)* (49,50). Rehabilitation after recovery is important physiologically and psychologically and requires the use of an individually tailored program, based on exercise, oxygen therapy, and coordinated support of family and health providers.

■ TUBERCULOSIS REVISITED

Tuberculosis, induced by *Mycobacterium tuberculosis*, was considered by the end of nineteenth century to be an infectious disease definitively eradicated by chemotherapy and improved socioeconomic and hygienic conditions. However, it remains, even today, a health problem, worldwide, primarily among individuals with acquired immunodeficiency syndrome (AIDS) but also among the elderly (58,59). The disease is chronic in nature, and the agent that causes it may remain dormant for many years but may be reactivated as immune defenses are reduced in old age (Chapter 14). The disease may be more widespread and severe in the elderly due to unfavorable conditions such as malnutrition, alcoholism, and superimposed diseases. Tuberculosis is generally viewed as one of the most easily treatable serious infectious diseases likely to occur in adults, including old adults. Its current reemergence has been ascribed, in part, to an increase in drug-resistant bacterial strains (60,61).

In the late 1980s, several outbreaks of tuberculosis occurred in nursing homes, where reactivation and primary contact infection may occur. Elderly persons in nursing homes are at greater risk for tuberculosis than those living in the community, because crowded living conditions facilitate transmission of the disease. It is important that all nursing home residents be tested for tuberculosis upon admission to the facility and that vigorous preventive measures be taken to stop the spread of the infection. Even in the elderly living in the community, the geriatrician must be alert to the possibility of the infection, which is often masked by atypical (simulating cold, influenza, pneumonia) symptoms.

■ HEMATOPOIESIS AND ERYTHROCYTES

Massimo De Martinis

The maintenance of a blood-forming or hematopoietic system (from the Greek *hemato*, blood, and *poiesis*, producing) is a requirement for survival. A common view of hematopoiesis holds that a pluripotent hematopoietic stem cell (PHSC) gives

origin to committed progenitors of myeloid bone marrow (BM), erythroid (erythrocytes or RBCs), and megakaryocytic (platelet) lineages (Table 7); these progenitor cells would, in turn, give origin to the recognizable hematopoietic precursors of the BM. A complex web of growth factors—colony-stimulating factors, stem cell factors, some interleukins (ILs), erythropoietin (EPO), thrombopoietin—as well as specific conditions of the hematopoietic microenvironment act in concert to achieve a normal blood picture (62). The present section is concerned with RBCs (Table 8) (Box 3). White blood cells are discussed with the immune system in Chapter 14, and platelets, with the cardiovascular system in Chapter 15.

Hematopoiesis is the study of the origin and development of blood cells, all derived from a pluripotent stem cell, but with distinct trajectories in their developmental potential, depending on the different tissues of origin. Hematopoiesis develops early in fetal life, and the primary source of blood cells changes several times during development. Prenatally, it changes from (*i*) the yolk sac to (*ii*) the liver and then to (*iii*) the spleen and, postnatally, to (*iv*) the BM.

The hematopoietic system, responsible for efficient oxygen delivery, hemostasis (responsible for stoppage of bleeding through blood clotting and vascular contraction), and all phases of the inflammatory response, has an astonishing capacity to respond to environmental stimuli (Table 9). The responsiveness of each hematopoietic lineage results from coordinate increases in the production and functional activity of appropriate hematopoietic cell types, without expansion of irrelevant ones. For instance, a hypoxic stimulus (i.e., exposure to low oxygen) will induce a specific expansion of the erythroid (erythrocyte-forming) BM and a subsequent increase in RBC number (erythrocytosis), as occurs in populations living at high altitudes. However, the BM, in response to hypoxia, will not increase production of neutrophils, monocytes, eosinophils, mast cells, or T- or B-lymphocytes (Table 9).

■ THE STEM CELL POOL IN AGING

During a normal life span, humans will have produced, cumulatively, red and white blood cells with a mass many times larger than their bodies, but in such a manner that, at any given time, blood counts are maintained within narrow limits. *In fact, the short life span of many of the mature blood cell types, as compared to the life span of whole organisms, requires rapid, continuous, and precise recruitment of new cells to maintain each population at its appropriate size* (63).

Whether the hematopoietic system undergoes significant functional changes associated with aging has been a matter of considerable controversy over the past decades. The previously accepted concept that hematopoietic function becomes progressively impaired with aging has been more recently challenged by experimental evidence indicating that steady-state hematopoiesis is not consistently affected in the elderly.

The current opinion is that aging-related modifications of BM function are rather subtle and most probably irrelevant for the hematopoietic function of normal older individuals. These aging-related changes, however, may become clinically evident under conditions of severe hematopoietic stress, such as administration of repeated courses of chemoradiotherapy.

The mechanisms underlying an aging-dependent decline in the hematopoietic reserve are not fully clarified, and different hypotheses have been proposed to explain the reduced ability of recovery from hematologic stress that is associated with aging (64). It is now well established that a replenishment of relatively short-lived blood cells is maintained by a small population of primitive, self-renewing stem cells. The question of whether hematopoietic stem cells (HSCs) are altered in aging has been the subject of considerable controversy. Earlier studies in old individuals focused on changes in the capacity of these cells to provide hematological rescue from lethal doses of total body irradiation as well as on their potential for sequential replications (65).

Hematopoietic stem cells (HSCs) balance self-renewal and differentiation in order to sustain lifelong blood production and simultaneously maintain the HSC pool. In stem cell homeostasis, a delicate balance exists between self-renewal and terminal differentiation: self-renewal may initiate cancer (i.e., leukemia), and increased differentiation ultimately may lead to premature exhaustion of the stem cell pool. It is likely that during replicative stress (which can be experimentally induced by serial transplantation but may also result from normal aging), this balance weighs in favor of terminal differentiation, thereby resulting in exhaustion of the HSC pool. *Under normal conditions, the HSC pool is large enough to provide an organism with a sufficient number of committed progenitors to ensure homeostasis, even after serious bleeding or chemotherapy.* Old people, or mice, usually do not die because they run out of HSCs. It has also been documented that HSCs can outlive their original donor, upon repeated serial transplantation in lethally irradiated recipients (66). Evidence from mouse studies shows that the aging program of HSCs is largely intrinsically regulated (67). Nevertheless, there is ample evidence that stem cell quality actually does decrease with each self-renewal division (Table 10).

Hematopoietic activity differs considerably in young children from that of adults, and, in adults, from that of the elderly. Studies of the proliferative capacity of a colony-forming unit of granulocytes and macrophages (CFU-GM) in BM and blood from donors of different ages indicate that a functional decline in hematopoietic progenitor (stem cell precursor) cells begins at birth and continues throughout life (68).

The decrease with old age in the number of peripheral blood-cluster of differentiation (CD34+) cells and of peripheral blood CFU-GM suggests a decrease of pluripotent blood stem cells with aging (69). This is consistent with the reduction

TABLE 7 Lineages of Mature Blood Cells Derived from Bone Marrow Stem Cells

White blood cells
Granulocytes
Neutrophils
Eosinophils
Basophils
Lymphocytes
B cells
T cells
Monocytes
Erythrocytes (red blood cells)
Platelets

TABLE 8 Some Unique Characteristics of Red Blood Cells

Red blood cells represent a cell population that
is easily accessible
is in continuing renewal
has a well-defined life span
has become a popular model for the study of cell function at all ages, including old age

BOX 3 *Function and Origin of Red Blood Cells*

The bone marrow, located in the cavities of the long bones, remains active throughout life; it contains the stem cells from which at least eight lineages of mature blood cells, including red blood cells (RBCs), are ultimately derived through a series of steps involving differentiation and proliferation (Table 7).

RBCs are biconcave disks. In mammals, they lose their nuclei before entering the circulation. Their function as carriers of oxygen depends on their content in hemoglobin, an iron-containing protein that binds strongly to oxygen and transports it from lungs to cells, where oxygen is needed for metabolic processes. Hemoglobin also plays a minor role in the transport of carbon dioxide from the tissues to the lungs. The average RBC counts (in humans) are 5.4 million/ L in men and 4.5 million/ L in women. Human RBCs survive in the circulation for an average of 120 days. When RBCs are no longer flexible enough to squeeze between the endothelial cells that line the blood sinuses of the spleen, they are removed from the circulation, die, and are phagocytized in the spleen.

Erythropoiesis, i.e., RBC formation, is subject to feedback control: it is inhibited by a rise (above normal values) in RBC number and it is stimulated by RBC reduction below normal values, as in anemia, or by a decrease in blood oxygen content, as in hypoxia. Control of erythropoiesis is regulated by several growth factors among which is the circulating protein hormone, *erythropoietin,* secreted from the kidney (85%) and liver (15%); erythropoietin production is stimulated primarily by hypoxia, and, to a lesser extent, by *androgens* and by *cobalt salts (cobalt is part of vitamin B_{12}, necessary for normal blood cell* formation). RBCs represent a cell population with unique characteristics, some of which are listed in Table 8.

Reference will be made here, whenever possible, to studies of hematopoiesis in healthy centenarians, taken as examples of successful aging. Comparison of function at progressive ages may improve our understanding of developmental and aging processes in these cells and extend this knowledge to other cell types.

in the number of myeloid progenitor cells in the BM of 88-year-old apparently healthy individuals, compared to young controls (70).

A progressive reduction of hematopoietic tissue mass is observed with aging. In fact, the great majority of BM areas show hematopoietic activity at birth, but starting from childhood, a progressive fatty replacement of active marrow areas in the long bones takes place. In early adulthood, hematopoiesis is confined to the central skeleton and proximal ends of femura and humeri. By age of 70 years, the hematopoietic cellularity of BM in the iliac crests is reduced to about 30% of that usually found in young adults. Nevertheless, under conditions of steady-state hematopoiesis, peripheral blood counts do not appear to be significantly decreased with aging in the majority of the population. *These findings suggest that aging is associated with changes in the dynamics of the hemopoietic stem and progenitor cell compartments.* Although functionally irrelevant in healthy individuals, such aging-related changes may influence the BM response to pathologic events, such as leukemic transformation, and chemoradiotherapy-related stress.

Age-related modifications of the hematopoietic system may involve (*i*) intrinsic changes of stem/progenitor cells (i.e., quantitative or qualitative defects), (*ii*) defective response to physiologic local concentration of cytokines, or (*iii*) alterations in BM microenvironment, resulting in impaired cytokine production and/or cell contact–dependent support for hematopoietic stem/progenitor cells (64).

The BM in newborns is very active and fills most of the cavities of the skeleton, whereas in adults, it is mainly found in the pelvis and sternum. In older people, the number and distribution of blood cells in BM decline together with the marrow reserve. Evidence that stem and progenitor cell function may decline with age is further supported by the several observations summarized in Table 10.

■ HEMATOPOIETIC POTENTIAL AND REMODELING

Whereas hematopoietic potential is maintained in aging, under basal (steady-state) conditions, the capacity of stem cells for self-renewal and recovery after administration of repeated courses of chemoradiotherapy appears to decline gradually with advancing age. Therefore, the developmental and aging potential of stem cells under basal conditions must be differentiated from that under stress conditions (65).

Basal hematologic parameters show little change with aging, although some aging-associated changes have been

TABLE 9 Major Functions of Blood Cells

Efficient oxygen delivery to tissues and cells (erythrocytes)
Hemostasis: prevention of blood loss (e.g., through blood clotting, platelets)
Immune response, primarily white blood cells (Chapter 14)
Responsiveness to environmental stimuli (e.g., increase in erythrocyte number in response to hypoxia
Specificity of responses to demands (only relevant lineage is stimulated without expansion of irrelevant ones; e.g., hypoxia selectively stimulates erythroid bone marrow and subsequent erythropoiesis)

TABLE 10 Evidence for the Decline in Function of Progenitor Cells with Old Age

Decline begins at birth and continues throughout life
Bone marrow from old donors is more likely to fail when transplanted than bone marrow from young donors
Ability of old people to respond to hematologic stress is decreased
Recovery after inhibition of proliferation of bone marrow cells (myelosuppression) is slower in older persons
Reduction of telomere length

reported in a few subpopulations of blood cell groups. Conversely, *under conditions of severe hematopoietic demands and in response to stress, the reserve capacity of the BM for recovery may become significantly curtailed in old age.*

■ Changes with Old Age Under Steady-State Conditions

The ability of stem cells derived from old donors to reestablish erythropoiesis as measured in competitive repopulation assays of lethally irradiated young mice is as active as, or even more active than, that from young donors. However, in long-term studies, stem cell function is less efficient in old than in young animals. Quantitative analysis of the data suggests that the hematopoietic compartment of aging mice contains a higher proportion of precursor, also called "progenitor," cells, i.e., cells preceding the stage of stem cells. Proliferation of precursor cells in old people may represent an attempt to reestablish normal cell number, although these precursor cells are less efficient in doing so than the succeeding stem cells. A study of autologous stem cell transplantation has shown comparable neutrophil and platelet recovery times between young and old patients (71).

Despite the general assumption that important functions may deteriorate with old age, careful examination of the healthy elderly suggests that many physiologic parameters may be preserved throughout life. *In particular, most hematological parameters, such as absolute number and percentage of peripheral blood cells (e.g., erythrocytes, with normal hemoglobin levels, neutrophils, monocytes, eosinophils, basophils, and platelets) are well preserved in healthy elderly, including centenarians, and remain quite similar to those of young normal subjects. Thus, the mechanisms responsible for steady-state hematopoietic functions appear to be well conserved in the later decades of human life.*

Hematopoiesis is finely controlled by a complex cytokine network, cytokines being hormone-like chemical messengers involved in immune cell communication (Chapter 14). Changes in cytokine production and response occur with aging, as illustrated by (*i*) the decrease in the production of granulocyte-macrophage colony-stimulating factor (GM-CSF) and of IL-3 by phytohemagglutinin-stimulated peripheral blood mononuclear cells, but (*ii*) an increase in serum levels of stem cell factor (SCF) (Table 11). Yet, despite significant changes in the in vitro production of hematopoietic cytokines (IL-3 and GM-CSF) and in the serum level of hematopoietic growth factors (SCF), stem cells from old subjects remain responsive to hematopoietic cytokines in vitro, and are able to form different types of colonies in a way indistinguishable from that of young subjects.

The profile of cytokine production changes during aging (67). An increased concentration in peripheral blood of inflammatory cytokines that suppress hematopoiesis, such as IL-6 and TNF, occurs with aging. Indeed, in older individuals, anemia (of unknown origin) bears partial resemblance to the changes of anemia of chronic disease (ACD). Increased levels of proinflammatory cytokines are encountered in geriatric individuals with no discernible chronic disease or inflammation; this cytokine dysregulation results in alterations of erythropoiesis such as (*i*) inhibition of the erythropoietin hormone (EPO) synthesis and colony-forming unit–erythroid maturation and (*ii*) disruption of normal iron metabolism. As a result, whether an "anemia of aging" exists has been, and remains, a controversial topic.

Hypoxemia (low blood oxygen content) stimulates EPO gene expression, primarily in kidney interstitial cells and to a lesser extent in the liver and the brain. Under physiological conditions, peritubular interstitial cells in the kidney detect low levels of oxygen in blood, causing an increase in the secretion of EPO, a hematopoietic growth factor that regulates erythropoiesis, into the blood. EPO *stimulates the erythrocyte progenitor cells in the BM to proliferate and differentiate into mature erythrocytes.* When the BM of elderly patients with anemia of unknown cause was examined, a markedly lower level of erythrocyte progenitor cells was observed compared to in young subjects or elderly subjects without anemia. In addition, erythrocyte progenitor cells in elderly anemic patients were less responsive to endogenous EPO.

At the molecular level, the hypoxia inducible factor (HIF) is the master regulator for hypoxia-induced gene expression. Recent studies demonstrated age-related changes in the HIF system, which might explain the reduced ability to cope with hypoxia in the elderly. There is also some evidence that HIF is functionally connected to the aging process itself (72). It is possible that the overall mechanisms responsible for the complex remodeling of hematopoiesis depend on modifications of the network of hematopoietic cytokines, rather than on the unresponsiveness of hematopoietic progenitors to these growth factors. These considerations are supported by previous studies showing that the in vitro production as well as the plasma levels of cytokines involved in hematopoiesis, such as IL-6, increase with age in healthy donors and centenarians. Indeed, the increase of SCF, as well as that of IL-6, could be interpreted as a compensatory mechanism with which to maintain the CD34+ cell pool and to stimulate erythroid cell differentiation (Table 11).

TABLE 11 Changes of Hematopoietic Remodeling in Elderly Subjects

Decreased absolute number of CD34+ progenitor cells in peripheral blood
Well-preserved competence of CD34+ cells to respond to cytokines and to form erythroid, granulocyte-macrophagic, and mixed colonies indistinguishable in number, size, and morphology from those formed from similar cells of young subjects
Decreased in vitro production of granulocyte-macrophagic colony-stimulating factor and interleukin-3
Increased serum levels of stem cell growth factor
Delayed recovery from hematological stress
Centenarians exhibit few hematological changes

Abbreviation: CD, cluster of differentiation.

■ Changes in Old Age Under Stress Conditions

Hematopoietic competence decreases in old age under various experimental conditions, including stress and stem cell transplantation. In both cases, it is important to distinguish between long-term versus short-term effects of stress (Chapter 9) *on the aging-associated decline in recovery* (70). The reduced potential for sequential replication is not readily expressed in the aged individual, but it may play a role under conditions where the stem cells are subject to replicative stress (that is, stress-stimulating cell

TABLE 12 Changes in Stem Cells Proliferation in Old Age

Proliferative potential decreases
Telomere length shortens despite telomerase presence and upregulation by cytokines

replication). Therefore, the proliferative ability of stem cells must be evaluated before a clinical decision is reached on whether transplantation of BM is appropriate, in which case, the stem cells must be able to replicate extensively. However, replicative stress may render the cells susceptible to mutations and thereby represent a possible cause of leukemia secondary to BM transplantation.

Although basal hematopoietic potential is well preserved in healthy centenarians, the hematopoietic cytokine network may undergo a complex remodeling with aging. Although significant changes do not occur under steady-state conditions, under pathological conditions (e.g., bacterial infections), or during periods of increased hematopoietic demand (e.g., hemorrhage, hypoxia), an impairment of the hematopoietic response may emerge even in the healthy elderly and in centenarians. Indeed, the well-preserved basal CD34+ cell function in centenarians probably arises from a new equilibrium between hematopoietic cytokines and their hematopoietic target cells, as a result of continuing adaptive processes (65).

The hypothesis that the aging process is slowed down in HSCs, as compared to other cells, is strengthened by the recent observation that telomerase activity is present in HSCs from adult human BM (Chapter 4). It can be speculated that the aging process follows diverse timetables in the various cells of the body and that the cell type responsible for maintaining a reservoir of pluripotent cells can be spared, at least in part, from the aging process, thus contributing to individual longevity (■ Chapter 2).

■ STEM CELL PROLIFERATIVE CAPACITY

Stem cells are usually quiescent, and, at any time, very few are cycling, perhaps as protection against (*i*) exhaustion of the pool and (*ii*) development of mutations. The BM of aged mice contains stem cells that are ready to cycle and to replicate upon incubation with cytokines or seeding onto a thymic stroma; however, replications cease soon after termination of the experimental intervention. *In the elderly, induction of stem cell proliferation would depend on the action of cytokines in the BM, as part of normal stem cells replication. With advancing age, once the cells have entered the division cycle, they may become more susceptible to mutations and undergo neoplastic processes, particularly in view of the reduced fidelity of DNA repair.*

Analyses of isolated long-term reconstituting stem cells show a high frequency of the cells in the growth phase (70). *With aging, the increased proportion of the stem cells about to enter the cell division cycle may represent a compensatory process consequent to the reduction in number of active stem cells.* A quantitative analysis of the frequency and proliferation of five subsets of primitive hematopoietic cells in the BM (73) revealed that the relative and absolute numbers of the most primitive stem cell subsets is three- to fourfold higher in old than in young mice (70). The negative correlation between maximum life span and the capacity for proliferation, manifested by changes in the frequency and cell-cycle kinetics of primitive HSCs, reflects effects that may be both age and strain dependent.

The findings that somatic cells have a limited capacity for replication have raised the question of whether the potential for self-renewal is gradually reduced with age, due to a possible finite cell replication programming (Chapter 4). Serial transplantation of hematopoietic cells from young mice to healthy irradiated recipients showed that cells originating from the transplanted BM donor could not be recovered from the recipients after a limited number of generations. The stem cells have a limited capacity for replication, and the potential for self-renewal would be exhausted in old age (Table 12).

■ Telomeres and Telomerase

Telomeres, the structures protecting chromosome ends, have received much attention as a potential cell-intrinsic trigger to induce replicative senescence. *The jury is still out as to what role telomeres may play during stem cell aging.* Length of the telomeres, the terminal section of chromosome involved in chromosomal replication, and stability correlate with replication of somatic cells and residual replication potential (■ Chapter 4). Thus, the sequential loss of telomeric DNA from the ends of the chromosome with each somatic cell division would eventually reach a critical point capable of triggering cellular senescence, and of influencing the balance between stem cell renewal and proliferation (74).

The loss of telomeric repeats in hematopoietic cells is a dynamic process that is differentially regulated in young children and adults. Human stem cells with a CD34+ CD38 low phenotype—that characterize the immature hematopoietic progenitors, purified from adult BM—have shorter telomeres than cells from fetal liver or umbilical cord blood. Cells produced in cytokine-supplemented cultures of purified precursor cells show a proliferation-associated loss of telomeric DNA. *These findings strongly suggest that the proliferative potential of most, if not all, HSCs is limited and decreases with aging* (Table 12).

Telomerase, the key enzyme that catalyzes the addition of telomeric sequences at the end of chromosomes, is not expressed in somatic cells, but is expressed in germ-line cells, where telomere length is maintained so that viable chromosomes can be transmitted to the following generation (■ Chapter 4). If stem cells are to maintain their integrity during replication, for the progeny to manifest the same properties as the parental cells, their telomere length must remain intact through self-renewal. However, experimental evidence shows a decline with aging in the capacity of the stem cells to replicate (replicative senescence). The highest telomerase activity can be detected in immature BM hematopoietic progenitors, and telomerase is downregulated with cellular maturation.

Telomere loss in normal tissues begins in early adult life and progresses gradually with advancing age. However, rates of telomere loss, surprisingly rapid in young children, are highly variable at subsequent ages. The telomere loss, differentially regulated in leukocytes from young children and adults, may serve as a model for telomere dynamics of other somatic cells (75).

Telomerase, expressed at basal levels in primitive HSCs, is upregulated in response to cytokine-induced proliferation and cell cycle activation, and downregulated with decreased proliferation and transition to subsequent developmental subsets. Telomerase-deficient HSCs showed reduced long-term repopulating capacity, concomitant with an increase in genetic instability. *Humans suffering from the rare inherited disorder dyskeratosis congenita, which results from a mutation in the hTR gene, have reduced levels of telomerase activity and shortened telomeres. In these patients, BM failure is the principal cause of death.*

It should be noted that telomere shortening occurs rapidly in cell lines derived from patients who suffer from premature aging disorders, such as *Werner syndrome and ataxia telangiectasia* (Chapters 3 and 4). A strong argument against a direct role for telomerase in preventing stem cell aging is the observation that

HSCs from murine telomerase reverse transcriptase (mTERT) transgenic mice, in which telomerase is overexpressed and telomere length is preserved, cannot be serially transplanted more often than wild-type cells. This indicates that other mechanisms must be involved in regulating stem cell exhaustion.

■ DNA Damage

Each replication round of the genome during cell division results in numerous copy errors, but elaborate proofreading and editing mechanisms have evolved to correct these. The appropriate cellular response after detection of DNA damage is an initial attempt at repair, but if damage is too extensive or compromises DNA metabolism, a signaling cascade triggers cellular senescence or death. *The cumulative extent of DNA damage during the lifetime of a stem cell may potentially result in its demise.* Indications that DNA damage can actually result in HSC exhaustion originate from an example from recent studies. In mice deficient in Ercc 1 (a protein essential in nucleotide excision repair), reduced responses to hematopoietic stress; the associated exhaustion of hematopoietic progenitor activity suggest premature senescence of the HSC (76).

DNA lesions may be induced by oxidative damage resulting from free radical production. The aging process may therefore be influenced by energy restriction through a reduction in the metabolic "rate of living," ultimately leading to reduced oxidative damage.

■ The Bone Marrow (BM) Environment

The ontogeny of hematopoiesis during fetal life and the differentiation of blood cells in adult life depend upon a fully competent microenvironment to provide appropriate signals via production of soluble factors and cell contact interactions. The cellular constituents of the microenvironment, also defined as "the hematopoietic niche", a highly organized three-dimensional structure, largely derive from a common progenitor of mesenchymal origin.

Hematopoiesis occurs in unique microenvironments that facilitate the maintenance of HSCs as pluripotent and support the maturation of progenitors. Each of these activities may require different growth factors and microenvironments, the identities of which are yet to be determined. In vitro, BM stromal cells (BMSCs) serve as a rich source of growth factors for a variety of hemopoietic processes. BMSCs are composed of several different populations, including fibroblasts, macrophages, endothelial cells, and adipocytes. It is difficult to discern the relative importance of each of these cells. However, (*i*) direct stromal cell–blood cell contact, (*ii*) BMSC production of the extracellular BM matrix, and (*iii*) cytokine synthesis are all relevant to the formation and maturation of blood cells in vitro. The role of BMSCs in vivo is less clear. In the BM, the most important elements of the niche appear to be osteoblasts (77).

Osteoblasts have long been known to play a central role in skeletal development (Chapter 20). In the BM, osteoblasts constitute part of the stromal cell support system, but little is known about their functional relevance to HSCs. Primary human osteoblasts express many molecules known to modulate hematopoiesis. For example, granulocyte colony-stimulating factor (G-CSF), GM-CSF, macrophage colony-stimulating factor, IL-1, IL-6, IL-7, leukemia inhibitory factor, osteoprotegrin, receptor activator of NF-kB ligand (RANKL), stroma-derived factor, TNF-, and vascular endothelial growth factor (VEGF) have all been detected using human cells. Aging is associated with decreased maximal life span and accelerated senescence of BMSCs (78). *The changes during aging within the BM hematopoietic microenvironment most likely are linked to the physiology of aging bone*. Bone degrades with age (osteoporosis) due to decreased formation of new bone by osteoblasts. Marrow stem cells are considered the progenitor of adipocytes, osteoblasts, and hematopoietic stromal cells. Further, a controlled reciprocal regulation of osteoblasts versus adipocyte differentiation exists; with age, adipocytes increase, and osteoblasts decrease. It is possible that stromal cell generation from marrow stromal cells is compromised during aging (79). Mobilization of hematopoietic stem and progenitor cells from BM into peripheral blood in response to G-CSF requires deadhesion from the niche.

In a mouse model of G-CSF–induced mobilization, the ability to mobilize HSCs is approximately increased fivefold in aged mice and this enhanced mobilization ability is intrinsic to the stem cell. The stroma–stem cell interaction seems to be dynamic over the lifetime and results in physiologically relevant changes in the biology of primitive hematopoietic cells with age (80). Changes in the stem cell compartment may be related to primary intrinsic processes or to induction by the stroma—that is, the collagen matrix of the bone that supports the cells concerned with bone formation (osteocytes, osteoblasts, and osteoclasts) and with BM function—and by other neighboring cells in the microenvironment. Indeed, age-related changes have been noted in stroma cells as well as in mature lymphocytes in BM. The capacity of the hematopoietic stroma cell lines for replication is reduced unless the cells are cocultured with stroma cell lines that promote stem cell maintenance. Stem cells from aged mice do not show any change in their ability to maintain (70) or to form colonies on stroma cultures, indicating that their capacity to interact with stroma elements is not changed with aging. Persistence of normal function may be related to compensatory changes with aging in the cytokines produced by stroma cells, as well as by neighboring macrophages and mature lymphocytes. The increased proportion of lymphocytes in the BM of aged mice and their altered pattern of function may be of particular importance. Relevant aging-related changes in cytokines include the following:

- Reduced availability of stroma cell–derived IL-7
- Shift in the cytokine profile from T-helper 1 to T-helper 2 (81)
- Increased production of prostaglandin (PGE2) by macrophages
- Impaired expression of hematopoietic growth factors in aged humans and mice (82)

The shift toward T-helper 2 type and proinflammatory cytokines in BM cells of aging mice is similar to the shift detected in the peripheral lymphoid tissues (83). Cytokine production by BM stroma cells (in vivo and in vitro) is also dependent on estrogen levels: for example, IL-6 secretion is significantly higher in postmenopausal than in nonmenopausal adult women, an increase prevented by estrogen replacement therapy (84).

■ ERYTHROPOIESIS AND AGING

The primary function of the end product of erythropoiesis, the mature RBC, is to transport oxygen efficiently through the circulation to all cells and tissues of the body and to respond quickly and appropriately to increased oxygen demands, either acute (e.g., rapid and severe blood loss) or chronic (e.g., hypoxia from

pulmonary disease). The overall BM response, however, is complex, and requires the following:

- The participation of erythroid cells responsive to EPO
- A structurally intact microenvironment
- An optimal iron supply within the marrow

■ Anemia in the Elderly

Aging may favor the development of anemia, a disease in which the blood is deficient either in the amount of hemoglobin or in the number of RBC or both. It is still not clear if aging affects erythroid homeostasis and is responsible for low hemoglobin levels (84). The two reasons for considering anemia in the elderly to be a sign of disease and not a physiologic parameter are: (*i*) most older people maintain a normal RBC count, normal hemoglobin, and normal hematocrit, and (*ii*) in most elderly subjects, an underlying cause of anemia can be found when hemoglobin levels are decreased below 12 g/dL (normal values: 14–16 g/dL). Frequently, patients are already affected by a disorder (e.g., congestive heart failure, cognitive impairment, dizziness, and apathy) that may be worsened by the anemia (85).

Using the World Health Organization definition of anemia—hemoglobin less than 12 g/dL in women and less than 13 g/dL in men—the prevalence of anemia in the elderly ranges from 4.4% to 48%, with higher frequency in men than women, and the highest prevalence in men 85 years and older (86–88). Sex differences in hemoglobin levels tend to narrow significantly in old age and virtually disappear in the very old. It is unclear if the higher anemia incidence in old men is due to more frequent illnesses or to physiologic changes. Race and ethnicity affect the prevalence of anemia, which is significantly higher in elderly blacks. The common causes of anemia in the elderly are listed in Table 13. Hemoglobin levels decline with age, and anemia is considered to be an important health problem among older individuals, but estimates of the prevalence of this condition vary substantially.

Causes of anemia are divided into three broad groups: (*i*) nutrient deficiency, most often iron deficiency anemia (IDA); (*ii*) anemia of chronic disease (ACD), perhaps better termed as anemia of chronic inflammation (Table 14); and (*iii*) idiopathic anemia (i.e., unexplained or of unknown cause). Unexplained anemia accounts for approximately one-third of all anemia in individuals ≥65 years of age. Several theories have been put forward to explain the high rate of unexplained anemia in elderly individuals, including the following:

- Reduced pluripotent hematopoietic stem cells (PHSC) reserve
- Decreased production of hematopoietic factors
- Reduced sensitivity of stem cells and progenitors to growth factors
- Marrow microenvironment abnormalities
- Androgen deficiency
- Unrecognized chronic kidney disease
- Undiagnosed myelodysplasia, or early stage ACD

TABLE 13 Hematological Profile of Some Older Individuals

Decreased hemoglobin
Decreased hematocrit
Decreased red blood cell number
Delayed onset of erythropoiesis after severe bleeding
Decreased erythropoietic responses to erythropoietin administration
Most of these changes are not experienced by centenarians

The feedback between hemoglobin and EPO is maintained in the elderly, but EPO secretion in response to IDA is decreased in elderly individuals. In contrast, EPO levels are not elevated or increased inappropriately in elderly individuals with unexplained senile anemia. It is possible that age-associated increases in levels of proinflammatory cytokines, such as IL-6, may reduce the responses of stem cells and hematopoietic progenitors to growth factors, including EPO. Plasma levels of IL-6 are elevated secondary to infection, trauma, or stress, and regulation of this cytokine becomes less precise with age. The mechanisms underlying increased IL-6 levels in the elderly are not clear, but they may involve elevated production by monocytes, T-cells, endothelial cells, or bone cells (87,88).

As individuals approach 100 years of age, the hematological profile may undergo significant changes (Table 13) (87,88). *In humans, several observations suggest a progressive exhaustion of PHSC.* The hematopoietic tissue of the BM contracts progressively with aging. The concentration of erythroid colonies in the BM of older individuals is decreased (a decrement not associated with clinical anemia). The concentrations of PHSCs in the peripheral blood of persons older than age 70 and younger than age 30 have similar baseline values, but the response to the administration of growth factors (GM-CSF) is greater in younger individuals. The reduction in the production of burst-promoting activities in the BM of older individuals implies that although aging may not be regarded as a cause of anemia, it may increase susceptibility to the disease.

The main hormonal modulators of erythropoiesis are EPO, testosterone, and IL-3 (89) (Table 14). Some (but not all) investigators have reported normal testosterone levels and normal cell responsiveness to testosterone in the elderly. The responsiveness of progenitor cells to androgen does not appear to change with old age, and the age-related differences found in IL-3 levels do not appear to change (69,90,91).

With respect to EPO, levels in healthy elderly subjects have been described as unchanged, lower, or even slightly higher, compared to younger controls. EPO secretion is usually not compromised in elderly individuals, although it may be reduced in acute or chronic anemia as the result of concomitant disease or exhaustion of its production (91).

■ RED BLOOD CELLS AND AGING

Given the short, 120-day life span of the RBCs, the continuous renewal of the RBC population from the BM should require their functional and structural integrity even at very old ages (Fig. 3). However, unsuccessful aging accompanied by either physiologic impairment or degenerative disorders, such as *diabetes mellitus and hypertension, may cause alterations in circulating erythrocytes that may shorten their life* (Table 15). RBC removal from the circulation may be accelerated by changes in their membrane composition associated with old age that are capable of altering the rheological properties, i.e., dealing with the deformation and flow properties of these cells (92). Aging is closely associated with increased free radical production, which ultimately leads to devastation of normal cell function and membrane integrity.

TABLE 14 Hormonal Regulators of Erythropoiesis

Erythropoietin
Testosterone
Interleukin-3

■ Oxidative Stress Relationship to Aging-Related Changes in Erythrocytes

Erythrocytes, the unique carriers of oxygen, are highly susceptible to oxidative stress conditions. The rich polyunsaturated lipids of the cell membrane and the iron of hemoglobin, a potent catalyst for free radical reactions, make erythrocyte a good substrate for oxidative damage. *Membrane oxidation does affect the intrinsic membrane properties, by altering membrane fluidity, ion transport, and loss of enzymatic activities of the cell. Cell membrane is an important target for radical damage, and blood can reflect the liability of the whole animal to oxidative condition*. Thus, erythrocytes have been used extensively for determining the effect of aging in studies concerning the possible involvement of free radicals. The total negative electric charge of the erythrocyte membrane, determines the correct course of many processes such as (*i*) transport of metabolic substrates and products through ionic pumps, (*ii*) transfer of information through carriers and membrane channels, and (*iii*) prevention of aggregation of erythrocytes from each other. The aggregability of erythrocytes is mainly determined by glycocalyx (a cell coat structure consisting of a fuzzy layer of polysaccharide or glucoprotein) on their membrane, in particular by the amount of the sialic acid residues that are present on the membrane and bear a negative surface charge. *Oxidative stress or other damaging effects due to surface sialosaccharides may themselves play a role in the aggregation of erythrocytes,* stress may increase the adhesiveness of endothelial cells and contribute to the development of various pathologies including diabetes mellitus, atherothrombotic complications, and sequestration of circulating erythrocytes by macrophages. In normal conditions, the amount of oxy-repair enzymes or antioxidants would possibly be sufficient to cope with the amount of damage produced. However, during aging, an oxidant challenge exceeds the capacity of the cell's defense system, and membrane damage may occur. The erythrocyte surface charge in aged rats significantly decreases compared to in young rats (93,94). This decrease may be due to the increased protein oxidation leading to carbonyl formations in the erythrocytes of aged rats. *A disruption in the systemic modulation of oxidative stress in aging was demonstrated in platelets and erythrocytes (94).*

A recent study demonstrates a close correlation between oxidative stress and erythrocyte membrane glycohydrolases (94). A comparison between elderly (70 years) and young

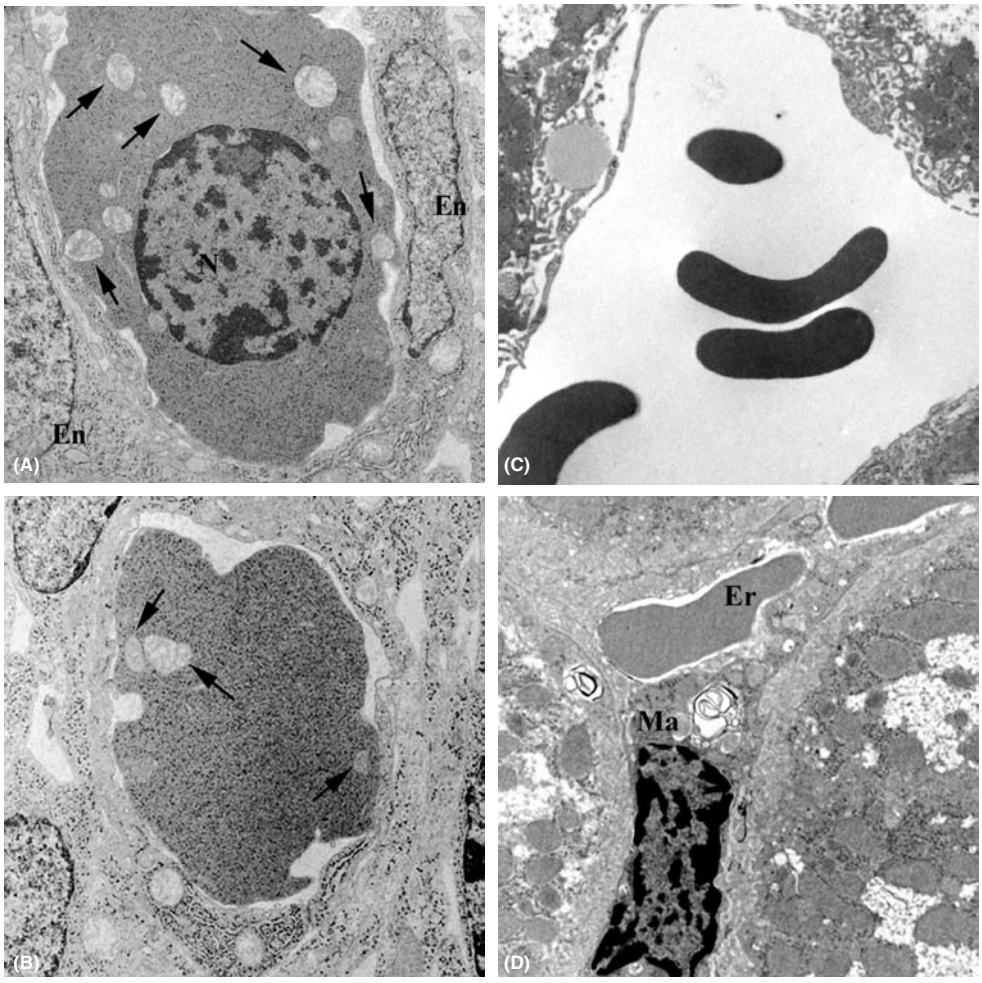

FIGURE 3 The life span of the red blood cells Original magnification 7000. (**A**) Electron micrograph of a nucleated (immature) erythroblast. Numerous mitochondria (*arrows*) are visible in the cytoplasm of the erythroblast, which is surrounded by endothelial cells. (**B**) A reticulocyte showing irregular cell shape, numerous ribosomes, and few mitochondria (*arrows*). (**C**) Mature, biconcave erythrocytes in a small vessel. (**D**) Macrophage in the act of phagocytizing an erythrocyte. *Abbreviations*: En, endothelial cells; N, nucleus; Ma, macrophage; Er, erythrocyte. *Source*: Courtesy of Drs. Roberta Nardacci, Laura Falasca.

TABLE 15 Factors Involved in Earlier and Faster (<120 days) Removal of RBCs from Circulation in Old Subjects

Causes	Mechanisms
Earlier and greater fragility	Accelerated desialylation of membrane glycoconjugates may promote RBC aggregation
Greater tendency to aggregate	Alteration in membrane lipids increases cell fragility as well as decreases glucose transport and utilization
Decreased availability of energy for metabolism	Decreased activity of NA^+K^+-ATPase reduces availability of energy (ATP for cell metabolism)
Altered ionic balance, especially in aging-associated diseases	Increased cytosolic Ca^{++} and decreased Mg^{++} change ionic balance (risk factors: hypertension or diabetes)

Abbreviations: RBCs, red blood cells; ATP, adenosine triphosphate.

subjects reveals that elder people are subject to a higher oxidative stress, which causes an increase in plasma hydroperoxide levels (18%) and a decrease in antioxidant defense (25%). Moreover, the marked decrease in erythrocyte membrane fluidity observed in the elderly subjects significantly decreased β*D*-glucuronidase and neutral sialidase activity by 30% and 50%, respectively. Activity differences were also observed when erythrocytes were further separated according to their biological age: β*D*-glucuronidase decreases with the subject's age, while neutral sialidase levels are higher in the elderly. This is presumably due to the localization of these enzymes in distinct plasma membrane microdomains, which are differently peroxidized. A possible role of these enzymes in signaling early membrane alterations has been suggested (95).

In conclusion, several hypotheses have been proposed concerning the mechanisms involved in the generation of senescence signals and the removal of aged RBCs by splenic macrophages. Among these, changes in membrane components, such as are indicated below, have been given considerable attention. They are as follows:

- Desialylation of glycoproteins (96) (i.e., breakdown of sialic or neuramic acid, a major building block of cell membrane)
- Decreased phospholipid asymmetry
- Alterations in membrane proteins, with the appearance of senescence surface antigens
- Unchanged cholesterol:phospholipids ratio despite increased cholesterol and phospholipids (96,97) (there are conflicting reports about RBC membrane lipids)
- Decreased cholesterol:phospholipid ratio with decreased membrane cholesterol
- Increased cholesterol:phospholipid molar ratio in healthy centenarians when compared with old subjects, due to a marked decrease in membrane phospholipids accompanied by a smaller decrease in cholesterol

The observed increase in the cholesterol:phospholipid molar ratio and the decrease in membrane cholesterol, together with the increased polyunsaturated:saturated fatty acid ratio in centenarians, are likely to provide the RBC membrane with better fluidity and, thereby, a greater flexibility that would allow RBCs to escape rapid destruction in the spleen. Free radicals accumulate in RBCs, as in other tissues, and oxidative damage may be responsible for some of the alterations in RBCs in old age (98,99) (Chapter 5).

The protein composition of the erythrocyte membrane in older healthy individuals (including centenarians) remains essentially unchanged except for a marked increase in content of the microtubular, contractile protein actin, the most abundant protein in mammalian cells. The structural organization of the membrane, responsible for the RBC shape and flexibility, is known to be susceptible to the attack of oxidant agents (100). An elevated actin content in the membrane skeleton might strengthen the spectrin-4.1-actin junctional sites and, consequently, increase the resistance to alterations in shape and to increased fragility by mechanical stress. *Longevity is thought to be associated with a well-preserved membrane structure, and a more viable RBC may contribute to a longer life span.* It remains to be clarified whether the increase in actin is a change brought about by the aging process or whether it is a characteristic of individuals genetically predisposed to become centenarians. In the latter case, some biochemical characteristics of the RBC membrane may be taken as an index of individual life expectancy.

With respect to RBCs' survival in the old individual, a number of changes have been reported that may be responsible for an earlier and greater fragility in old age (but with a certain degree of persistence of normal flexibility in centenarians):

- Degradation of band-3 protein calpain, a calcium-dependent protease involved in glucose transport and utilization, is enhanced in RBCs of old people; reduced band-3 protein would be responsible for decreased glucose transport and utilization in the elderly (while oxidative metabolism would be spared) (101).
- Accelerated desialylation of membrane glycoconjugates may promote RBCs' aggregation (by reducing electrostatic repulsion among cells) and trigger the clearance of senescent cells from circulation (102,103). The maintenance of sialic acid membrane content in centenarians would prevent RBCs from aggregating, preserve their flexibility, and prolong their survival.
- The activity of the enzyme Na^+K^+-ATPase, which regulates transport of sodium and potassium through the membrane, decreases progressively with aging in older individuals, including centenarians. The decrease of this enzyme, responsible for one-third of the total intracellular energy consumption at all ages, may lead to decreased intracellular ATP content during senescence (104).
- The progressive increase in cytosolic free calcium (Ca) and the decrease in magnesium (Mg), two divalent cations vital to cellular homeostasis, have been correlated with pathological conditions such as hypertension (Chapter 16) and diabetes (Chapter 13). Thus, these ionic changes may be clinically significant, and underlie the predisposition of older subjects to cardiovascular and metabolic diseases (105,106).

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18

The Kidney, Lower Urinary Tract, Body Fluids, and the Prostate

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As with all other body systems, the urinary tract is directly and indirectly affected by aging (1–5).

- Direct effects are exemplified by intrinsic, molecular, and cellular changes involving the nephron (the functional unit of the kidney), the bladder, and the prostate (both parts of the lower urinary tract) (6,7).
- Indirect effects may be secondary to cardiovascular, endocrine, or metabolic alterations that occur with aging and have vital repercussions on urine formation and excretion.

Reciprocally, physiologic decrements in renal function and disturbances of the lower urinary tract in the elderly individual may not only alter the elimination of end products of metabolism but also impair other functions such as regulation of body fluids, acid–base balance, and blood pressure. Additionally, given the metabolic and excretory functions of the kidney with respect to drugs of environmental, recreational, and therapeutic use, the decline of renal function with aging may alter the renal handling of drugs and thereby modify drug pharmacokinetics, including toxicity and therapeutic effectiveness (Chapter 22). Drug–kidney relationships are important at any age but their impairment in old age is particularly dangerous for the elderly, for whom the prescription of multiple drugs, simultaneously, increases the dangers of polypharmacy and of toxicity (Chapter 22).

In the first section, the aging-related changes in the urinary system will be grouped as follows:

- Aging-related changes in renal function
- Aging-related changes in the function of the lower urinary tract
- Water and electrolyte distribution and acid–base balance

In the second section, aging of the prostate will be discussed given the anatomical locations and the functional interrelations of the prostate and the lower urinary tract.

AGING-RELATED CHANGES IN RENAL FUNCTION

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All parameters of renal function may be affected by aging, but the age of onset, rate and course of changes, and consequences vary. Thus, glomerular filtration, closely dependent on the efficiency of the renal blood flow and the integrity of the glomerular basement membrane, appears affected at an earlier age and more severely than tubular reabsorption and secretion. After the age of 30, renal function in humans, as measured by several tests (see below), gradually decreases, and, by 85 years, has been reduced by half. This decline has been ascribed to

- gradual loss of nephrons,
- diminished enzymatic and metabolic activity of renal tubular cells, and
- increased incidence of pathologic processes, primarily atherosclerosis, which affects the renal blood circulation (Chapter 15), an essential factor in determining renal competence.

A brief summary of the structure and function of the kidney is presented in Box 1, Figure 1, and Table 1, and several tests, routinely used to measure renal function, are listed in Table 2.

Glomerular Function

The function of the glomerulus is to selectively filter plasma to produce a glomerular filtrate that, under normal, healthy conditions, is practically free of proteins. Glomerular filtration is determined by measuring the clearance of plasma and the excretion in the urine of a substance such as inulin or creatinine that is freely filtered through the glomeruli but not secreted or reabsorbed by the tubules. This technique establishes the filtration rate in an average young man as approximately 125 mL/min. *According to early and now classic studies, commencing as early as 30 years and continuing into old age, glomerular filtration rate and renal blood flow decrease progressively and significantly* (8–11).

Creatinine Clearance

Both inulin (a polymer of fructose), a polysaccharide administered intravenously, and creatinine, a normal endogenous product of protein metabolism, are usually utilized to assess glomerular function. Creatinine clearance, which does not involve any drug administration, provides an acceptable index of renal function; it is most frequently used for assessing renal function and determining the dosage of drugs excreted through the kidneys (Chapter 22). Unfortunately, this method requires the collection of a timed urine sample, often difficult to obtain in elderly individuals. To avoid the need for timed urine samples, creatinine clearance is often calculated from easily obtained information on weight, gender, age, and serum creatinine (Scr) concentration, as shown in the following formula:

$$\text{Creatinine clearance} = \frac{(140 - \text{age}) \times \text{body weight(in kg)}}{72 \times \text{Scr(in mg\%)}}$$

A 20-year-old individual, with an Scr of 1 mg% and a body weight of 72 kg has a creatinine clearance of 120 mg%, whereas a

BOX 1 *Major Structural Characteristics and Functions of the Kidney*

The kidneys are paired retroperitoneal organs that lie on either side of the descending (abdominal) aorta and receive their blood via the renal arteries, two main branches of the aorta. A smooth outer capsule covers the cortex, which contains the majority of the glomerular portion of the individual nephron (Fig. 1). *The nephron represents the functional unit and is formed of the glomerulus, in the cortex, and renal tubule*, which dips down and occupies the medulla, the inner portion of the renal parenchyma. Running parallel to the tubules in the medulla is a network of blood vessels, the *vasa recta*, which participate, with the *tubular loop of Henle*, in controlling the osmolality of the medulla through a counter-current mechanism. The urine formed in the nephron flows into *collecting ducts* and out through the *calyces* and the *pelvis* to the *ureter* and from there to *the bladder*.

The *glomerulus* is formed by a tuft of capillaries between the entering, the afferent, and the exiting, efferent arterioles. Filtration is through a fenestrated glomerular endothelium that is separated by a basal lamina from the interdigitated epithelial cells, the so-called podocytes, of the tubular epithelium. The so-called ultrafiltrate has the same composition as plasma, except for the absence of protein. The epithelial cells lining the various segments (proximal tubule, loop of Henle, distal tubule, and collecting duct) of the *renal tubule* have been divided into types and subtypes on the basis of minor differences in histologic structure; there is some evidence that these differences correlate with differences in function, which is either reabsorption or secretion.

Cells of the afferent arteriolar wall and the abutting distal tubule form the *juxtaglomerular apparatus*, site of the formation and release of the enzyme renin (see below). Cells of the distal convoluted tubule and the collecting duct are sensitive to hormones, aldosterone for sodium reabsorption and antidiuretic hormone (also called vasopressin) for water reabsorption (Chapter 10).

The kidneys adjust the amount of secreted water and electrolytes. Including $H+$ in such a way that volume and composition of body fluid, including acid-base balance and blood pH are maintained in homeostasis. Kidneys also excrete end products of metabolism. They have certain endocrine functions: they secrete to the blood renin, which regulates the production of angiotensin and, hence, indirectly influence blood pressure and aldosterone secretion; they activate vitamin D and thereby play a role in Ca^{++} metabolism; they secrete erythropoietin and, thus, maintain the hemoglobin level of the blood (Table 1).

90-year-old with the same body weight and Scr has a creatinine clearance of 50 mg%, a greater than 50% reduction; values in women are usually 10% lower than those in men. The reduction with aging in the urinary output of creatinine, a muscle-specific metabolite, has been interpreted as reflecting the reduction in lean body mass (LBM) (demonstrated by decreased radioactivity, in whole body, of labeled potassium, the major intracellular electrolyte) that occurs with old age. Although creatinine clearance may provide a useful index, recent studies have shown great variation in the rate of decline of renal function. It should be noted that a glomerular filtration rate of 120 mL/min represents 7.2 L/hr or 172.8 L/day whereas normal urine volume is about 1 L/day; thus, under optimal conditions of glomerular function, 99% of the filtrate is reabsorbed. Despite a decreased clearance in the majority of the elderly, in some individuals, no decrease in renal clearance can be detected with advancing age (11).

Creatinine clearance is often measured in elderly individuals not only as a test of renal function but also as a guide for adjusting dosage of administered drugs to Scr and creatinine clearance (Chapter 22). However, a survey of several creatinine-clearance-estimating equations in common clinical use suggests that they are more imprecise and have a narrower range of applicability than hitherto believed (12). Therefore, it is more appropriate to estimate creatinine clearance using an equation that takes into account not only age and Scr but also lean body weight (LBW). A commonly used equation is that developed by Cockcroft and Gault (Equation 1) where the weight used is the individual's LBW; the equation provides a formula for calculating LBW, including for individuals who are overweight (Equation 2) (13,14).

$$\mathrm{CrCl_{men}} = \frac{(140 - \mathrm{age})\mathrm{LBW(kg)}}{72 \times \mathrm{Scr(mg/dL)}}, \quad \text{(Eq. 1)}$$
$$\mathrm{CrCl_{women}} = \mathrm{CrCl_{men}} \times 0.85,$$

$$\mathrm{LBW_{men}} = 50\ \mathrm{kg} + 2.23\ \mathrm{kg/inch\ above\ 5\ feet}, \quad \text{(Eq. 2)}$$
$$\mathrm{LBW_{women}} - 45.5\ \mathrm{kg} + 2.3\ \mathrm{kg/inch\ above\ 5\ feet}.$$

The Cockcroft–Gault equation is useful when calculating doses for drugs that are eliminated by glomerular filtration such as aminoglycosides and vancomycin (antibiotics for the treatment of infections), digoxin (for the treatment of cardiac failure),

TABLE 1 Major Functions of the Kidney

Water and electrolyte regulation
Metabolic products excretion
Hydrogen ion excretion and maintenance of blood pH
Endocrine functions
Renin-angiotensin secretion (blood pressure)
Vitamin D activation (Ca^{++} metabolism)
Erythropoietin secretion (hematopoiesis)

TABLE 2 Tests of Renal Function

Routinely used tests
Urine volume (per 24 hr)
Analysis of urine constituents
Urine concentration and dilution tests
Clearance tests
Complex tests
Radiologic
Isotopic
Ultrasonic
Magnetic resonance spectroscopy and imaging
Tomographic imaging
Biopsies

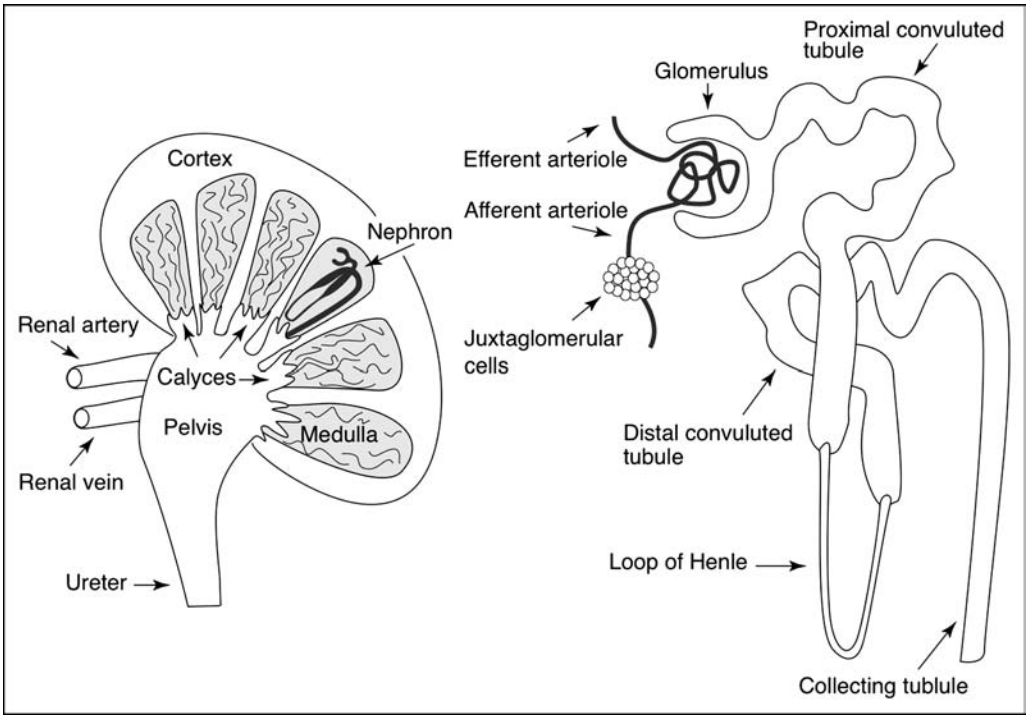

FIGURE 1 Diagram of the kidney (*left*) and of a nephron (*right*).

lithium (a mood-stabilizing drug), and histamine antagonists (Chapter 22).

Drugs that are eliminated by tubular secretion also exhibit decreased excretion with age. This can occasionally be clinically significant for drugs that rely on tubular secretion for elimination (Chapter 22).

Aging-Related Changes in Glomerular Morphology and Function

Characteristics of the glomerular wall and of renal blood flow are the most important factors that affect glomerular function. *In humans, the decrease in glomerular function in old age may be due primarily to a loss or alteration of glomeruli and secondarily to alterations in blood flow.* The age-related decrease in blood flow is often reversible and responsive to improved renal hemodynamics. Irrespective of the primary site of the damage, studies in rats show a 30% incidence of necrotic glomeruli with age caused primarily by oxidative stress (15–17) and/or accumulation of glycation end products (18,19); in contrast, under conditions of restricted food intake (Chapter 23), the incidence is reduced to 2%. In humans, widespread glomerular necrosis is rare: rather, a thickening of the glomerular basement membrane is associated with concomitant biochemical changes such as a progressive decrease (e.g., 3.7% reduction per decade) in some amino acids, which suggests a reduction in collagen (20,21).

Microscopic and ultramicroscopic studies of glomeruli in rats reveal a progressive thickening (from 1300 Å, neonatally, to 4800 Å, at the advanced age of two years) of the basement membrane in some areas (focal or segmental thickening) and collapse (with loss of distinct layers) in others. Podocytes are cells of the glomerular epithelium; they have numerous feet-like projections (pseudopodia) that interdigitate to form filtration slits along the capillary wall. With aging, some of these cells are lost or undergo swelling or atrophy. Proliferation of interstitial collagen leads to progressive sclerosis in rats (22). In humans, atherosclerosis of renal blood vessels is frequent, especially in diabetes mellitus, Type 2, associated with severe microangiopathy that accelerates the onset and increases the severity of the vascular damage (Chapters 13 and 15).

Studies in individuals affected by a congenital loss of urinary proteins have shown that the proteins, *nephrin and podocin, are major components of the kidney filter that keeps vital proteins from escaping from the blood in the urine* (23–25). This filter, also known as the "slit diaphragm," is a zipper-like structure of molecules that forms between the podocytes, wraps around the glomerular capillaries, and prevents leakage of proteins from the blood into the glomerular filtrate. The gene that encodes nephrin is located on chromosome 19, together with other genes similarly involved in the regulation of the kidney filter. Podocin is an integral membrane protein exclusively expressed in glomerular podocytes; mutations of the podocin gene produce an autosomal-recessive nephritic syndrome, associated with proteinuria. Congenital, adult, or, more frequently, old-age mutations of the nephrin gene might predispose one to alterations in the structure and function of the kidney filter with consequent alterations of the glomerular capacity to prevent protein loss.

Aging-Related Changes in Day/Night Urine Excretion (Nocturia)

In the normal adult, urine and electrolyte excretion follow a day/night pattern, with higher levels in the daytime. This pattern may have evolved to permit undisturbed sleep. *In the elderly, associated with changes in sleep pattern (Chapter 7), the rhythm of urine excretion is reversed, with increased water and electrolyte excretion during the night (nocturnal polyuria or nocturia)* (26,27). This shift may have multiple renal causes: decrease in renal concentrating capacity and sodium-conserving ability as well as changes in the function of the renin–angiotensin–aldosterone system. It may also be regulated by extrarenal, neuroendocrine factors (26), such as:

- Alterations of antidiuretic hormone (ADH) receptor expression (Chapter 9) (28)
- Deficiency of ADH production and secretion (29)
- Changes in production and function of the atrial natriuretic peptide (ANP) (30). ANP is secreted by the cardiac atrial muscle cells and stimulates natriuresis (i.e., excretion of sodium in the urine) by increasing sodium glomerular filtration rate

Nocturnal polyuria may lead to urinary incontinence and is more severe in the elderly with Alzheimer's disease. Treatment of nocturnal polyurea with ADH often reduces nocturnal urine production, with improvement in symptoms of frequency, nocturia, and incontinence (26).

Aging-Related Changes in Glomerular Function and Dietary Proteins

Changes with aging in glomerular function do not pose any serious threat to well-being; likewise, renal function may not be seriously compromised with aging, even in octogenarians and older persons (11,31). If, however, intrinsic renal disease or surgical removal of renal tissue or other factors, such as inappropriate diet, add to the glomerular burden of old age, then the course of glomerular sclerosis and impaired glomerular filtration rate may be hastened appreciably (31,32).

Under normal conditions, little protein is filtered in the glomeruli and excreted in the urine. *One manifestation of impairment in glomerular filtration is a greater permeability of glomerular cells with consequent proteinuria, that is, more than the usual trace amounts of protein are present in the urine.* As a consequence of altered glomerular molecular structure, abnormal proteinuria appears in about 25% of male rats at a young age, and its incidence and severity increases progressively with age, although less so in female rats.

High-protein diets make it more difficult to prevent filtration of protein. Diets high in proteins may induce glomerular damage, and conversely, reduction of dietary protein intake reduces this damage. For example, feeding rats a diet low in protein or restricted in total calories (Chapter 23) reduces aging-related proteinuria (Fig. 2). Dietary restriction prolongs the life of laboratory animals, and such extension may involve the beneficial effects of a low-protein diet on renal function (32,33).

In humans, the usual diet offers sustained (three meals a day) rather than intermittent (feast or famine of animals in the wild) intake of protein. Such sustained "excess" would impose rigorous demands on glomerular filtration rate and renal blood flow, thereby contributing to the decline of these functions with aging. It may also be responsible for the inexorable progressiveness of renal functional deficits and the greater incidence of renal pathology in the elderly (34,35). Calorie or protein restriction fairly early in the course of renal disease slows the rate of decline in glomerular filtration. *This is true not only for aging but also for metabolic and renal disorders; this is the case, for example, of obesity, in which diet modification leading to body weight loss reduces the proteinuria often associated with glomerular sclerosis* (Chapter 13).

■ Tubular Function

Although tubular function remains quite efficient in most individuals 65 years and older, *in some elderly persons, the kidney is unable to concentrate urine as well as it does in younger persons; because of this, maintenance of appropriate water and electrolyte metabolism is potentially critical in the elderly,* even in those without overt signs or laboratory tests indicating renal dysfunction (36–38).

Aging-Related Changes in the Ability to Concentrate Urine

The ability to concentrate or dilute urine may be lost gradually, with the result that the elderly individual is unable to cope optimally with either dehydration or water load (36–38). This inability is manifested by stimulation or inhibition of the secretion of the

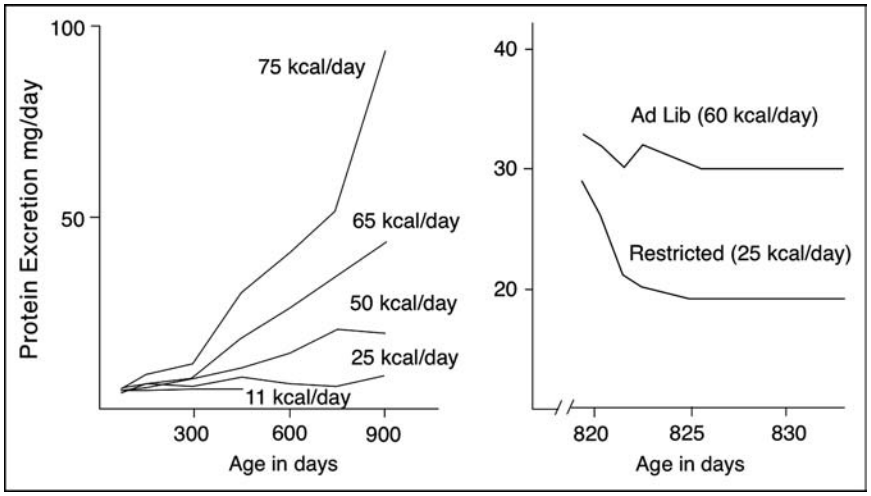

FIGURE 2 Dietary caloric restriction reduces aging-related proteinuria in male rats. (*Left*) Proteinuria is reduced proportionately to the severity of the caloric restriction: severe food restriction (12.5 Kcal/day) initiated at a young age (70 days) abolishes the steep rise in protein excretion with aging (but is not compatible with long life), and a less severe restriction (25 or 50 Kcal/day) markedly inhibits it. (*Right*) This effect of caloric restriction is also observed in older (820 days) rats in which about one week of restriction was sufficient to reduce proteinuria significantly. Thus, rats consuming 75 Kcal of food per day, a really large quantity, show high protein excretion throughout life from 4 mg/day at 70 days, just after sexual maturation, to 88 mg/day at the old age of 900 days. Normal food consumption is more in the range of 50 to 65 Kcal/day and, with this diet, compared to values of 75 Kcal consumption, protein excretion is halved. If food is severely restricted to 12.5 Kcal/day, the rise in proteinuria with age is abolished (19). The same modification is apparent even with short-time food restriction in old animals. When the diet is reduced from 60 to 25 Kcal/day, protein excretion decreases by 40% in one week and continues at this level for the next week, even after discontinuation.

ADH, also known as vasopressin (Chapter 9). Administration of ethanol (alcohol) reduces circulating ADH levels for as long as 120 minutes after administration, in young individuals. In old subjects, ADH levels are reduced immediately after alcohol administration, but they increase thereafter as shown at 120 minutes (Fig. 3). In the young individual, inhibition of ADH secretion is associated with the expected diuresis and increased water clearance. In old individuals, inhibition of ADH secretion occurs early after alcohol administration and then disappears, with diuresis absent or only minimal (39–41).

Likewise, stimulation or inhibition of ADH secretion in response to appropriate stimuli differ in the elderly as compared to the young (Chapter 9). In response to a given osmotic challenge (i.e., administration of hypertonic sodium chloride solution), ADH plasma levels increase more in old subjects than in young. However, in the elderly, the increase in ADH levels is not accompanied by the expected increase in water retention observed in the young (Fig. 3) (39–41).

Identification of the site(s) of the altered function, whether renal or extrarenal, is still unclear. Administration of a standardized dose of ADH shows a decline in the ability of the collecting tubules to perform osmotic work and increase water retention in old individuals as compared to young controls. Further studies suggest that decrements in the ability of cellular membranes of the collecting duct to become more water permeable under ADH influence occur primarily in old individuals with renal infections or hypertension; these decrements should not, therefore, be viewed as the usual accompaniment of aging. *Experiments in aged rats show that decreased responsiveness of collecting tubular epithelium to ADH is the most likely explanation for the impairment of urine-concentrating ability,* whereas reduced secretion and plasma levels of ADH may not play an important role in this impairment.

Whether the defect in renal regulation of water excretion lies in the hypothalamus, the pituitary, or the kidney, there is no doubt about the decreasing ability of the aged kidney to concentrate or dilute urine and the consequent ease with which the elderly person may develop acute or chronic renal failure. As long as the total water intake is adequate—2.5 to 3.0 L/day—renal function will be adequate (Chapter 23). Crises will arise when water intake is diminished due to loneliness, immobility, confusion, fright of incontinence, etc. This may also occur with unnecessary use of diuretics (Chapter 22).

Aging-Related Changes in Electrolyte and Urea Excretion

Failure to concentrate urine may also be related to sodium loss, particularly in the distal nephron. *Elderly persons are more prone to develop hyponatremia (low blood levels of sodium) and hypokalemia (low blood levels of potassium) during diuretic therapy or due to an inadequate diet* (Chapter 23). Body potassium content is less in the elderly than young controls, even when measured in terms of lean (fat-free) body weight. This lower potassium content may be due to renal loss as well as insufficient potassium intake. *Urea, a product of amino acid deamination in the liver, contributes to the establishment of the osmotic gradient in the renal medulla and to the ability to form a concentrated urine in the collecting ducts*. Blood urea may be reduced due to (*i*) diminished protein intake, often present in the elderly, (*ii*) liver disease and insufficient urea production, or (*iii*) abnormal regulation of the urea transporters (42).

■ Renal Disease in the Elderly

The relatively minor impairments of renal function in the healthy elderly are aggravated by

- the increased incidence of renal diseases with advancing age and
- the reduced ability of the kidney to handle the excretion of drugs.

The major renal disorders of the young and middle-aged—acute nephritis, collagen disorders, and malignant hypertension—are uncommon in old age. If they occur, treatment in the old, as in the young, aims at protecting water and electrolyte balance.

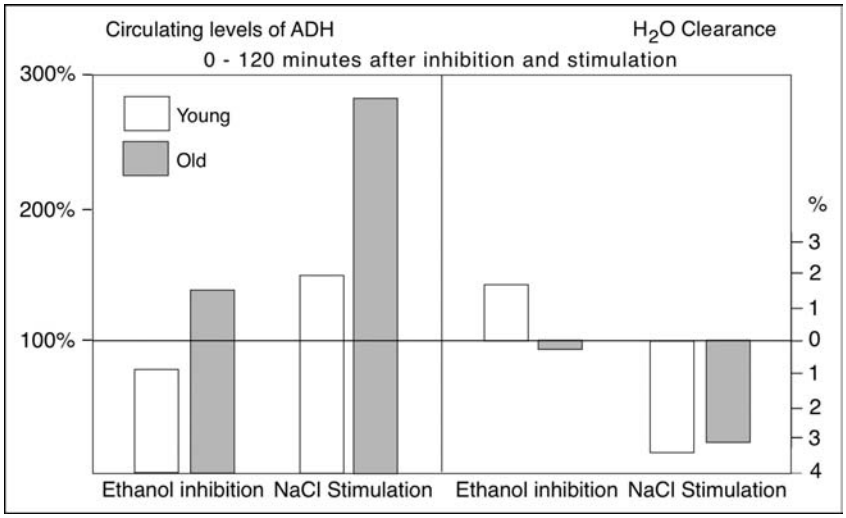

FIGURE 3 Age-related changes in tubular responses to ADH and to hypertonic sodium chloride solution. Ethanol inhibits ADH less efficiently in the elderly than in the young; low ADH levels are associated with increased diuresis to ethanol in the young, but diuresis is minimal or absent in the old. Hypertonic sodium chloride increases ADH more in the elderly than in the young, but the expected increase in water retention is less marked in the elderly. *Abbreviation*: ADH, antidiuretic hormone.

In the aged population, the common problems affecting renal function are related to damage induced by infections, drugs, hypertension, or miscellaneous disorders such as tuberculosis, nephritis, diabetes mellitus, amyloidoses, and collagen disorders (Table 3). If untreated, these disorders may lead, perhaps more easily than in the young and with more life-threatening consequences, to dysfunction and, finally, to renal failure.

■ Failure of Renal Function: Pathogenesis and Management

Renal failure refers to the decline in renal excretion of sufficient severity to result in retention (in blood and extracellular spaces) of nitrogenous waste products, acids, potassium, sodium, and water. Clinically, it is designated as either acute or chronic. Normally, one-third of the nephrons can eliminate all normal waste products from the body and prevent their accumulation in body fluids. When the number of active nephrons falls to 10% to 20% of normal, urinary retention and death follow.

Acute Renal Failure with Aging

The acute form, frequent in the elderly, occurs in a matter of hours or days. Its consequences depend to a great extent on the food and water intake of the individual. With moderate intake, the most important signs are the following:

1. Generalized edema, resulting from salt and water retention
2. Acidosis, resulting from failure to excrete normal acidic products
3. High concentration in the blood of nonprotein nitrogen, especially urea, resulting from failure to excrete metabolic end products (creatinine, uric acid, phenols, etc.)

This condition of failing urine excretion is called uremia; it is characterized by the presence of high concentration of urinary constituents in the blood and the toxic condition produced thereby (Table 4).

The causes of acute renal failure are numerous and are divided into prerenal, renal, and postrenal, depending on whether they are traced to alterations of the kidney or extrarenal alterations (Table 5). *Prerenal causes*, depending on factors unrelated to renal pathology and most likely to be found in the aged, include loss of body fluids (e.g., vomiting, diarrhea); inadequate fluid intake (often associated with the overuse of diuretics and laxatives); and surgical shock or myocardial infarction.

Renal causes, depending on renal pathology are relatively rare in the elderly; they include drug toxicity due to certain antibiotics (sulfonamides, aminoglycosides, amphotericin B); X-ray contrast materials (in a rather dehydrated individual); drug-induced immunologic reactions; infectious diseases: gram-negative bacteremia with shock or peritonitis (Chapter 14); thrombosis and other circulatory alterations due to atherosclerosis (Chapter 15); and intravascular hemolysis (i.e., destruction of red blood cells with liberation of hemoglobin) such as may follow surgical resection of the prostate (see below).

Postrenal causes, depending on factors occurring after normal urine formation, are primarily due to urinary tract obstructions such as those that occur with stones, tumors, or prostatic enlargement (see below).

While the outcome of acute renal failure is generally poor in the elderly, a decrease in mortality from 70% to 50% has been reported in the years 1975 to 1990 and continues to date. A similar trend has been observed in young individuals. *Prevention is of paramount importance: maintenance of an adequate extracellular volume and drug dosages tailored to the degree of glomerular filtration efficiency are essential* (43,44).

Chronic Renal Failure with Aging

Chronic renal failure results from slow progressive and generally irreversible deterioration of renal function due to destruction of nephrons. It is more often a disease of young and middle-aged individuals, as survival is reduced in the major instances, such as in glomerulonephritis, and polycystic disease. *In the elderly, causes of the disease differ from those in younger subjects; they are progressive renal sclerosis (due to atherosclerosis), chronic pyelonephritis (i.e., inflammation of the kidney and pelvis), and obstructive uropathy due to slow but progressive enlargement of the prostate*.

The pathology includes decreased urinary output to 20 to 200 mL/day (oliguria), renal tubular necrosis, scattered basement membrane disruption, presence of protein, red blood cells, epithelial cells, and brown casts in the urine, and signs of uremia (nausea, vomiting diarrhea, hypertension, and others). Management depends on identification of the mechanism that is responsible for the failure. Pivotal measures of management include (*i*) treatment of the underlying cause; (*ii*) monitoring of fluid, electrolytes, and acid–base balance; (*iii*) prevention of infections, and (*iv*) alterations of the diet: not more than 40 g/day protein with sufficient (at least 3000) calories to prevent endogenous catabolism. Other interventions include dialysis treatment and kidney transplantation.

TABLE 3 Common Renal Problems in the Elderly

Renal failure
Impaired drug excretion
Urinary tract infections
Hypertension
Miscellaneous disorders
Tuberculosis
Nephritis
Diabetes, etc.

TABLE 4 Some Signs of Renal Failure

Generalized edema
Acidosis
Increased circulating nonprotein nitrogen (urea)
Increased circulating urinary retention products

TABLE 5 Selected Causes of Acute Renal Failure

Prerenal
Loss of body fluids
Inadequate fluid intake
Surgical shock or myocardial infarction
Renal
Drug toxicity
Immune reactions
Infectious diseases
Thrombosis
Postrenal
Urinary tract obstruction

Dialysis, Kidney Transplantation, and Stem-Cell Therapy in the Elderly

The question often arises as to what extent "heroic interventions" are justifiable and advisable in the elderly (Chapter 3). In the case of renal failure, dialysis and kidney transplantation represent interventive measures, which are widely utilized with considerable success in the young and the adult. Evidence for their rational use with a favorable outcome in the elderly has emerged from the cases treated so far and from animal experimentation. *Clearly, while all contraindications and immediate and long-term risks of these measures must be taken carefully into consideration, as they may be magnified in the elderly, age alone should not deter their appropriate use*. Today, up to one-third of new patients entering dialysis throughout the world are older than 65 years (43,44).

Indication and success of transplantation of organs such as the kidney depend on meeting several criteria such as normal function and competency of the organ to be transplanted and the age (young) and health (good) of the donor. With respect to age, although the use of kidneys from older donors is controversial, a number of data suggest that age alone should not eliminate using older kidney donors when their renal function and tissue matches are good (45–49). Pregnancy puts a strain on kidney function, but a report of a successful pregnancy in a renal transplant recipient with a kidney from a 75-year-old donor supports the view that an old kidney may function normally.

Kidney transplants in older individuals (with kidney from young donors) seem to fare as well as in the young, once the surgical and pharmacologic measures appropriate to the age of the individuals are taken into account. Until the beginning of the 1980s, older patients were considered a high-risk group. Even today, the reality is that only 4% of patients aged 65 to 74 years under treatment for end-stage renal disease receive a renal transplant and that the proportion of transplantation recipients varies with the country.

Efforts to utilize bone marrow stem cells and progenitor cells as therapeutic strategy have demonstrated, so far, the safety of the procedures and have provided some hints of efficacy. However, current preliminary encouraging results await subsequent trials for validation (50).

■ Kidney Susceptibility to Drugs

The kidneys are particularly susceptible to the toxic effects of drugs and other chemical agents because:

1. blood flow (carrying drugs) to the kidney is high: 20% of cardiac output passes through the kidneys;
2. drugs tend to accumulate in the renal medulla as water is removed from the glomerular filtrate; drug accumulation increases when renal function is impaired;
3. reduced hepatic enzyme activity in the elderly increases circulating drug levels and renal toxicity; and
4. incidence of autoimmune disorders increases with aging, with consequent hypersensitivity reactions in the kidney.

The manifestations of renal drug intoxication are not unique to the elderly; they are, however, more frequent and, when they occur, they may be more severe than in the adult (Table 6) (Chapter 22). In the kidney of old individuals, toxic effects are seen with lower doses than in the adult, and the consequences are more dangerous, taking into account the multiplicity of drugs taken by the elderly, the generally long duration of treatment, and the often impaired conditions of the kidney (51–53). A few examples of drug-induced renal damage include

TABLE 6 Drugs and the Aging Kidneys

Questions
Is the drug excreted primarily by the kidney?
How competent are the kidneys?
What are the side effects?
What are the consequences of drug toxicity when the kidney is impaired?
Etiopathology of renal drug toxicity
High renal blood flow
Increased drug concentration and accumulation in kidney
Increased hepatic enzyme inhibition in the elderly
Increased autoimmune disorders in the elderly

1. dehydration-induced uremia due to use of diuretics and laxatives,
2. obstruction of urinary tract due to deposition of crystalline matters such as calcium from excessive administration of vitamin D,
3. vascular lesions produced by thiazide diuretics,
4. glomerular damage by penicillin-like antibiotics,
5. interstitial and tubular damage from radiological contrast media, and
6. necrosis due to analgesics.

■ AGING-RELATED CHANGES IN THE FUNCTION OF THE LOWER URINARY TRACT

The major health problems of the elderly can be easily recalled by listing them under five words—all of them starting with the letter "I."

- *I*nstability
- *I*mmobility
- *I*ncontinence
- *I*mpaired cognition
- *I*atrogenic diseases (induced by the physician) (54)

Of these, one of the most embarrassing and distressing is urinary incontinence. *Urinary incontinence can be defined as the involuntary loss of urine sufficient in amount and frequency to be a social or health problem*. It is most frequent in the elderly; it is stressful not only for those affected but also for their family and caregivers and can play a decisive role in the decision to place an elderly subject in a nursing home (55–57).

Failure of urinary continence often results from alterations in the function of the structures of the lower urinary tract (58,59). Incontinence, therefore, is the major topic discussed in this section. Major structures and functions of the lower urinary tract are presented in Box 2 (Figs. 4 and 5).

Numerous surveys show that incontinence occurs in 10% to 30% of community-dwelling elderly and in 50% to 60% of those living in institutions. Regrettably, such statistics are not accurate inasmuch as people often fail to disclose this condition.

Many consider it inevitable, and many refuse to admit to it. Normal control of bladder and urethral functions are taken for granted by the majority of individuals and, in fact, remain efficient in many individuals well into old age. However, failure of this control is considered a main threat to the welfare of those affected; it conjures up fears of rejection, which are often real and further restrict the social interactions of the elderly. Urinary incontinence may result not only from aging of the urinary tract

BOX 2 *Structures and Functions of the Lower Urinary Tract*

The structures involved in transfer, storage, and excretion of urine are the ureter, the bladder, and the urethra (Fig. 4). These retroperitoneal and pelvic organs are similar in both sexes except for the urethra and associated structures, such as the prostate, which are sex specific.

All these structures consist essentially of smooth muscle lined by mucosa: their contraction depends on an intact nervous system. *The largest is the bladder, which is a smooth-muscle chamber comprising a body, formed of the detrusor muscle with a web-like structure, a neck and, near the neck, the orifices through which the ureters and urethra pass*. When the detrusor muscle contracts, the bladder neck opens and the bladder empties. Release of urine from the bladder is also controlled by internal and external sphincters. The internal sphincter responds to pressure built up in the bladder by stimulation of stretch receptors; the external sphincter, composed of striated muscle, is under voluntary control (Fig. 5).

The main function of these structures is micturition, that is, the process by which the bladder empties when it becomes filled with urine. Basically, micturition may be considered a special reflex, stimulated or inhibited by higher brain centers. Like defecation, it is subject to voluntary facilitation or inhibition. The events of micturition consist of progressive filling of the bladder with urine from the kidneys through the ureters. Once the bladder is filled, there is a tension that initiates a reflex to micturate, or, at least, a conscious desire to urinate.

The innervation of the bladder consists of three components, the two branches of the autonomic nervous system and the somatic nerves. The autonomic fibers, both sympathetic and parasympathetic, act on smooth muscles (detrusor, trigone, and internal sphincter) and the somatic fibers travel to the skeletal (striated) muscle (external sphincter). The act of micturition involves a complex coordination of neural and muscular responses leading to:

1. Relaxation of the internal sphincters to the urethra under sympathetic control and of the external sphincter, under somatic control
2. Constriction of the sphincters of the two ureters under sympathetic control to prevent urine retrograde flow to the kidney
3. Contraction of the detrusor muscle under parasympathetic control

The entire sequence is under voluntary control and can be initiated or inhibited at will. In the absence of inputs from the cerebral cortex and other high brain centers, spinal reflexes may take over control and induce automatic emptying of the bladder whenever the retained volume of urine reaches a critical level. When the voluntary system is active, the desire to urinate becomes apparent after approximately 150 to 300 mL of fluid has collected.

but may also reflect and, as such, may be viewed as an indicator of overall functional decline and harbinger of frailty and death (60–63).

In view of the complexity of the mechanisms regulating micturition (i.e., the excretion of urine), the high prevalence of incontinence in the elderly should not be surprising. Continence depends on a long list of physiologic requirements, several of which undergo changes with aging (Table 7). In addition, the administration of drugs for a variety of ills and their effects, particularly on cognitive functions, may also contribute to incontinence.

■ Causes and Course of Urinary Incontinence in the Elderly

Although urinary incontinence is not an inevitable consequence of aging, certain aging-related changes may contribute to the etiology of incontinence (Table 8). For example, in postmenopausal women, the reduction in estrogen levels

- weakens the tissues around the pelvic floor and bladder outlet,
- decreases the tone of urethral smooth muscle, and
- facilitates the occurrence of atrophic vaginitis (i.e., inflammation of the vagina).

All of these changes contribute to the decrease in urethral contractility and pressure with aging. In elderly men, hypertrophy of the prostate represents one of the major causes of incontinence, as it leads to a decline in urinary outflow rate, increased risk of urine retention, and increased instability of the detrusor muscle (see below). Other causes include delirium, effects of drugs such as anticholinergics (inducing urinary retention) and diuretics (inducing polyuria), and diseases such as infections and diabetes. A mnemonic device to remember the major causes of acute incontinence is the word DRIP, where D stands for delirium, R for urinary retention due to anticholinergic drugs and restricted motility, I for infection, and P for polyuria due to diuretics or diabetes (Fig. 6).

Many of the causes of acute incontinence may be easily diagnosed and reversed. However, when they have been addressed without any significant improvement, the incontinence is considered persistent, and its management is considerably more difficult than the acute form. Persistent urinary incontinence is categorized into four types to which correspond specific etiologies and interventions.

- *Stress incontinence*, due to increased intra-abdominal pressure as in coughing and laughing, is caused by weakness of the pelvic floor musculature and is common in multiparous (having had two or more pregnancies) women.
- *Urge incontinence*, due to the inability in delaying voiding after perception of bladder fullness, may be caused by mild outflow obstruction or by a central nervous system disorder such as stroke.

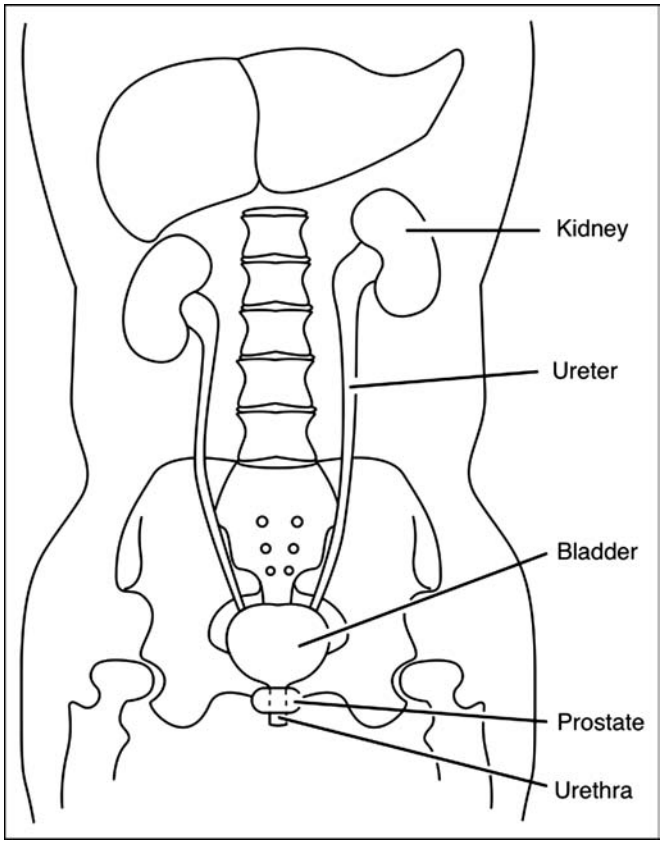

FIGURE 4 Diagram of the major components of the urinary tract in man.

- *Overflow incontinence*, due to a leakage of urine resulting from mechanical forces of an overdistended, a-contractile (i.e., failing to contract) bladder, may be due to prostatic obstruction or neurogenic disturbances.
- *Functional incontinence*, due to inability or psychologic unwillingness to get to the toilet, is caused by cognitive deficits, psychologic conditions, or unavailability of caretakers.

■ Management of Incontinence

Management varies with the type of incontinence (Table 9). It is based on the differential diagnosis of incontinence types not only in terms of urinary symptoms but also in terms of a complete history and physical examination, including evaluation of the mental status, physical activity, and ambulation (walking). An abdominal exam should look for signs of bladder distension; a rectal exam for prostate size, rectal sphincter tone, and the presence of fecal impaction; a pelvic exam for uterine prolapse or atrophic vaginitis. Laboratory tests should include a urinalysis, a urine culture, and measurement of postvoid residual bladder urine volume (with the use of an indwelling catheter). Treatment methods include: (*i*) strengthening of abdominal and pelvic muscles by specific exercises, (*ii*) administration of estrogen, and surgery for stress incontinence; (*iii*) administration of bladder relaxants for urge incontinence; (*iv*) intermittent catheterization or removal of the prostate for overflow incontinence; (*v*) for functional incontinence, habit training, scheduled toileting, undergarment devices, and indwelling catheterization (taking into account the risks involved, such as danger of infection) (55,63–65). As indicated above, urinary incontinence is a widespread problem among the elderly. And yet, despite its prevalence and its serious consequences for those affected, it receives little to no attention because of social taboos and ignorance of its physiologic and pathologic causes. Awareness of the condition is important, as even advanced cases may respond well to treatment. *Urinary incontinence in the elderly can and must be treated in each and every case*. Specific questioning as to urinary continence must be carried out in all routine evaluations of elderly patients and, in this, as in all disorders, an understanding of the physiopathology is essential for a rational diagnostic evaluation and treatment.

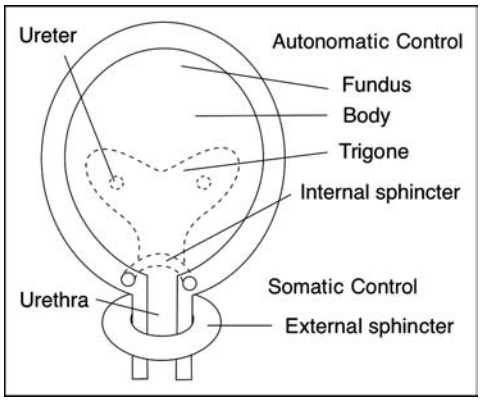

FIGURE 5 Schematic of urinary bladder with structures under autonomic and somatic nervous control.

TABLE 7 Physiologic Requirements for Continence

Motivation to be continent
Adequate cognitive function
Adequate mobility and dexterity
Normal lower urinary tract function
Storage
No involuntary bladder contractions
Appropriate bladder sensation
Closed bladder outlet
Low-pressure accommodation of urine
Emptying
Normal bladder contraction
Lack of anatomic obstruction
Coordinated sphincter relaxation and bladder contraction
Absence of environmental or iatrogenic barriers

■ Other Bladder Dysfunctions in Old Age

In addition to urinary incontinence, other major dysfunctions of the lower urinary tract are listed in Table 3. Some of these

TABLE 8 Age-Related Changes Contributing to Incontinence

In females
Estrogen deficiency
Weak pelvic floor and bladder outlet
Decreased urethral muscle tone
Atrophic vaginitis
In males
Increased prostatic size
Impaired urinary flow
Urinary retention
Detrusor muscle instability

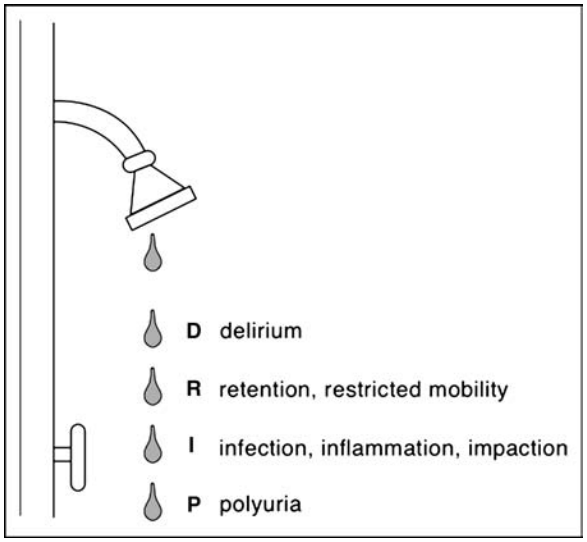

FIGURE 6 Mnemonic device for the major causes of acute urinary incontinence.

dysfunctions are amenable to pharmacologic interventions, especially infections, efficiently treatable with antimicrobial agents; however, high urinary antibody levels are often associated with decreased survival rates (66). Urinary retention responds to treatment with cholinergic agonists that facilitate bladder emptying by stimulating contraction of the detrusor muscle and relaxation of the internal sphincters. The characteristics of neural control of micturition are presented in Table 10. While a number of drugs may act on other parasympathetic targets and, therefore, induce side effects, they are preferred to bladder catheterization with its attendant danger of infection.

In addition to being the target of specific drugs administered for therapeutic effects, the lower urinary tract, particularly the bladder, may bear the consequences or side effects of many prescribed as well as over-the-counter drugs (Chapter 22). Thus, the bladder may become a victim of polypharmacy (51–53). Drugs in this category include decongestants, antihistamines, antidiarrheals, antipsychotics (e.g., phenothiazines), and antidepressants. Even if taken as prescribed, these drugs may add up to increasing toxicity. Most of these substances have some autonomic activity. The greater number has anticholinergic actions, that is, they block the parasympathetic responses: for example, over-the-counter sleeping, asthma, and antidiarrheal medicines contain the "belladonna alkaloids" such as atropine or scopolamine or synthetic substitutes. They block the parasympathetic responses, inhibit micturition, and induce a degree of urinary retention.

Sympathomimetics (mimicking the actions of the sympathetic branch of the autonomic nervous system, Chapter 9), such as some decongestants and α-adrenergic blockers, relax the detrusor muscle, constrict the sphincters, and promote urine retention. Some of the α-adrenergic blockers, extensively utilized as antihypertensive drugs, have some additional side effects, distressing in the older male, for they also prevent contraction of the vas deferens and inhibit ejaculation.

TABLE 9 Management of Urinary Incontinence

Type	Management
Stress	Exercises α-adrenergic agonists Estrogen Surgery
Urge	Bladder relaxants Surgery
Overflow	α-adrenergic antagonists Catheterization
Functional	Habit training Scheduled toileting Hygienic devices

TABLE 10 Neural Control of Micturition

Muscle (type)	Parasympathetic nerves (cholinergic)	Sympathetic nerves (adrenergic)[a]	Somatic nerves
Detrusor (smooth muscle)	Contraction +++	Relaxation +	No effect
Internal sphincter (smooth muscle)	No effect	Contraction ++	No effect
External sphincter (striated muscle)	No effect	No effect	Relaxation ++

Note: Number of "+"s indicates strength of relaxation or contraction.
[a] In the male, adrenergic stimulation of smooth-muscle sphincter causes contraction to prevent retrograde flow of semen into the urinary bladder at ejaculation; the same stimulation relaxes the detrusor muscle to prevent coincidental contraction of the muscle during ejaculation.

■ BODY COMPOSITION, WATER AND ELECTROLYTE DISTRIBUTION, AND ACID-BASE BALANCE

The kidney of the normal, healthy, older individual is capable of maintaining water and electrolyte distribution and acid–base balance within homeostatic limits, despite changes in body composition. In old individuals as compared to young, fat-free body weight and lean body mass (LBM) as well as body mineral are reduced, in contrast to body fat, which increases (Chapters 13 and 20). These age differences carry over to sex differences: women show a greater increase in total body weight due to increased body fat, whereas men maintain their body weight. Maintenance of body weight is due to reciprocal changes in LBM (decreased) and body fat (increased). Some gross comparisons in "reference man" between 25 and 70 years show the following percent changes: at age 25, fat 14%, water 61%, cell solids 19%, and bone mineral 6% and at age 70, 30%, 53%, 12%, and 5%, respectively.

Aging-related changes in body composition may vary depending on variability among individuals or on methodology. For example, gain in body fat often increases until the age of 60 years but declines thereafter and, at all ages, depends on a number of variables, among which the degree of physical activity is critical (Chapter 24). In contrast, lean body mass (LBM) or body cell mass (as measured by body density through water displacement or helium dilution) continues to decrease with aging, more in males than in females. Some studies show a 3.6% decrease per decade from age 30 to 70 and, thereafter, 9% per decade. LBM as well as nonfat mass (measured by total body potassium) show an early decrease in potassium with aging; but, after 70 years of age, potassium decrease is more

pronounced than that of LBM. At older ages, tissues that are low in potassium and have a lower metabolic rate replace, in part, the lost lean tissue. An alternative explanation with respect to regression of metabolic rate with aging is that the oxygen uptake of cells in old individuals is not significantly different from the young; rather, there are fewer functioning cells (3).

Total body water is diminished with age (36–41). Extracellular water remains unchanged, whereas intracellular water decreases. The reduction in total body and intracellular water in the absence of change in extracellular water can be taken as further support for a loss of functioning cells (LBM) with increasing age (66).

Under normal physiologic conditions and in young adult individuals, acid–base balance is maintained by renal excretion of hydrogen ions (eliminated as acids or ammonia); these ions are generated during metabolism of dietary protein and other metabolic processes. A disturbance in systemic pH (taken as an index of H+ ions in a solution) with an excess or loss of acid or base shifts body buffers and promptly stimulates respiratory excretion of carbon dioxide; such adjustments correct the pH until it can be stabilized by the appropriate changes in renal excretion of acids. Healthy adults, young and old, manifest a low-grade diet-dependent metabolic acidosis; in some old individuals, the severity of acidosis may increase significantly due to diet, medications, or disease as well as the inability of the kidney to increase the hydrogen ion excretion to match the increased metabolic acidosis (3,67).

■ THE FUNCTION OF THE PROSTATE

Joyce Leary

The prostate gland is a secondary sex organ of the male reproductive system (Box 3) (Chapter 11). During ejaculation, the prostate gland secretes stored prostatic fluid into the urethra; this fluid contributes one-third of the seminal fluid volume. Prostatic fluid consists of water, zinc, citric acid, acid phosphatase, fibrolysin, prostate-specific antigen (PSA), prostaglandins, and proteases. Prostatic enzymes liquefy ejaculated semen and neutralize the acidic vaginal environment to facilitate fertilization (Table 11).

Approximately the size of an almond at birth, the prostate gland does not resume growth until puberty when testosterone levels in the male rise dramatically (Box 3, Fig. 7). Growth, differentiation, and secretion of prostatic fluid depend on the presence of testosterone and dihydrotestosterone (DHT). Thus, *the prostate is said to be androgen dependent*. The amount of testosterone synthesized in the testes is regulated via a feedback loop with the hypothalamus and anterior pituitary gland (Chapter 11). The hypothalamus releases gonadotropin-releasing hormone (GnRH), which signals the anterior pituitary to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH). In the testes, FSH induces upregulation of LH receptors on testicular Leydig cells. LH then acts on Leydig cells to induce testosterone production. Finally, testosterone negatively feeds back to both the hypothalamus and the anterior pituitary to inhibit the release of GnRH, LH, and FSH. In the prostate, DHT is made from testosterone by the enzyme 5 α-reductase. DHT promotes epithelial growth, differentiation, and secretion (Table 12).

During puberty, the prostate grows approximately to the size of a walnut. Once adulthood is reached, prostate growth ceases, but prostatic fluid continues to be produced. With age, the outer zones of the prostate progressively atrophy while the inner zones begin to grow again. *Unlike any other organ, the prostate grows until death* (Table 13) (68).

■ THE PROSTATE AND URINARY FUNCTION

Because the prostate surrounds the neck of the bladder and the urethra, any pathology of the prostate gland can contribute to urinary dysfunction. The three most significant pathologic conditions affecting the prostate gland all have the potential to cause urinary dysfunction.

- Prostatitis
- Benign prostatic hyperplasia (BPH)
- Prostate cancer

Prostatitis is an inflammation or infection of the prostate and is the only one of these three conditions that is not associated with aging, occurring most frequently in men between the ages of 20 and 40. In contrast, BPH and prostate cancer occur most often in men over 50. While prostatitis is an inflammatory process, BPH and prostate cancer involve cellular proliferation.

BOX 3 *Anatomy and Histology of the Prostate*

The prostate is divided into zones by the traversing urethra: the posterior glandular zone and the anterior fibromuscular stroma. The posterior zone is further divided into four zones: the transition zone, central zone, peripheral zone, and periurethral zone. The transition zone surrounds the proximal prostatic urethra and the central zone surrounds the ejaculatory ducts. The peripheral zone surrounds the distal prostatic urethra, makes up the bulk of prostatic volume, and is the most common site of prostate cancer. Finally, the small periurethral zone is embedded in periurethral smooth muscle and is the most common site of benign prostatic hyperplasia (Fig. 7). Although these zones are not clearly separated from one another anatomically, they are useful because pathologic conditions of the prostate have a predilection for occurring in specific zones. The prostate is an encapsulated organ on its posterior and lateral sides, but anteriorly and apically, the anterior fibromuscular stroma makes up the outermost portion of the organ (68–71).

Histologically, the prostate gland is made up of branched tubuloalveolar glands arranged concentrically around the urethra. The secretory epithelium of the prostate gland is heterogeneous, exhibiting cuboidal, columnar, and pseudostratified cells and secretes prostatic fluid. The stroma of the prostate gland is composed mainly of dense collagen, fibroblasts, smooth muscle, and immune cells. Innervation of the prostate is autonomic, with both sympathetic and parasympathetic components. During ejaculation, the stored secretory products of the prostate epithelial cells are expelled into the prostatic urethra.

TABLE 11 Prostatic Fluid

A slightly alkaline fluid that increases sperm motility and aids in fertilization by neutralizing acidic secretions of the vas deferens and vagina
Major components:
Water
Zinc
Citric acid
Prostaglandins
Acid phosphatase
PSA
Other proteases

Abbreviation: PSA, prostate-specific antigen.

■ BENIGN PROSTATIC HYPERPLASIA

With age (around 45 years), the inner zones of the prostate begin to proliferate. These late changes of the inner prostate transitional and periurethral zones can lead to enlargement of the prostate. If the enlargement is significant enough, the urethra can become constricted. Men with significant BPH often complain of

- increased frequency of urination,
- difficulty starting and stopping urination,
- weak urine stream,
- feeling that the bladder has not emptied completely,
- urinary retention (Fig. 8), and
- painful urination (72).

In severe cases, the inability to void the bladder can lead to distention and hypertrophy of the bladder, bladder infections, kidney infections, and even kidney failure (73). While 80% of males have histologic evidence of a hyperplastic prostate by age 80, only up to 25% of these men require treatment for the condition (74).

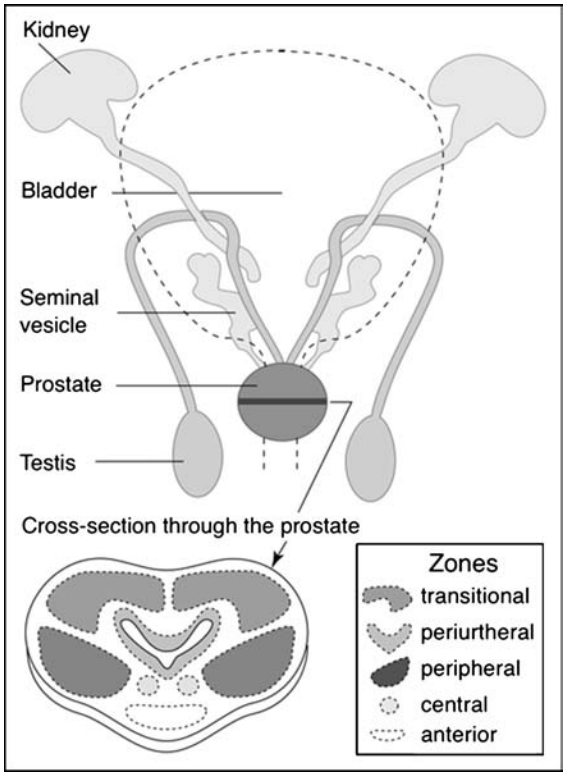

FIGURE 7 Diagrammatic location and structure of the prostate with major zones.

TABLE 12 The Prostate and Testosterone

The healthy prostate is dependent on androgens for growth
In the prostate: testosterone ⟶ DHT
The enzyme catalyzing this reaction is 5 α-reductase
DHT stimulates growth of the prostate

Abbreviation: DHT, dihydrotestosterone.

■ Etiology

A specific etiology for BPH has been difficult to identify. However, it is clear that two important factors must be present for BPH to develop.

1. Men with BPH are over the age of 40.
2. Men with BPH always have testosterone-producing testes.

Studies have shown that castration results in a decrease in prostate size (75), and androgen ablation therapy has been shown to cause a decrease in number and a shrinkage of luminal epithelial cells in the prostate (76). Recall that the prostatic epithelial cells are dependent on the androgen DHT for growth. A wide variety of interacting intrinsic and extrinsic factors may be responsible for abnormal prostate growth. Intrinsic factors (Table 14) include:

- Proliferation of stromal elements
- Fibroblasts
- Smooth-muscle cells
- Extracellular matrix proteins, hereditary predisposition

Extrinsic factors include:

- Dietary factors
- Environmental toxins
- Endocrine factors (77,78)

With age, rising estrogen levels in males may act synergistically with androgens by inducing transcription of the androgen receptor (79). Although the prevalence of BPH does not differ racially (74), some studies suggest African-American men often require treatment at younger ages than men of other racial groups (80). Moreover, men of Asian ancestry appear less likely to undergo surgery for BPH as compared with white men, which may reflect a reduced disease severity or higher tolerance for symptoms (81). In addition, the effects of diet on BPH are evidenced by the fact that BPH is not seen in Asian countries to the same degree as it is seen in the United States. However, on entering the United States, Asian immigrants develop evidence of BPH mirroring that of the American population (82).

■ Treatment

The first strategy for BPH treatment is observation, in which the patient is monitored by his physician but receives no other

TABLE 13 Normal Aging of the Prostate

After age 40
Outer regions
Atrophy of smooth muscle and proliferation of connective tissue
Flattening of secretory epithelium
Inner region
Increase in the number of cells present (hyperplasia)
After age 60
Slower, but more uniform changes
Accumulation of prostate concretions

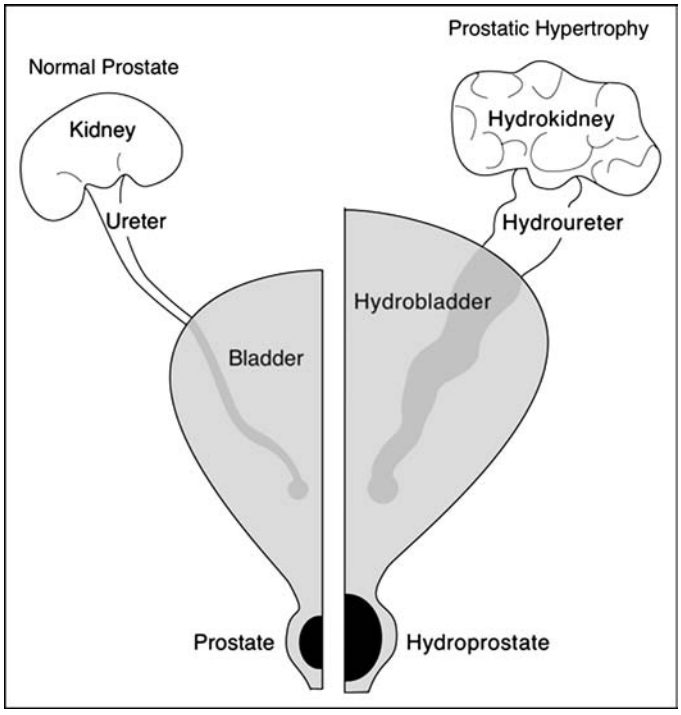

FIGURE 8 Consequences of prostate hypertrophy. The enlarged prostate constricts the urethra with consequent difficulties in bladder emptying and the occurrence of retrograde filling of the ureters and the renal pelvis (hydronephrosis).

treatment (83). Simple observation is undertaken if the patient's symptoms are minimal. Beyond this, 5 α-reductase inhibitors and α_1-adrenergic antagonists are drugs of choice for minimizing the symptoms of BPH. 5 α-reductase inhibitors block the conversion of testosterone to DHT within the prostate, thereby limiting further growth of the gland. α-adrenergic antagonists inhibit the contraction of smooth muscle within the prostate, thereby diminishing the strangulation of the urethra by the prostate. Studies examining these two main pharmacologic therapies for BPH have revealed the heterogeneity of the condition. The variable effectiveness of these drugs in different patients suggests there may be two distinct forms of BPH, one dominated by epithelial growth and the other by smooth-muscle proliferation. In all cases, 5 α-reductase inhibitors are ideal for slowing growth of the prostate. In patients with a high degree of smooth-muscle overgrowth, however, α_1-antagonist drugs can further reduce constriction of the urethra through relaxation of prostatic and nonprostatic smooth muscle (84–88). *It is also important to note that BPH patients with a high degree of smooth-muscle proliferation may exhibit symptoms of BPH without a significant increase in prostate volume* (89). *This finding can be explained by the fact that smooth-muscle proliferation may not add volume but may still have the potential to strangulate the urethra via contraction*. Excessive epithelial and stromal proliferation, on the other hand, will inevitably result in a large prostate.

When observation and drug treatment are not successful, surgical procedures may be required to reduce symptoms. *Surgery to correct BPH in men over 65 years is a leading cause of surgical intervention, second only to cataract surgery* (73). Accessing the prostate via the urethra, incisions can be made in the prostate or the inner portion of the prostate can be removed, reducing urethral constriction. Prostatectomy is another common procedure in which the entire prostate is surgically removed (83). Other possible treatments include androgen suppression, thermotherapy, and high-intensity focused ultrasound.

Recently, some specialists have begun to study the potential use of the PSA test as a surrogate marker for the presence of BPH. *PSA is a product of the prostate gland that contributes to seminal fluid and helps anticoagulate semen following ejaculation. Normally, a small amount of PSA is found in the blood, usually less than 4 ng/mL* (90). The amount of PSA in the blood varies linearly with the prostate volume (91). PSA is, therefore, elevated in patients with BPH and could be used as a marker for the likelihood that the patient will develop symptoms of BPH in the future (92,93), and eventually prostate cancer (see below). Some recommend treating asymptomatic patients with 5 α-reductase inhibitors prior to the develop of symptoms in the hope that preventing growth stimulation of the gland by DHT will prevent development of symptomatic BPH.

TABLE 14 Synopsis of BPH Characteristics

Caused by growth of the prostate from about age 45 until death
Affects 50% of men > 50 yr old
Affects 95% of men > 70 yr old
Clinical symptoms due to obstruction of the urethra are present in up to 25% of men with histologic evidence of BPH
BPH tissue resembles normal prostate tissue with increased amounts of smooth muscle, glandular, and/or stromal components
An enlarged prostate can strangle the urethra
BPH is not found in men who have been castrated or men who lack 5 α-reductase

Abbreviation: BPH, benign prostatic hyperplasia.

■ PROSTATE CANCER

Prostate cancer is the second most common cancer diagnosed in men, the second most common cause of cancer death, and the fifth overall cause of death in men over age 45 (94). As men age, their risk of developing prostate cancer increases: 75% of diagnoses are made in men over 75 years of age. While up to 30% of men have microscopic evidence of prostate cancer when they die, only about 10% to 16% (one in six) are diagnosed with the disease during life (94,95). *In most cases, prostate cancer is a slow-growing cancer compared to other forms of cancer.* The lifetime risk of dying from prostate cancer is about 3% (94). For these reasons, it has been said that men die with prostate cancer far more frequently than they die of prostate cancer. In most cases, prostate cancer is an adenocarcinoma that arises from the glandular epithelium, the secretory cells of the gland. Approximately 80% of cases are located in the posterior peripheral zone.

■ Risk Factors for Prostate Cancer

The incidence of prostate cancer differs racially. African-Americans show the world's highest incidence in prostate cancer and tend to be diagnosed with more advanced stage disease than do white or Hispanic men (94). Conversely, is much more rare, however, for Asian-Americans to develop prostate cancer. Moreover, prostate cancer is more common in white men than in Hispanic, Asian, Pacific Islander, and Native American men (94). Racial differences in prostate cancer incidence have helped motivate research on genetic contributions to its etiology.

Racial differences in prostate cancer incidence may be explained in part by differences in the androgen receptor gene. A region of CAG repeat sequences in the gene varies in number from person to person. On average, African-Americans show

the fewest number of CAG repeats in the gene, while Asians have the highest number. Activity of the androgen receptor has been shown to be inversely correlated with the number of CAG repeat sequences in the gene. Thus, Asians have more CAG repeats, less androgen receptor activity, and a lower incidence of prostate cancer (96). These findings suggest that an increase in androgen effect is necessary for the development and progression of prostate cancer (96). Indeed, the majority of prostate cancers are androgen dependent or at least androgen promoted. In advanced stages, however, genetic alterations allow prostate cancer to become androgen independent. The development of androgen independence appears to be due to an accumulation of mutations in the androgen receptor gene, preventing the receptor from functioning normally within cancer cells (97,98). Once a state of androgen independence occurs, treatment and management become increasingly difficult.

Another genetic predisposition is exemplified by the 10-fold increase in cancer risk found in men with three affected relatives, and several alleles associated with familial clustering have been located (96,99). In addition, studies have shown that certain genetic alterations are common on the path to malignancy. The loss of tumor suppressor genes (including *p53* and *Rb*), and the amplification of oncogenes (including *MYC* seen in a variety of other tumors and *HER2/neu* seen in breast cancer) give prostate cells the ability to proliferate and avoid apoptosis.

Immigration studies have suggested that diet may play a role in the development of prostate cancer. Asian immigrants to the United States experience an increase in incidence of prostate cancer, but the rate of cancer development in these populations remains lower than that for Native Americans. Such evidence argues against genetics as the only influence. Studies have linked high consumption of animal fat to prostate cancer (95), and higher testosterone levels in men who eat animal fat may be a mechanism (100). The evidence for these risk factors and protective agents is controversial, and thus far, none is as consistently associated with development of cancer as is genetics.

A list of environmental risk factors and potential beneficial factors associated with prostate cancer is presented in Table 15. The evidence for these risk factors and protective agents is controversial, and thus far, none is as consistently associated with development of cancer as is genetics.

■ Diagnosis and Treatment

Rarely, prostate cancer is detected after a subject complains of symptoms. Symptoms of prostate cancer can be similar to symptoms of BPH. More often, prostate cancer is suspected after a patient's digital rectal exam (DRE) is found to be abnormal and/or after an elevated serum level of PSA has been detected (see below). The patient then undergoes a biopsy. Twenty percent of prostate biopsies will show cancerous-appearing glandular tissue (94). Biopsy can miss small prostate cancer, and if a patient has a persistently high or rising PSA, he may require repeat biopsy (101).

Cancerous changes in prostate glandular tissue include a missing outer basal layer of cells, or large, vacuolated nuclei with one or more nucleolus (73). Pathologists grade prostate cancer using Gleason's scoring system, wherein the most normal-looking tissue obtains a score of 1, and the most cancerous-looking tissue obtains a score of 5. Because biopsies are rarely uniform in structure, a score is given to the predominant pattern visible, and a second score is given to the second most predominant pattern. These two scores are added together to yield the Gleason score, which can thus be between 2, closest to normal, and 10, most cancerous. Thus far, the Gleason score is the most significant prognostic factor for prostate cancer morbidity and mortality (95).

Once the diagnosis of prostate cancer has been made, there are multiple treatment options. The appropriate option(s) for an individual depends on his life expectancy, overall health status, personal preferences, size of his prostate, and extent of the disease. Treatments include

- watchful waiting,
- surgical procedures to remove prostate tissue,
- radiation therapy,
- hormonal therapy, and
- cryotherapy (freezing the prostatic cells).

Young men are often encouraged to pursue aggressive therapies such as radical prostatectomy, a complete removal of the prostate, so as to guarantee a normal life span if possible. On the other hand, the slow-growing nature of most prostate cancers allows men over 70 to focus less on long-term effects and outcome, so therapies like watchful waiting may be more realistically considered. If the cancer is fully contained within the prostatic capsule, surgery can often eradicate it in its entirety. However, if the cancer has spread beyond the capsule or metastasized to other areas, surgery is usually not a tenable option, leaving radiation and hormonal therapies as the most effective treatments. Researchers continue looking for new prostate cancer therapies. Gene therapy, vaccinations, and new drugs may be on the horizon (102).

■ PSA: the Controversy Over Screening for Prostate Cancer

As mentioned above, *PSA is one of the products of the prostate gland that contributes to seminal fluid. PSA is an enzyme that helps anticoagulate semen following ejaculation*. Normally, a small amount of PSA is found in the blood, usually less than 4 ng/mL (90). However, prostatitis, BPH, and especially prostate cancer can lead to elevations in serum PSA levels. In addition, serum PSA levels increase with age, so a normal PSA level for a 70-year-old man might be a high PSA level for a 50-year-old man. Some studies have suggested that PSA may also vary racially, African-American men showing significantly higher levels than white men (103). Other factors that can contribute to an elevated serum PSA include recent ejaculation, finasteride medication (a 5 α-reductase inhibitor usually used for BPH), urinary tract infection, or recent prostate procedure [not including the DRE (104,105)].

While PSA levels can be elevated in this wide variety of conditions, none causes as dramatic a rise as prostate cancer (Box 4). It is suspected that blood levels of PSA rise more

TABLE 15 Possible Risks and Beneficial Factors for Prostate Cancer

Possible risk factors	Possible beneficial factors
Tobacco	Intake of vegetables
Cadmium	Soybeans
Zinc exposure	Omega fatty acids
Vasectomy	Selenium
Vitamin D imbalance	Vitamin E
Sexual activity	Nonsteroid anti-inflammatory agents
History of sexually transmitted infection	
History of prostatitis	

BOX 4 *Prostate Cancer Screening with the PSA test*

In an attempt to improve the specificity of the prostate-specific antigen (PSA) test, researchers are experimenting with variations on the basic PSA test. There are two types of PSA in the bloodstream, protein-bound and unbound (free). *In cancer, there is a higher percentage of bound PSA, whereas in benign prostatic hyperplasia and other conditions, there is a higher percentage of free PSA*. Thus, a free-PSA test would show a reduction in percentage of free PSA with prostate cancer. A more direct measurement of bound PSA is also under investigation. Furthermore, the PSA velocity test will measure the rate of increase with time while the PSA density test will measure PSA in relation to overall prostate volume. These tests have not been validated, have not been shown to be superior to simple PSA testing, and are not routinely in use (105–109).

One of the most controversial topics in medicine surrounds the use of the PSA test to screen for prostate cancer. Since its advent in the early 80s, a surge in prostate cancer screening has led to an increase in diagnosis of the disease, most notably in smaller, earlier-staged tumors (110,111). The PSA test detects more tumors confined to the gland than does the digital rectal exam (112). Proponents of prostate cancer screening argue that finding cancer early allows a wider variety of treatment options and, therefore, will decrease mortality from prostate cancer. However, there is not yet enough evidence to show early detection of prostate cancer increases overall survival (113). This being the case, opponents of widespread screening fear the increase in diagnosis may simply cause unnecessary, anxiety-provoking, and possibly life-altering procedures for men who otherwise might not suffer from their slow-growing, insignificant prostate tumors. In the next few years, some key studies should begin to provide definitive evidence whether a decrease in mortality from prostate cancer has occurred since the widespread use of the PSA test.

Various organizations have issued guidelines for the screening of prostate cancer. The American Cancer Society (114), American Urologic Association (115), and American Medical Association (116) recommend that PSA test be offered annually to men over the age of 50 with a life expectancy of at least 10 years and to men over the age of 40 who are African American or who have a positive family history for the condition. However, the U.S. Preventive Services Task Force (117) and the National Cancer Institute (118) recommend each individual patient and his physician discuss the pros and cons of PSA testing and jointly decide when to screen.

dramatically in cancer than in other conditions because in cancer, barriers to the bloodstream are often damaged, allowing PSA to escape the prostate (106). In 80% of cancer cases, a highly elevated PSA will be found (>4 ng/mL in a man over 60) (107,108). However, the inverse is also true: up to 80% of the time the PSA is elevated, no cancer will be found (109). Thus, PSA screening allows for a high sensitivity for cancer but a low specificity.

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19

The Gastrointestinal Tract and the Liver

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INTRODUCTION

Physiologic changes in the gastrointestinal (GI) function do occur with aging. These aging-related changes, as those that occur in other body systems, have a significant impact on human longevity and well-being in old individuals; hence the commitment of the Future Trends Committee of the American Gastrological Association to strengthen education, research, and medical practice on aging-related GI issues to better serve the growing population of the elderly (1). The current description of GI changes with aging focuses on cellular and molecular mechanisms, leading to functional alterations in secretory activity and motility of the major GI structures (2–5). In the absence of localized disease, function is usually maintained in line with requirements (6,7). Disorders and diseases, however, become more common with advancing age and involve all levels of the GI tract, starting with the mouth and extending to the rectum, anus, and pelvic floor musculature (Fig. 1) (8–11). In geriatric clinics, about 20% of all patients have significant GI symptoms and morbidity from GI diseases such as cancer of the colon, second only in incidence and mortality to lung cancer (12,13).

This chapter will present, first (see section entitled Aging-Related Changes of the Teeth, the Stomach, and the Intestine), aging-related changes in the major GI structures, the mouth, stomach and intestine, followed by a synopsis of the aging exocrine pancreas (see section entitled Aging-Related Changes of the Exocrine Pancreas) and of the aging liver (see section entitled Aging-Related Changes of the Liver). A brief discussion of the senses of smell and taste, because of their close association with the GI tract, will be presented in the section entitled Aging-Related Changes of the Senses of Smell and Taste.

The major function of the GI system is to provide the organism with nutritive substances, vitamins, minerals, and fluids (Chapter 23). This function is achieved by a series of chemical and mechanical processes involving digestion, storage or propulsion of food, absorption of water and nutrients, and transfer of nutrients to blood and tissues or excretion of unabsorbed components (Table 1). Secretion of hormones and immune activity represent other key functions.

As often stated in this textbook, the elderly constitute a heterogeneous group of individuals in many important aspects, including nutrition and GI function. This is true in relation not only to age but also to degree of health and disease, degree of physical activity as well as psychological and socioeconomic characteristics. Thus, "young elderly" (aged 65–75 years) should be distinguished from "old elderly" (over 75 years) with respect to nutritional needs. In the past, persons "aged 51 years and older" were classified together and, as aging progressed, the many individual differences were often disregarded. Current recommendations for "the dietary reference intakes of essential dietary constituents" have begun to take into consideration ages and sex differences: they distinguish males from females and separate the 51- to 70-year-old

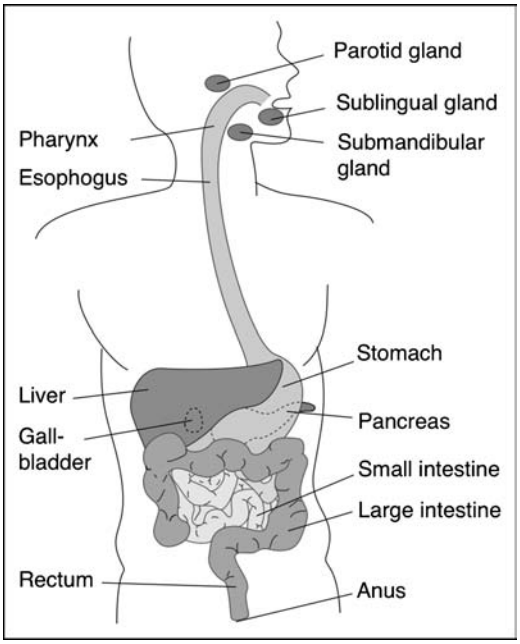

FIGURE 1 Schematic representation of the gastrointestinal tract, with the liver and pancreas.

TABLE 1 Major Functions of the GI System

The major goals of the GI system are:
- To provide the organism with food, vitamins, minerals, and fluids
- To ensure the absorption of nutrients in the intestine, their transfer into the blood and from the blood to the tissues
- To facilitate the transit of the GI contents along the GI tract and the removal of waste metabolic products

These functions are achieved through the following four key processes:
- *Digestion*: chemical (enzymes) and mechanical (teeth and muscles) breakdown of foods into small units that can be absorbed through the intestinal epithelium
- *Absorption*: active or passive transfer of substances from the GI tract to the blood and extracellular spaces
- *Motility*: circular and longitudinal smooth-muscle contraction and relaxation (peristaltic movements) regulate digestion and propel GI content along the tract
- *Secretion*: synthesis and release of hormones, enzymes, chemical mediators, mucus, intrinsic factors to promote food digestion absorption and elimination

Abbreviation: GI, gastrointestinal.

persons from those 70 years of age and older; centenarians represent a rapidly growing group and are also studied separately (Chapter 23) (1).

Of the two major appendices of the GI tract, the liver and to a lesser extent, the pancreas, both with endocrine and with exocrine functions, appear to be affected by aging (8,9). Given the numerous liver functions, alterations with aging have widespread repercussions on the well-being of the entire organism. However, in the liver as in the whole organism, not all functions are affected simultaneously or with equal severity. The bile, produced in the liver for optimal digestion and absorption of lipids, is important at all ages and is particularly crucial in the elderly whose poor diet may be deficient in several essential elements such as lipid-soluble vitamins (Chapter 23); *bile formation remains quite stable in healthy individuals well into old age.* Another important hepatic function, the detoxification of many (therapeutic and recreational) drugs, is progressively restricted with advancing age, and this restriction contributes, with the increased use of drugs (polypharmacy), to the greater susceptibility of the elderly to the potential toxicity of excessive or incorrect medications (9) (Chapter 22).

In the pancreas, the changes with aging of endocrine functions have been discussed in Chapter 13. Changes with aging of the exocrine functions that involve powerful protein-splitting (proteolytic) enzymes may lead to impaired digestion and absorption (11).

Regulation of GI function with aging depends not only on the type of diet but also on intrinsic factors such as the nervous system, hormones, and local chemical mediators that influence GI growth, secretory activity, and motility. The significant role of the immune system in preventing infections to which the GI tract is easily exposed throughout its length was overlooked until the recent discovery of the role of the bacillus, *Helicobacter pylori*, in diseases of the stomach (14). Until then, the GI tract was considered practically sterile. However, *recent studies show that of the 30,000 genes encoded in the human genome, more than 1000 derive from bacterial species living in the GI tract (15). For example, one-third to one-half of the human population carries H. pylori, and once infected, most persons remain infected for decades, if not for life. H. pylori* infection is associated with two major inflammatory processes, gastritis and peptic ulcer disease. Gastritis is so common, especially among the elderly, that it was thought to represent a characteristic of the aging stomach. We now know that eradication of *H. pylori* from the stomach results in clearance of the gastritis (16).

With respect to the influence of the diet, changes in sensory function with aging may result in depressed taste and smell. *The decline in olfactory and gustatory sensory functions negatively affects eating behaviors, resulting in less consumption of food, overall decline in health state, and decreased enjoyment of life (see below).*

Although, in this chapter, the focus will be on how late and how well GI digestion and absorption are retained, it must be kept in mind that limiting food intake may have beneficial effects. Since early studies, more than 60 years ago, evidence has been accumulating steadily that a long-term dietary reduction of caloric intake, or of specific food constituents, will markedly prolong life and improve health in old age in rodents; other studies are in progress in primates and other species with similar beneficial outcomes (Chapter 23). The earlier in life the restriction begins, the more successful is the subsequent prolongation of healthy life (Chapter 23). It will always remain unthinkable to perform such restrictive experiments in humans. However, encouragement to limit caloric intake for short periods of time and to prevent or treat obesity is easily justified (Chapters 13 and 23).

■ AGING-RELATED CHANGES OF THE TEETH, THE STOMACH, AND THE INTESTINE

■ Teeth, Gums, and Oral Mucosa

In the mouth, food is mixed with saliva, chewed, and propelled into the esophagus. *Chewing (mastication), a matter of breaking down large food particles is a function of the teeth. The saliva contains the digestive enzyme ptyalin, which plays a minor role in starch digestion (17). It also contains the glycoprotein, mucin, which lubricates the food and facilitates its passage through the esophagus on the way to the stomach.*

With aging, the teeth undergo characteristic changes (18–21). Major changes include the following:

- The teeth undergo yellowish-brown discoloration due to staining by extrinsic pigments from beverages, tobacco, and oral bacteria.
- The pulp recedes from the crown and the root canal becomes narrow and thread-like.
- The roots become brittle and fracture easily during extractions.
- The layer of odontoblasts (cells secreting dentin) lining the pulp chamber becomes irregular and discontinuous.
- The pulp undergoes fibrosis and calcification.
- Concomitantly, with faster destruction than reconstruction of the dentin, the mandibular and maxillary bones in which the teeth are imbedded undergo the same osteoporotic processes as all other bones (Chapter 20).
- The increased bone loss results in looser teeth, contributing ultimately to tooth loss.

The surfaces of the teeth involved in chewing become progressively worn down throughout life. This attrition is not only a consequence of chewing, but also, in some individuals, of the habit of grinding or clenching the teeth together (so-called bruxism), often during rapid eye movement (REM) sleep (Chapter 7). Abrasion (sometimes due to improper brushing) and erosion (often aggravated by the demineralizing action of soft drinks) are frequent. Although new caries (i.e., cavities with decay) are uncommon in the elderly, loss of interest in dental hygiene and a decline in dexterity needed for tooth brushing may lead to plaque accumulation and caries (20). About 50% of elderly in the United States have lost the majority of their teeth by age 65 and about 75% by age 75. Restoring appropriate hygienic measures self-managed or provided by a dental hygienist can prevent teeth loss.

Recession of the gingivae (gums) occurs in all elderly. The epithelial attachment that forms a cuff around the tooth at the interface with the gums recedes and opens the way to accumulation of particulate material with bacteria (i.e., plaque), swelling, inflammatory hyperplasia, or low-grade infection. Whether such a gum recession is a physiologic process or the result of chronic periodontitis (i.e., inflammation of the periodontal membrane) due to a variety of local irritative factors (e.g., ill-fitting bridges or partial dentures) remains to be clarified. Indeed, after the age of 40, chronic periodontitis is the major cause of tooth loss. Periodontal disease is not only common in aging humans, but also, it is usually found in aging experimental animals (e.g., mice, rats, dogs, monkeys, and baboons). In humans and experimental animals, the disease is due to local factors as well as some systemic predisposing disease (e.g., diabetes) or stress (19,20). Indeed, in germ-free animals, recession of gums does not occur with age.

With aging, the epithelium of the oral mucosa becomes relatively thin and atrophic. Specialized structures, such as the papillae of the tongue, also become atrophic, and this atrophy is associated with loss of taste (see below). Other structures, like the palatal mucosa, undergo edema and keratinization (i.e., accumulation of a highly insoluble protein, keratin), a condition which seems to be delayed or prevented by the wearing of dentures. Keratin, also a normal component of the skin, is the product of epidermal cells and undergoes characteristic changes with development as well as extensive cross-linking with age. The oral epithelium exhibits increasing amounts of glycogen and alterations in collagen (19).

Oral Diseases in the Elderly

Although the above changes in oral structures with aging are gradual and relatively benign, they may predispose the involved tissues to a variety of pathologic conditions. Although very few oral diseases are characteristic of old age, many pathologic states are seen with greater frequency in older than in younger individuals (21). Among these, chronic periodontal disease (discussed above), xerostomia, mucositis and mucosal atrophy, leukoplakia, and malignant neoplasias are the most common.

Xerostomia or dry mouth may be due to a large variety of etiologic factors. In the elderly, the major causes are:

- Primarily, atrophy of the salivary glands
- Decline of salivary secretion
- Systemic disease (e.g., diabetes)
- Heavy cigarette smoking
- Anxiety and depression
- Several medications (antihypertensives, antidepressants, and antihistamines) depending on the dose (Chapter 22)

In this condition, the oral cavity is extremely dry, the mucosa appears red, fissured, and often coated with food particles and sloughed-off cells. Therapy involves cessation of the underlying cause (e.g., smoking), treatment of the underlying systemic disease (diabetes), or the use of artificial saliva. The decrease in salivary volume is associated with enzymatic changes such as reduction in amylase activity and electrolytes.

Mucositis and mucosal atrophy are frequent occurrences in elderly individuals in whom the oral mucosa has become atrophic and less resistant to irritation by oral noxious stimuli such as trauma, hot foods, and smoking and less resistant to infections and chemotherapeutic agents or radiation therapy. These stimuli result in a chronic inflammatory process (oral mucositis) and, in more severe cases, in ulceration with pain.

Leukoplakia, or keratosis, represents a hyperplasia of the mucosa with accumulation of keratin, hence, the name of "white patch." It is rarely seen in young individuals but is frequent after 60 years. It may be caused by pipe or cigarette smoking, by ill-fitting dentures, or by infections (e.g., candidiasis). It may be associated with precancerous histologic alterations, and in this case, it must be treated as if it were a carcinoma (22).

Swallowing and Pharyngoesophageal Function

Dysphagia, or difficulty in swallowing, is a common complaint of elderly individuals. It can result from alteration of any of the components of deglutition, a complex motor activity, involving the mouth, the esophagus, and several levels of nervous control (23,24).

Deglutition or swallowing is a reflex response that pushes the contents of the mouth into the esophagus. The afferent stimuli are generated by the voluntary collection of the oral contents on the tongue and their propulsion backward into the pharynx; these stimuli are carried by several nerves to the brain in the medulla oblongata, where they are integrated. The efferent stimuli are carried, also by several nerves, from the medulla to the pharynx and the tongue, where they activate the corresponding musculature. Inhibition of respiration and closure of the glottis are part of the reflex. Swallowing is impossible when the mouth is open; it is rapid during eating but continues at a slower rate between meals. Upon swallowing, the upper portion of the esophagus relaxes to permit entrance of the swallowed material, which then progresses through the esophagus to the stomach by peristaltic movements (circular waves). In the standing position, liquids and semisolid foods may fall by gravity to the lower esophagus, where the musculature relaxes upon swallowing and permits the passage of food into the stomach.

The act of swallowing is divided into three stages, all of them affected by aging (25,26). The first stage, in which the material to be swallowed is passed from the mouth to the pharynx, is a voluntary act, mediated through stimulation of skeletal muscles. These undergo aging-related changes with atrophy and increasing weakness common to all skeletal muscles (Chapters 20 and 24). *The second stage,* reflex in nature, is short in duration but complex in its neural control and involves the relaxation of the sphincter between pharynx and esophagus. *In the third stage,* reflex transport sweeps the contents onward through smooth-muscle peristalsis. All three stages require a precisely timed contraction and relaxation sequence. With aging, they may become desynchronized and result in less efficient deglutition. *Dysphagia* of varying degrees of severity is a common complaint. Mild dysphagia may be found in otherwise healthy elderly. However, in the most severe cases, dysphagia is associated with symptoms of choking and drowning with aspiration or regurgitation of food. The regurgitated food may enter the respiratory airways and induce pneumonia, one of the most frequent and severe pulmonary infections of the elderly (Chapter 19) (27,28). Severe dysphagia is always a symptom of a systemic disease, either of the muscle or the nervous system. In addition to being an uncomfortable and unpleasant symptom, dysphagia leads to reduced and altered nutritional intake, particularly in the elderly. Malnutrition, weight loss, dehydration, and the possibility of secondary infections are common features of this condition.

Presbyesophagus, or old esophagus (29), is characterized by a disruption of the esophageal motility, of various degrees of severity. Radiologic and manometric studies reveal

- Increased incidence of polyphasic (i.e., nonperistaltic contractions)
- Reduced amplitude of peristaltic contractions (i.e., wave of contraction passing along the GI tube)
- Incomplete relaxation of the lower esophageal sphincter (i.e., a ring-like muscle that closes a natural orifice) probably due to concomitant diseases, e.g., diabetes and neurologic disorders

Additional motor disorders of the esophagus, occurring at all ages including old age, are *achalasia*, (failure of the GI smooth muscle fibers to relax) in which food accumulates in the esophagus and the organ becomes extremely dilated; *sphincter incompetence*, which permits reflux of acid from the stomach into the esophagus; *aerophagia*, or ingestion of air, which can be regurgitated or absorbed in the intestine or expelled as flatus.

Gastroesophageal-Reflux (or Regurgitation) Disease

Symptoms of dysphagia, regurgitation (i.e., backward or return flow), *chest pain, and heartburn are fairly common in the geriatric population, with a prevalence of 35% reported in the 50- to 79-year-old groups (30).* The ensuing condition, and its consequences, is

called gastroesophageal-reflux (or regurgitation) disease (GERD). The reflex involves reflux of gastric acid into the esophagus; GERD risk factors include

- decreased esophageal clearance,
- gastric factors such as decreased emptying of the stomach or increased gastric pressure,
- decreased low esophageal sphincter pressure or inappropriate low sphincter relaxation,
- the presence of an hiatal hernia, that is, protrusion of any structure through the esophageal hiatus (opening through the diaphragm for the passage of the esophagus to connect with the stomach), and
- increased gastric acid secretion.

Older individuals have a significant increase in esophageal acid exposure and longer duration of reflux episodes (31). This greater GERD severity in the elderly may depend more on decreased esophageal peristaltic movements than on changes of gastric emptying (31–34). Concurrent disease (in addition to the local esophageal disorder) and side effects of medications may play a greater role in the pathophysiology of GERD in older patients. Treatment may be surgical or pharmacologic (32,33). The latter, uses two different but highly successful pharmacotherapies: the H_2-receptor blockers and the proton-pump blockers, representing some of the most used health-care products (and expenditures) advertised in the media (35).

Another form of esophageal disease that is prevalent in the elderly is the so-called *Barrett's esophagus (BE). BE presents with symptoms similar, but, often more severe than those of GERD*. BE has been reported to occur in 10% to 15% of individuals with GERD; however, a number of other factors—smoking, diet, and alcohol consumption—may be implicated in the association of BE with cancer (adenocarcinoma) of the esophagus. In case of esophageal cancer, surgery is the recommended therapy (36).

■ The Stomach and the Duodenum

The stomach

- serves as a food reservoir,
- breaks down ingested food by its churning movements (due to the presence of three smooth-muscle layers),
- secretes gastric juice, which contains a variety of substances: digestive enzymes (e.g., pepsin), mucus (which lubricates the food), and hydrochloric acid [which destroys the ingested bacteria, aids in protein digestion, and is necessary for the transformation of iron (from the dietary ferric F^{3+} to the water-soluble F^{2+} ferrous form for the synthesis of hemoglobin)],
- secretes and is responsive to hormones: gastrin, glucagon, somatostatin (Chapters 9 and 13), and to special peptides also found in the brain: vasoactive intestinal polypeptide and substance P, and
- secretes an intrinsic factor (a glycoprotein) necessary for the absorption of vitamin B_{12} from the small intestine; vitamin B_{12} is necessary for the maturation of red blood cells, and deficiency of the vitamin or intrinsic factor results in a severe type of anemia (Chapter 17).

With aging, changes in the stomach and neighboring duodenum involve the mucosa cells and the hydrochloric acid and pepsin secretions; under basal conditions and in the "healthy" elderly, both are decreased (Fig. 2). This decrease may contribute to some of the difficulty of digestion (involving foods rich in protein such as meat) that affects a large proportion of the elderly (Chapter 23). However, more threatening to the health status of the elderly is the possible disruption of the so-called *"gastric mucosal barrier" that protects the mucosa from attack by acid and pepsin*; failure of this protection, usually associated with impaired immune function (37), results in injury and death of mucosal cells (37–39).

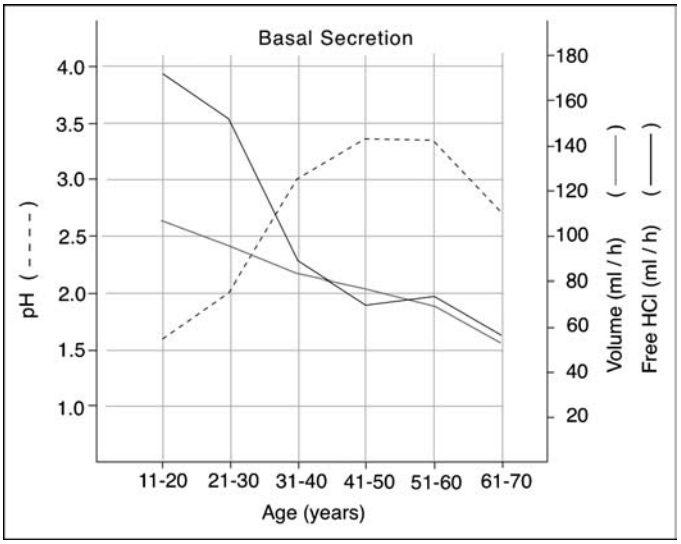

FIGURE 2 Changes with age under basal (preprandial) conditions of gastric secretions. Note, with advancing years, the moderate decrease in total volume and the greater decrease in free HCl. As the free HCl decreases, pH increases. *Abbreviation*: HCl, hydrochloric acid.

The protective barrier is formed of tight junctions between the cells and of a thick layer of mucus. Because a pH of 7 to 8 is present at the mucosal cell surface, and a pH of 2 is present in the gastric lumen, under normal conditions, a gradient from mucosa to gastric lumen is established to prevent back-diffusion of hydrogen ions into the gastric cells. Bile acids, nonsteroidal anti-inflammatory drugs (e.g., aspirin), other substances (ethanol and caffeine), and bacteria may disrupt this mucous protective layer and allow acid and pepsin to damage and destroy mucosal cells (40). *Prostaglandins*, bioactive lipids present in the stomach and duodenum (as well as in many other tissues), stimulate bicarbonate secretion and thus play a role in gastric mucosa defense. However, prostaglandin secretion decreases with aging. *Gastrin*, one of the GI hormones, secreted by the gastric mucosal cells as well as the pituitary, pancreas (fetal), and brain, stimulates gastric acid and pepsin secretion. Gastrin may also participate to some degree in regenerating processes by promoting mucosal growth; this growth-promoting action, beneficial in cases of mucosal damage, is mediated through the release of the epidermal growth factor (EGF) from the duodenal Brunner's glands. The production of EGF, a peptide, may have a protective action on the glycoprotein of the gastric mucous.

Changes with aging involve some of the digestive enzymes as well; they may be consequent directly to changes in the enzyme secreting cells and organs or indirectly to hormonal and neural regulatory alterations (Table 2). Failure of enzymatic activity may also depend on alterations of the regulatory mechanism of enzyme synthesis and release due either to a change in the hormonal or neural stimuli or to a change in response (altered receptors).

In addition to physiologic changes with aging, the stomach undergoes pathologic changes, among which the most common are gastritis and peptic ulcer disease.

TABLE 2 Possible Mechanisms of Aging-related Changes in Digestive Enzyme Secretion

Enzyme-secreting organs
Reduction in
Number of cells
Enzyme concentration
Enzyme synthesis and release
Hormonal and neural regulation of enzyme-secreting organs
Reduction in
Number of GI endocrine cells
Hormone concentration
Impairment of
Sensitivity of endocrine cells to digestive stimuli
Alteration of
Distribution and metabolism of GI hormones
Number and affinity of endocrine or neural receptors

Abbreviation: GI, gastrointestinal.

Gastritis and Peptic Ulcer Disease

Gastritis is an inflammatory process of the gastric mucosa; both its incidence and its prevalence increase with advancing aging. This is also the case of gastric and/or peptic ulcer disease. In this condition, one or several ulcers (i.e., break in the mucosa with loss of cells) may be situated in the stomach (usually near the pylorus, the region close to the duodenum) or the duodenum and will then be designated as gastric or duodenal ulcers. The incidence of peptic ulcer is on the rise in developing countries, particularly in India and Africa. In Western countries, including the United States, incidence is decreasing in the overall population (perhaps reflecting advances in treatment) but increasing in the elderly group (41,42). In this group, not only is the incidence on the rise, but the severity of the disease and its consequences are also greater than in younger individuals. Elderly patients tend to predominate among those admitted to hospitals for peptic disease; patients over 60 years of age account for nearly 50% of those with gastric and 40% of those with duodenal ulcers. In these individuals, age is the major mortality risk.

Excessive acid production was believed to be the major cause of gastritis and peptic ulcers until about four decades ago, when infection with the common microorganism, *H. pylori*, usually attached to mucosal cells, but rarely located intracellularly, was established as a major cause of gastritis and ulcer. The presence of *H. pylori* had been noted in the stomach since the end of the nineteenth century, but it is only in recent years that its association with gastritis and peptic ulcer led to one of the major medical revolutions of the twentieth century (14,16). One important aspect of the infection with *H. pylori* is the diversity of its consequences depending on

1. the virulence of the infection,
2. the genetic characteristics of the host: some have the infection but never develop the gastrointestinal problems,
3. the presence of environmental cofactors (e.g., nonsteroidal anti-inflammatory drugs, smoking), and
4. the age of the affected individual and the age when the infection was acquired.

Current studies of the molecular and cellular biology of *H. pylori* have provided two complete genome sequences; population genetics have been initiated and potential therapeutic targets with novel antibacterial drugs or vaccines are being developed (14,16).

Symptoms for gastritis and peptic ulcer are often indistinguishable, but generally, always more time is allotted for the onset of severe peptic ulcer. Symptoms consist primarily of epigastric pain, (over the region of the stomach) occurring periodically (more often during the night) and GI bleeding. Symptoms may be atypical in the elderly with vague abdominal pain and weight loss. Major life-threatening consequences are perforation of the gastric/duodenal wall and hemorrhage: these can occur, suddenly, even without previous symptoms. A summary of peptic ulcer management is presented in Box 1.

Vascular Alterations

As indicated above, bleeding in the elderly presents a special problem—incidence and mortality are high after the age of 60 years (43). In addition to bleeding and hemorrhage from GI ulcers, another cause of bleeding in the elderly is vascular malformations, or *ectasia* (i.e., dilatations), of the intestinal vessels, which are easily subject to bleeding. These ectasias occur throughout the GI tract, particularly the small bowel, stomach, and colon. They are associated with aortic stenosis (i.e., narrowing of the aorta opening in the left ventricular wall of the heart), age-related degenerative changes in tissues, inherited collagen, or ground substance defects.

Circulatory alterations are not confined to bleeding but also encompass atherosclerotic lesions (Chapter 15) that may result in ischemia (i.e., local and temporary deficiency of blood), and lead to mesenteric infarction. Ischemia produces symptoms of varying severity from transient intestinal discomfort to abdominal angina, infarction, and inflammation.

Carcinoma of the Stomach

For unknown reasons (perhaps a change of dietary and of other habits), *the incidence of carcinoma of the stomach, once one of the most frequent cancers in men, has been declining in the last 30 years.* It is still relatively frequent and is situated primarily in the lower regions of the stomach (antrum and pylorus). It has a very unfavorable outcome (prognosis). Peak incidence is reached in 80- to 90-year-old men and may have a familial occurrence. Diagnosis is made on the basis of gastroscopy with biopsy. Unfortunately, treatment, either by surgery, by radiation, or by chemotherapy shows a low (5–10%) five-year survival rate (12,13).

■ The Small and the Large Intestine

Aging-Related Changes in Intestinal Absorption

Intestinal absorption depends on the structural and functional integrity of the intestinal mucosa (Box 2). In a number of animal species, advancing age is accompanied by changes involving one or several of the following structures:

- Overall alterations of the shape of the villi (i.e., finger-like projections covering the mucosa of the small intestine) and the microvilli (thread-like projections on the luminal side of the intestinal mucosa cells) (Box 2)
- Increase in collagen
- Mitochondrial changes
- Lengthening of crypts (intestinal tube-like depressions opening on a free surface, Box 2)
- Prolonged replication time of the crypt stem cells

All of these changes are minor and none appears sufficient to explain the impaired absorption often found in the elderly. Other factors may intervene:

- Altered villus motility limiting functional surface area
- Inadequate intestinal blood supply (due to atherosclerotic involvement of major intestinal vessels)
- Impaired water "barrier" restricting diffusion and transport, and changes in small intestine permeability (44)

BOX 1 *Management of Peptic Ulcer*

Procedures used in the treatment of ulcers include:

1. dietary,
2. pharmacologic, and
3. surgical interventions.

Pharmacologic interventions were originally aimed at inhibiting acid secretion and enhancing mucosal resistance to acid. Currently, the use of antimicrobial agents, primarily antibiotics, (e.g., tetracycline and amoxicillin) is favored, eventually in association with antiacids and antisecretory agents.

A variety of antiacids, most of which contain aluminum and magnesium hydroxide or calcium carbonate are available. Inhibition of parasympathetic inputs (which stimulate acid secretion) by atropin gives variable responses and has many undesirable side effects. H_2 receptor blockers of histamine (an amine derived from the amino acid histidine and a powerful stimulator of gastric secretion), such as cimetidine and ranitidine (some of the most commonly prescribed drug in the US) are also often used. Drugs capable of inhibiting H^+K^+ATPase are also widely used. Epidermal growth factor to stimulate replacement of mucosal cell loss is still at the experimental stage. A number of substances increase the resistance of mucosal cells to acid by forming adherent protein complexes at the ulcer site. Usually the pharmacologic treatment is associated with special diets and cessation of cigarette smoking inasmuch as healing rates of duodenal ulcer are probably adversely affected by cigarette smoking and the incidence of ulcers is higher in smokers than in nonsmokers.

If pharmacologic, dietary, and hygienic measures fail, surgery is advisable, with the caution necessary for the older patient.

With increasing age, absorption of several substances (e.g., sugar, calcium, and iron) is reduced (Chapter 23), whereas digestion and motility remain relatively unchanged. Among the substances for which absorption has been studied in the elderly, calcium probably presents the best evidence for a gradual reduction with increasing age (Table 3). In addition to changes in bone calcium with aging, calcium absorption and transport are significantly reduced starting at about age 60 (Chapter 23). When comparing young and old subjects, the young individuals are capable of responding to a low calcium diet by increasing intestinal calcium absorption; a response no longer found in the elderly. Mechanisms responsible for reduced calcium absorption are listed in Table 3. There is conflicting evidence for other substances such as dextrose, xylose, iron, and vitamin B_{12}, fats, which may be reduced or may be absorbed normally at all ages.

Increased general frailty and weight loss may occur in the old, without evidence of any specific underlying cause, in the presence of a well-maintained appetite and a balanced diet. However, a relatively high percentage of old individuals suffer from malabsorption; at least 7% of residents in nursing homes are likely to have impaired absorptive ability.

Absorption of Nutrients and Malabsorption Disease

Adequate nutrition is indispensable at all ages but especially in the young, who must provide extra calories for growth, and in the old, whose GI function is only marginal. Indeed, dietary interventions to ensure a long and vigorous life have been popular for many centuries. Once achieved, old age has been recognized as a period requiring special attention to dietary habits (Chapter 23).

In the intestine, primarily the small intestine, the intestinal contents are mixed with mucus, pancreatic juice, and bile. Digestion, which begins in the mouth and stomach, is completed in the lumen and mucosal cells of the small intestine. Digestion depends upon a number of enzymatic processes under neural and hormonal stimuli, which can be affected in the elderly (Table 2). The products of digestion are then absorbed, along with vitamins and fluid in the small intestine (water also in the colon) and carried to the liver by the portal blood. The

BOX 2 *Structure of the Small Intestine Mucosa*

Throughout the length of the small intestine, the mucosa displays many folds and is covered with villi formed of a single layer of columnar epithelium and containing a network of capillaries and one lymphatic vessel. The free edges of the mucosal cells are divided into microvilli, which form a "brush border." Folds, villi, and microvilli augment considerably the absorptive surface. The mucosal cells are formed from mitotically active undifferentiated cells located at the bottom of the villi (in the so-called crypts of Lieberkhun). They migrate up to the tips of the villi where they are sloughed off into the intestinal lumen. The average life of these cells lasts two to five days, thereby representing a potential model (still little utilized) for the study of cellular aging. The crypts are also the site of active secretion of water and electrolytes. Studies in humans have reported, with aging, a reduced height and a frequent convoluted pattern of villi; changes were not observed in villus width, cell height, or mucosal thickness.

TABLE 3 Mechanisms of Decreased Intestinal Calcium Absorption with Aging

↓ Intake of vitamin D (poor nutrition)
↓ Vitamin D conversion in skin (reduced sunlight exposure)
↓ Intestinal absorption
↓ Vitamin D metabolism (hepatic) and activation (renal)
↓ Cellular calcium binding (less receptors)

small intestine, with its many folds, finger-shaped villi, and array of microvilli on the luminal side of the cells, is particularly well designed for this function of absorption. The rate of nutrient transport by the intestine is related primarily to the surface area that is functionally exposed to the luminal contents. Several factors are important for the maintenance of optimal function of the small intestine (Table 4) and may be affected by aging. Thus, any change in intestinal architecture or diffusion barrier of the mucosal cells greatly affects transport (44).

Many generalized conditions, such as rheumatoid arthritis (Chapter 20), afflicting the elderly may have some detrimental affects on absorption. A number of small bowel disorders may cause *malabsorption*, but their incidence is low and symptomatology vague. Other frequent causes of malabsorption include

- infections (e.g., after GI surgery, diarrhea),
- small intestine diverticula (i.e., small dilations or pockets leading off from the intestinal tube),
- pancreatic insufficiency, reduction of digestive pancreatic enzyme (rare),
- celiac disease (alterations of mucosa and cell transport),
- mental disorders (e.g., dementia).

Many old individuals with malabsorption are severely undernourished, weak, and debilitated. Management, therefore, includes treatment of the specific underlying disease and appropriate diet. With energetic treatment, even severely ill patients have a good prognosis.

Aging-Related Changes in the Large Intestine

Disorders of the large bowel are almost exclusive to the elderly (45,46). The physiopathology of such disorders is still little known, but given the multiple clinical problems, more attention is currently being dedicated to the study of physiologic changes with aging in this intestinal segment.

Anatomical changes are similar to those of the small intestine and include

TABLE 4 Important Factors for the Maintenance of Optimal Small Intestin Function

Anatomic integrity of the absorbing small intestine and normal cell replication of intestinal mucosal cells
Normal gastrointestinal secretions including basal and postprandial secretions from salivary, gastric, pancreatic, and hepatic cells
Coordinated gastrointestinal motility
Normal intestinal uptake and transintestinal transport
Adequate intestinal blood supply to maintain cell oxygenation and cell nutrient supply
Normal defense mechanisms against toxic injurious agents from the intestinal lumen:
Normal clearance of bacteria from the intestinal lumen
Immunological responses to injury
Mucosal cell-wall integrity
Mucosal cell detoxification of toxic absorbed materials

1. Atrophy of the mucosa
2. Proliferation of connective tissue
3. Vascular changes, mostly of an atherosclerotic nature

The large intestine major functions are storage, propulsion, and evacuation of the intestinal content (feces). Especially important are those conditions associated with bowel motility, such as constipation or diarrhea (46,47).

In the colon (the last portion of the large intestine before the rectum), the most obvious aging-related change is the increased prevalence of *diverticula*, small, pocket-like mucosal herniations through the muscular wall (48,49). They vary in diameter from 3 mm to more than 3 cm and are present in 30% to 40% of persons over the age of 50 and with increasing incidence thereafter. *Diverticula are often responsible for severe bleeding from the rectum and often become inflamed, causing diverticulitis.* A highly refined, low residue, diet as often consumed today, may be responsible for the formation of diverticula. The lack of dietary fiber and bulk is associated with spasm of the colon. The intraluminal pressure builds up, and the mucosa eventually pushes through the muscular coat at weak points, usually where the colonic blood vessels pierce the muscle to supply the mucosa. Diverticula become filled with packed feces and may ulcerate into the thinned mucosa, causing infection and inflammation.

The presence of diverticula may induce nonspecific abdominal pain, diarrhea, or constipation. A diet to increase the fiber content may alleviate these symptoms. Major complications include diverticulitis, hemorrhage, and colonic obstruction or perforation requiring surgery, and rigorous pharmacologic and dietary treatment (49).

Carcinoma of the large bowel, colorectal cancer, is the second (after lung carcinoma) most common malignancy in individuals over 70 years of age. Cancer of the colon appears to be more frequent in women and cancer of the rectum in men (12,13). Polyps (small-tissue mass) resulting from hypertrophy of the intestinal mucosa and extending into the intestinal cavity are also frequent. They may be benign tumors or possible precursors of carcinoma.

Constipation

The major cause of constipation (infrequent and/or difficult evacuation of the feces) is decreased motility of the large intestine, but diet (unbalanced with respect to bulk) and lack of exercise may also be implicated in its etiology (50,51). Constipation is considered one of the most common GI complaints of the elderly. Its prevalence seems to be greater in women, although this sex difference may not be real but rather due to the larger number of old women than old men and the overall degree of old women's disabilities (Chapter 3). Treatment involves increasing the bulk in the diet and increasing physical activity (Chapter 23).

Incontinence

As discussed in Chapter 18, urinary incontinence is one of the major afflictions of old age. The same tragic consideration applies to fecal incontinence (Fig. 3) (52–58). The maintenance of normal control on fecal evacuation, or defecation, is regulated by complex neuromuscular functions (Fig. 4). Should any physiologic decrement occur in the activity of the intestine, in the muscles of the pelvic floor, or in the neural inputs, the control of defecation may break down.

Distension of the rectum, the last portion of the intestine, initiates reflex contractions of its musculature. In humans, the internal involuntary sphincter is excited by the sympathetic but

FIGURE 3 Relationship of age and sex to fecal incontinence. *Source*: From Ref. 51.

inhibited by the parasympathetic nerve supply. The external voluntary sphincter is innervated by somatic nerves. The urge to defecate begins when the rectal pressure increases to a certain level (about 55 mmHg), at which time both sphincters relax and the rectal contents are expelled. At a lower rectal pressure, defecation can be initiated by voluntary relaxation of the external sphincter and contraction of the abdominal muscles. Thus, defecation is a spinal reflex that can be voluntarily inhibited by contraction of the external sphincter or facilitated by its relaxation.

A person with efficient sphincters may find control impossible during an attack of severe diarrhea (abnormal frequency and liquidity of feces). In the elderly, loss of sphincter muscle strength (Fig. 5), merely as a consequence of aging, creates a more difficult problem when confronted with diarrhea. Certain neurologic conditions affect the pelvic floor muscles, and these may be so severe that, even with a normal stool, continence cannot be preserved. At all ages, there may be organic deficiencies in the muscle ring due to trauma, and such deficiencies are more likely to occur in the elderly person.

With aging, the rectal muscle mass is decreased in size, and the sphincter is weakened. The external sphincter is always the most affected of the pelvic floor muscles. The high incidence of incontinence in the elderly makes it essential to exclude any possible underlying GI infection or systemic disease. One classification is shown in Table 5 and may be compared with the causes of urinary incontinence (Chapter 18).

Fecal incontinence may be caused by the following:

1. Neurogenic alterations involving the cortex (e.g., dementia) or the spinal cord (e.g., failure to inhibit defecation upon entrance of feces into rectum)
2. Muscle atrophy (due to trauma as in prolonged and difficult newborn delivery), a direct analogy to stress incontinence of urine (Chapter 18)
3. Constipation (due to immobility, poor reflexes, and difficulty in reaching the toilet)
4. Diarrhea, which may cause incontinence at all ages but more frequently at older ages

Knowledge of the etiology of incontinence leads to some practical interventions. A first step is to rule out constipation or diarrhea and, if present, to treat them. If fecal incontinence persists, then other causes must be sought and appropriate treatment established.

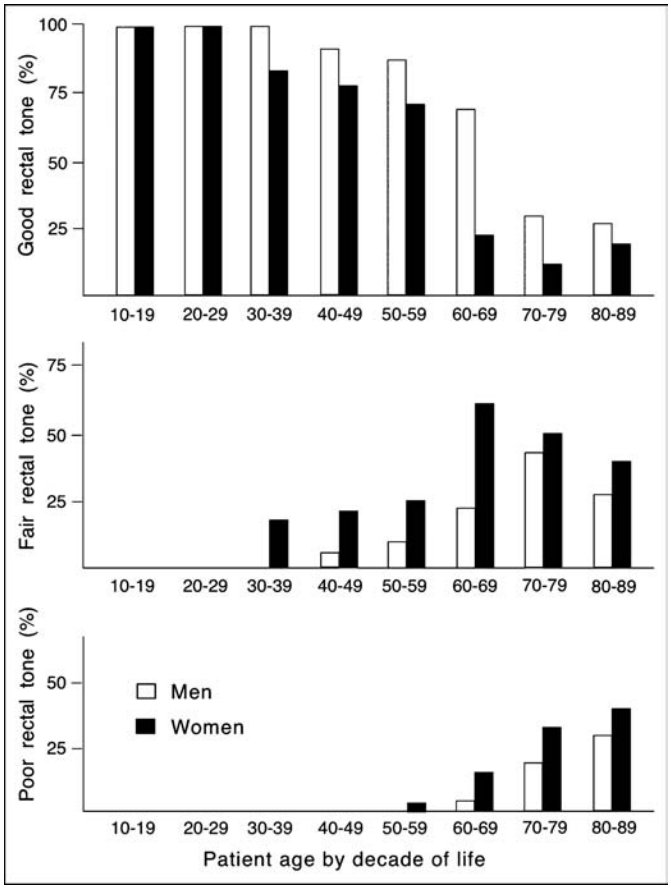

FIGURE 4 Relationship of sphincter tone to age. *Source*: From Ref. 51.

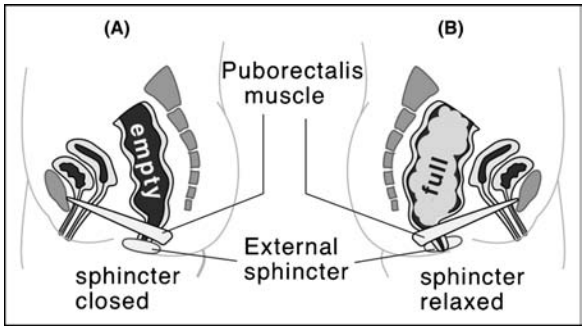

FIGURE 5 Diagrammatic representation of some of the structures involved in defecation (here, in women). Note that the external sphincter and the puborectalis muscle play an important role in maintaining normal fecal continence: (**A**) when the rectum is empty and the sphincter is closed, and (**B**) when it is full, and the sphincter is relaxed. Note that one important action of the puborectalis muscle is to maintain angulation (bend) between the lower rectum and the anal canal, upon which continence is largely dependent. Also note that with increased rectal pressure, the activity of the sphincter increases, thereby protecting the individual from involuntary defecation. However, above a certain pressure (fecal volume), this protection is lost.

TABLE 5 Classification of Fecal Incontinence

Cause	Consequence
Neurogenic	
Cerebral cortex	Loss of inhibitory control
Spinal cord	Reduced reflex activity
Muscle atrophy	"Stress" incontinence
Retention	Constipation
Overflow (bacteria, virus, and allergies)	Diarrhea

■ AGING-RELATED CHANGES OF THE EXOCRINE PANCREAS

In addition to its hormonal secretions, the pancreas produces a pancreatic juice containing enzymes—amylase, lipase, and proteases—important for digestion. The enzymes are discharged by exocytosis, and their secretion is controlled by a reflex mechanism and by the hormones, secretin and cholecystokinin. These hormones are both secreted from the duodenum and, in addition, cholecystokinin stimulates contraction of the gallbladder. *The major digestive pancreatic enzyme is trypsin,* which is secreted as an inactive proenzyme, trypsinogen. The active form, trypsin, has a proteolytic action (i.e., catalyzes the hydrolysis of peptide bonds in the basic amino acids arginine and lysine). Some uncertainty exists regarding the effects of advancing age upon pancreatic secretion (11,59). The senile gland is smaller, harder than normal (due to increasing fibrosis), and yellow-brown (due to accumulation of lipofuscin). Of the major enzymes, some (amylase) remain constant, whereas others (lipase, trypsin) decrease dramatically. Secretin-stimulated pancreatic juice and bicarbonate concentration remain unchanged. Little is known so far about age-related changes in the hormones that regulate pancreatic function. Although a decline in some functions of the pancreas occurs with aging, the genesis of this decline is unknown but has been related to the following:

1. Diet
2. Drugs (e.g., alcoholism)
3. Vascular sclerosis
4. General fibrosis
5. Lack of cell regeneration

However, as only one-tenth of pancreatic secretion is needed for normal digestion, it is not probable that only age is responsible for a significant pancreatic insufficiency capable of inducing severe digestive disorders. In general, these age-related changes do not seriously compromise pancreatic function, but their presence may increase the incidence of pancreatic disease (acute and chronic pancreatitis and cancer) in the elderly (11,59–61).

■ AGING-RELATED CHANGES OF THE LIVER

The liver is an organ with many functions; to mention only a few:

- Bile formation
- Carbohydrate storage and metabolism
- Ketone body formation
- Reduction and conjugation of steroid hormones
- Inactivation of polypeptide hormones
- Detoxification of many drugs and toxins
- Manufacture of plasma proteins
- Urea formation
- Regulation of lipid metabolism

As is the case with other multifunctional organs, not all liver functions age at the same pace (7,8,59,62,63). This section will focus on changes in morphology and function, particularly, of bile formation and excretion (64). Changes in enzyme activity with aging and hepatotoxic effects of various drugs are considered in Chapter 22.

■ Structural Changes

Major changes with aging in liver size and liver cells are listed in Table 6. Changes with aging may reflect degenerative processes (e.g., reduced liver weight, cell loss, and decreased mitochondrial number) or compensatory processes (e.g., increased cellular and mitochondrial size). That this is the case and that aged hepatic cells are active is supported by the increased activity of some enzymes (e.g., succinic dehydrogenase).

Hepatic cells regenerate throughout life, but their turnover slows down with aging, perhaps due to the absence or deficit of growth factors for cell replication or to the excess of growth inhibitory factors (64). The decelerated regeneration may also be due to cellular alterations such as binucleation (two nuclei in a cell) and polyploidization (increased nucleus size with more than two full sets of homologous chromosomes). Administration of growth-promoting hormones, such as thyroxine, does not appear to normalize regenerative processes. Indeed, the compensatory cell hypertrophy when regenerative power is more limited, would accelerate cell loss and, thereby, result in a vicious cycle of cell destruction and compensatory hypertrophy.

Smooth endoplasmic reticulum is decreased in the rat hepatocytes, a morphologic correlate underlining the age-related reduction in the hepatic capacity to metabolize drugs (Chapter 22). Other aspects of hepatic structure are not markedly altered, and maintenance of normal morphology agrees with minimal or no alteration of hepatic metabolic functions such as protein synthesis.

Hepatic Cells

The liver is organized in lobules formed of hepatic cells, hepatocytes, lined up in rows irradiating from the center of the lobule to the periphery. The hepatic veins are situated in the center of the lobule and the biliary ducts, the portal veins and

TABLE 6 Some Structural and Functional Alterations Associated with Aging in the Liver

Atrophy and ↓ weight (cell loss beginning around 60 yrs and accelerating in the 80-yr-old group and older),
Cell size remains unchanged or may ↑ (in contrast to malnutrition with cell size ↓)
↑ Collagen with aging-associated changes (Chapter 21)
Alterations of the usual cycle of hepatic cell degeneration/regeneration:
↑ Binucleate cells
↑ Ploidy (more than two full sets of homologous chromosomes)
↓ Regeneration (perhaps due to growth inhibition)
↑ Number of degenerating cells
↑ Compensatory hypertrophy of remaining cells
↓ Number but ↑ size of mitochondria (suggesting compensatory attempts to maintain function)
↓ Size of endoplasmic reticulum and ↓ ability to metabolize drugs (Chapter 22)
Unchanged liver function tests involving metabolism and elimination of specific dyes and radioisotopes, protein synthesis, metabolic functions

hepatic arteries are at the periphery. The specialized capillaries, the sinusoids, are lined with phagocytic cells (Kupffer's cells) that engulf bacteria or other foreign particles (Chapter 14). The lobules are separated by a small amount of interlobular connective tissue in which are found the blood vessels and the beginnings of the biliary ducts and lymphatic vessels (Fig. 6).

■ Functional Changes

Alteration of hepatic structure and enzymatic functions with aging is moderate. In the healthy elderly, routine tests of liver function involving the metabolism and elimination of specific dyes (e.g., bromsulphalein) and radioisotopes as a test of hepatic clearance do not show significant differences between individuals aged 50 to 69 and 70 to 89 years; likewise, tests of protein synthesis (e.g., plasma albumin levels) are not altered with aging (63–65), Furthermore, tests of liver dysfunction, including cholestasis (blockage of bile flow), cell necrosis, inflammation, and impaired detoxification are usually negative in the absence of specific liver damage and systemic disease (65).

In contrast to humans, studies in experimental animals (rats) show consistently reduced removal from the blood of many of the dyes used to measure hepatic function, storage, and transport (66). These changes have been ascribed to both circulatory alterations and increased collagen deposition (and hence, impaired transport) in the liver.

■ Bile Formation

Bile formed in the hepatic cells is carried, by the bile canaliculi, to the right and left hepatic ducts; the two ducts join to form, outside the liver, the common hepatic duct. The common duct joins the cystic duct that drains into the gallbladder. The *gallbladder,* located on the undersurface of the liver, serves as the bile reservoir. The common bile duct, derived from the union of the hepatic and cystic ducts enters the duodenum, usually united with the pancreatic duct, and pours the bile into the duodenum.

The bile, primarily through the action of its salts, has several important functions in the digestion: it emulsifies the lipids and activates the lipases, fast-splitting enzymes. Bilirubin, the major pigment of the bile, results from the breakdown of hemoglobin, myoglobin, and respiratory enzymes in the reticuloendothelial system of the liver and spleen. Unconjugated bilirubin, thus formed, is carried to the liver cell, where it is conjugated and bound to proteins; this conjugated form is water soluble. In the colon, conjugated bilirubin is hydrolyzed to urobilinogen and other oxidized compounds that give feces and urine their characteristic color.

Bile production is a major function of the liver. The hepatic cells form the bile that is considered as a product of both secretion and excretion. It is a greenish-yellow fluid composed of water, bile salts, conjugated bilirubin, cholesterol, and various inorganic salts; its many important functions are listed in Table 7.

Little is known of changes with aging in bilirubin metabolism. However, biliary disease is common, and its incidence increases steadily with age (Fig. 7) (67). *Biliary disease (gallstones)*, a common problem of most Western societies, affects 15% to 20% of adults of all ages and 30% to 50% of elderly persons by age 75, with a ratio of 2:1 for females to males (59,62,67–69). As people live longer, the incidence of biliary disease has increased in the last 30 years. About 40 to 60 of patients with gallstones show no symptoms, and it is possible that the condition starts this way in most individuals.

In the gallbladder, the bile is concentrated by absorption of water (water is 97% in the liver bile and 89% in the gallbladder bile). Stones form in the gallbladder or bile ducts when a substance that is not normally present appears in the bile or the composition of the bile changes so that a normal constituent precipitates. For example, cholesterol stones form when the proportions of cholesterol, lecithin, and bile salts in the bile are altered.

Several aspects of biliary disease are characteristic of the elderly. These include the following:

1. A greater incidence of acute versus chronic inflammation of the gallbladder
2. The presence of stones in the bile duct
3. The recurrence of the disease after a previous operation
4. The greater severity of the disease and the higher mortality

An increasing incidence of stones in bile ducts and gallbladder with aging has been well documented. With this increase, the incidence of related complications (e.g., jaundice,

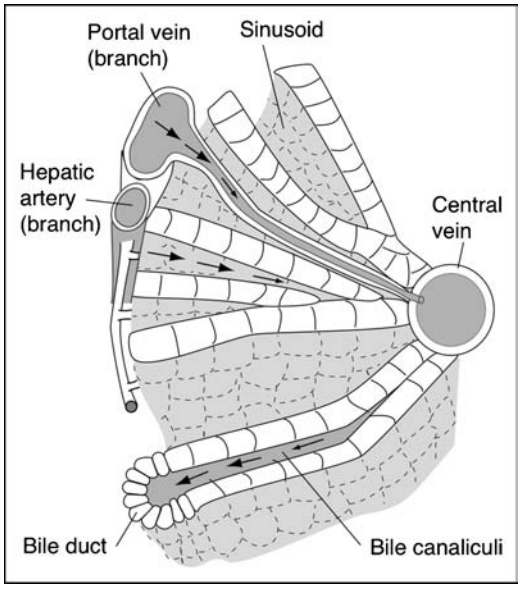

FIGURE 6 Diagrammatic representation of the liver, which is organized in lobules formed of hepatic cells, hepatocytes, lined up in rows irradiating from the center of the lobule to the periphery. The hepatic veins are situated in the center of the lobules and the biliary ducts, the portal veins, and the hepatic arteries at the periphery. The specialized capillaries, the sinusoids, are lined with phagocytic cells (Kupffer's cells) that engulf bacteria or other foreign particles. The radial disposition of the liver cell plates and sinusoids around the terminal hepatic venule or central vein show the centripetal flow of blood from branches of the hepatic artery and portal vein and the centrifugal flow of bile to the small bile duct.

TABLE 7 Major Functions of the Bile

Emulsification of lipids
Activation of enzymes for digestion of lipids
Conjugation of bilirubin (derived from hemoglobin breakdown) to form a water-soluble product for excretion
Excretion of cholesterol (Chapter 16)
Neutralization (by $HCO3^{-}$) of acid delivered to duodenum from stomach
Excretion of drugs, heavy metals, and environmental toxins (Chapter 22)

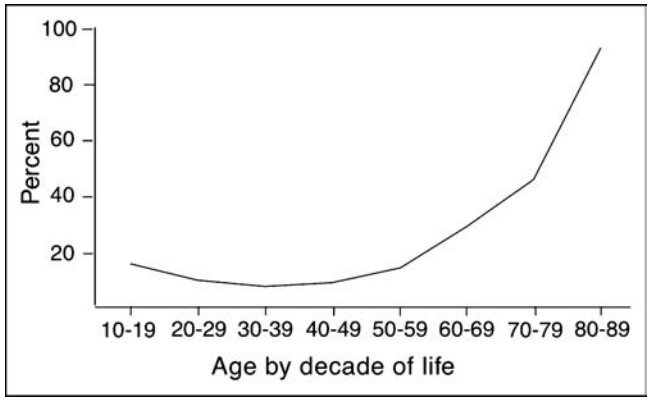

FIGURE 7 Incidence of bile duct stones at cholecystectomy in relation to age. Note the sharp increase from 60 years on.

pancreatitis, cholecystitis, liver abscesses, and systemic sepsis) also rises. The treatment of choice is surgical and consists in the removal of the stone(s); it entails, in the elderly, more complicated procedures to control or correct the disease (62,66–69).

AGING-RELATED CHANGES IN THE SENSES OF SMELL AND TASTE

Smell

The senses of smell or olfaction are important in food selection and nutrition, in social interaction, and enjoyment of life (70–74). Olfaction helps in the promotion of health by helping us to avoid the putrid smell of rotten matter. In addition to these values, olfaction warns against certain dangers posed by modern living, such as odiferous atmospheric pollutants. The olfactory system is comprised of peripheral chemoreceptor cells situated in the nasal mucosa and stimulated by molecules in solution in the mucus, in the nose (and saliva, in the mouth, for taste). The central pathways include the olfactory bulbs, the olfactory cortex (in humans, the piriform cortex), and the limbic system, where, presumably, olfactory discrimination and conscious perception of odors are mediated.

With aging, olfactory ability declines (hyposmia) or may be completely lost (anosmia). Decline in olfactory function can be demonstrated by two kinds of evaluations:

- Decreased sensitivity to odor thresholds, that is, decreased ability to identify various odors
- Decreased discriminatory ability to identify odor constituents in a mixture

The decrease in olfaction worsens with progressing age and may culminate in failure of smell detection by age 80 years and older; it is greater for some odors than others, and, overall, females score better than males (74–76). The decline in the ability to recognize odors has been related to alterations of peripheral and central nervous control as well as to some oral and pharyngeal diseases. In the nasal cavity, aging changes begin to occur at a relatively early adult age and steadily progress with advancing age (74). They include

- loss of cilia from cells of the nasal mucosa, followed by loss of cells, and by slower replacement of lost cells and
- loss of neurons in brain olfactory centers such as the olfactory bulb. Neuronal losses may be secondary to the loss of the sensory cells from the nasal mucosa (and hence, to loss of sensory stimulation) or to cerebral degenerative changes (e.g., in Parkinson's and Alzheimer's diseases).

Deficiency of olfactory function in old age may be one of the causes of poor appetite and irregular eating habits (75). Adding flavor to foods enhances meal satisfaction and improves dietary intake and body weight in the elderly (76,77).

Taste

Taste or gustatory sense is served by special taste buds located in the tongue and other regions of the oral cavity (mouth and pharynx). Taste buds contain taste cells, chemoreceptors similar to the olfactory cells and like them, in constant renewal (i.e., they have a 10-day cycle). Taste cells are in synaptic contact with the gustatory sensory neurons carrying, via the taste nerves, the information to the taste center in the medulla, and from there, gustatory stimuli are relayed to other sensory centers in the brain.

Classically, there are four primary tastes: sour, sweet, salty, and bitter, localized in different regions of the tongue: sweet at the tip, bitter at the back, and sour and salty on the sides. With aging, a spectrum of changes has been described in the sense of taste, although *it is generally accepted that, compared to olfaction, taste is less affected (77–81).* As for smell, (*i*) threshold sensitivity to specific food stimuli and discriminatory ability to identify flavors in a mixture decline with aging and (*ii*) this decline may be ascribed to peripheral (e.g., loss of taste buds) and, possibly, central degeneration of neural centers of the gustatory system.

Smell and taste losses in the elderly can reduce appetite and food intake. These losses may fail detection of noxious substances and may alter neural-immune connections as well (77–81). Stimulation of taste and smell by increasing salivary flow and excitability of olfactory cells may improve eating habits and satisfaction of life. They may also stimulate immune responses and thereby may help to remedy the immune deficiencies and the dry mouth frequently affecting the elderly (76,77).

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The Skeleton, Joints, and Skeletal and Cardiac Muscles

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INTRODUCTION

The musculoskeletal system and the cardiac muscle, as other systems and organs of the body, pass through stages of growth, maturation, and aging. The first two stages have been studied extensively. The postmature stage, often associated with declining functional competence, is less well understood. The present chapter, after a short introduction, considers the aging-related changes in the skeleton, including both bones and joints (see section entitled Aging of the Skeleton). In the section entitled Aging of Muscle, sarcopenia is presented as a fundamental manifestation of the aging skeletal muscles. Sarcopenia, in association with the aging-related changes of the skeleton presented here and those of the nervous system presented in Chapter 7, represents one of the causes of impaired mobility in the elderly. The possibility of recuperative effects on muscle size and strength by nutrition and physical exercise are discussed in the respective Chapters 23 and 24. Some aspects of the aging circulatory system presented in previous chapters have focused on the progression of atherosclerosis (Chapter 15) and the role of lipoproteins (Chapter 16) in aging. In the section entitled Aging of Cardiac Muscle, the discussion centers on the aging-related changes in the cardiac muscle as well as some of the pathologic consequences of cardiac dysfunction and their impact on survival and functional competence of old age.

Compared with other systems, the skeleton is rugged and durable; it usually carries on its tasks into advanced years, resists damage well, and has an efficient self-repair capability; indeed, normal and/or abnormal aging of the skeleton is seldom the direct cause of death. However, bones, the major components of the skeleton, are subject, as are other parts of the body, to various hazards as well, primarily trauma, but also deficient metabolism and nutrition and multiple degenerative changes. Major functions of the skeleton are summarized in Table 1 and bone structure and major factors responsible for bone maintenance are presented in Figure 1 and Box 1.

While aging of the skeleton usually occurs without conscious awareness on the part of the individual, *aging of the articulations (joints) induces considerable physical pain and causes severe disability.* Arthritic diseases, some of the most common expressions of joint aging, are among the most frequent and debilitating diseases of old age. The functional impairment and pain resulting from normal or pathologic aging of the joints limit the movement of elderly individuals, thus hindering their ability to care for themselves and eroding their independence (Chapter 3). Loss of mobility contributes also to the decline in competence of other systems (such as the circulatory system). Some pathology of the joints begins at a relatively young adult age (rheumatoid arthritis may begin in adolescence) but shows increasing prevalence with advancing age: prevalence of rheumatoid arthritis is less than 1% before the age of 30 years and thereafter rises in each decade to 1% to 3% in the late 50s and to 8% to 11% in the late 60s. Other manifestations of joint aging occur at later ages; *osteoarthritis,* which affects 85% of persons 70 to 79 years of age, is one of the major causes of invalidism, confining the affected individuals to bed or to the wheelchair. Major types of joints and their structure and function are presented in Figure 2 and in Box 2.

The study of aging processes in muscle is greatly complicated by the fact that muscle fibers do not constitute a homogenous tissue (e.g., differences between skeletal and cardiac muscles). In addition, the state of the tissue at any time depends on the extent and nature of many influences (e.g., nutrition, neural control, and hormones), some of which are specific to muscle (i.e., physical exercise). Thus, the characteristic decline of muscular performance associated with advancing age is variable and may be caused not only by primary aging changes in the muscle fibers but also by aging of other body systems such as nervous, vascular, and/or endocrine systems.

The function of the heart is a major determinant of blood circulatory competence, and any changes with aging in cardiac function are likely to play a central role in aging of the organism. The debate of whether atherosclerosis (Chapters 15 and 16) and its cardiovascular consequences (e.g., cardiac infarct, stroke, aneurysm, and gangrene) are superimposed on the aging process itself or merely occur in older persons due to their longer exposure to risk factors continues to generate considerable interest. Cellular and molecular changes with aging in cardiac function, and the possibility of preventive or

TABLE 1 Major Functions of the Skeleton

Major functions of the skeleton provide:
Body support
Body movement
Storage of calcium and other minerals, thereby aiding in mineral homeostasis: storage of calcium provides, upon request, calcium to blood and blood calcium is important for regulating essential cellular activities
Maintenance of acid–base balance in association with the lungs and kidneys
Regulation of phosphate and carbonate for buffers
Storage for bone marrow, where white and red blood cells and platelets are produced; the important functions and the changes with aging of these cells are discussed in Chapter 14 (the immune system), in Chapter 17 (hematopoiesis and red blood cells), and in Chapter 15 (platelets)

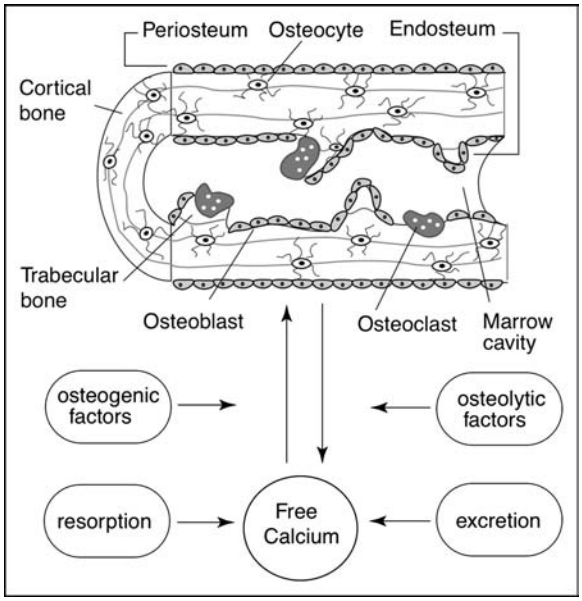

FIGURE 1 Factors responsible for maintenance of bone structure. Calcium in bone is derived from circulating free calcium, which depends on calcium absorption and excretion. Formation of new bone (osteogenesis) depends on osteoblasts and destruction of old bone (osteolysis) depends on osteoclasts. New bone is continuously formed in the periosteum and endosteum. Active bone is formed of osteocytes, which are part of the cortical and trabecular bone. In the bone marrow, blood cells are continuously produced.

therapeutic interventions, have been studied primarily in experimental animals (rodents). There is good reason, however, by incorporating basic knowledge with new technology, to look optimistically to progress in our understanding of cardiac function as well as in prevention and treatment of cardiovascular diseases in old humans.

■ AGING OF THE SKELETON

The skeleton, the heaviest and most durable part of the body, provides the body framework and derives its properties from the unique characteristics of bone remodeling. *Maintenance of bone structure and function is a dynamic two-phase process by which bone mass is regulated throughout adult life and involves bone resorption by osteoclasts (a process of breaking down of differentiated tissue and assimilation of resulting particles) and new bone formation by osteoblasts* (1–4). These processes are influenced by a number of factors, as illustrated in Figure 1. *Bone is a special form of connective tissue* made up of microscopic crystals of phosphates of calcium (particularly hydroxyapatites), within an extracellular matrix, consisting primarily of collagen and various proteins (i.e., glycoproteins) and proteoglycans (5). Proteoglycans are high molecular weight complexes of proteins and polysaccharides that form ground substances in the extracellular matrix of connective tissue and serve as lubricants and support elements.

The calcium of bone is derived from circulating free calcium; blood calcium levels partly depend upon calcium absorption, mainly through the gastrointestinal tract (Chapter 19). Levels of circulating calcium also depend on its rate of excretion, mainly through the kidneys (Chapter 18). A number of osteogenic factors promote bone formation through stimulation of osteoblastic activity or inhibition of bone resorption through osteoclastic activity (see below).

Although osteoblasts and osteoclasts are involved in bone remodeling, osteocytes are occupied with the maintenance of bone function and are part of the machinery guarding the integrity of the structure and function of bone itself. They are the most numerous and longest-lived bone cells. They probably sense the occurrence of bone deformation and signal the need for adaptive remodeling of bone size, shape, and strength to accommodate the prevailing loads. The death of osteocytes by apoptosis in estrogen deficiency, corticosteroid therapy, and advancing age is associated with the loss of bone strength, even before any actual loss of bone mass. The number of osteocytes that undergo apoptosis may then provide the topographic information needed to target removal by osteoclasts, as the latter cells must be informed where to go and how much to reabsorb (6). However, net bone formation during growth and the negative balance or bone loss in advancing old age are small. For these reasons, the rate of bone gain or loss are driven more by a high remodeling rate than by the magnitude of the actual changes in the bone unit. Largely on the basis of cross-sectional data, bone loss appears

BOX 1 *Bone Structure and Histology*

Bone is a hard form of connective tissue (Chapter 21) consisting of bone cells or osteocytes imbedded in a collagenous protein matrix that has been impregnated with mineral salts, especially phosphates of calcium. The matrix consists of two phases: an organic one that comprises collagen, proteins, and glycosaminoglycans (polysaccharide and protein complexes) (Chapter 21) and an inorganic one that contains mainly hydroxyapatites (calcium phosphate) and minor amounts of other minerals. The collagen fibers provide resilience and the minerals hardness. Given individual variations, it can be stated that, in childhood, about two-thirds of the bone substance is composed of connective tissue, whereas in aged individuals, about two-thirds consists of minerals. This transposition in content results in decreased flexibility and increased brittleness with advancing age (1).

Histologically, bone is distinguished as (*i*) "compact bone" found in shafts of long bones and outer surfaces (periosteum) of flat bones, (*ii*) "cancellous bone" making up the trabecular space containing the bone marrow, and (*iii*) "woven bone," which is an immature form of bone also involved in fracture repair. Bone cells are primarily concerned with bone formation and resorption; *osteocytes,* mainly in cancellous bone, maintain bone structure, *osteoblasts,* cells in the periosteum and endosteum (tissue surrounding the inner bone cavity) are bone forming cells, and *osteoclasts,* found in the same regions as the osteoblasts, resorb bone by phagocytosis and digestion in their cytoplasm. Bone is well vascularized and contains an abundant nervous network.

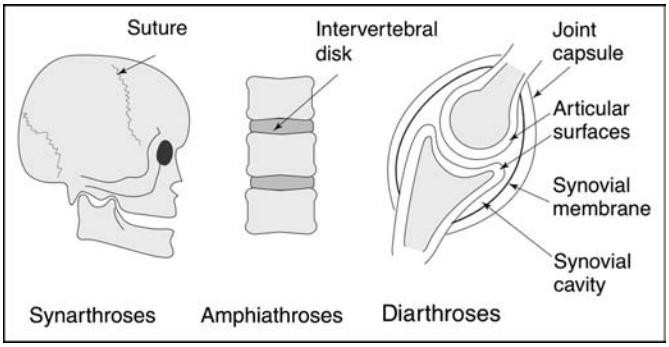

FIGURE 2 The three major types of joints.

to begin between the ages of 18 and 30 years but the process is slow because the remodeling is also very slow (7).

■ Patterns of Bone Remodeling

Throughout life, the purpose of the skeleton is a persistent attempt to adapt the material composition and structure of the bones to the different stresses (e.g., imposed by gravity) and prevailing loads that challenge the body in its daily activities. Bone remodeling is influenced by hormones in the systemic circulation [glucocorticoids, sex hormones, parathyroid hormone (PTH), and calcitonin], by local growth factors, and by vitamin D. Although net bone mass does not change throughout much of adult life, bone is never metabolically at rest, but, rather, it is in a continuous state of flux and constantly remodels and reapportions its mineral stores along lines of mechanical stress (Box 1). Bone maintenance depends on:

- Formation of new bone by the osteoblasts
- Resorption of old bone by osteoclasts
- Carrying out of mature bone functions by osteocytes

During growth, the processes of building new bone and of resorption of old bone fashion a structure able to accommodate even Herculean loads and to maintain strength. With advancing age, the balancing of adaptive and compensatory responses to loads is altered by the accumulation of abnormal changes in the bone remodeling machinery. Excesses or deficiencies of hormones, growth factors, declining muscle mass and mobility, nutritional deficiencies, and other factors tend to overwhelm the declining remodeling ability to adapt bone to prevailing loads (8).

The mechanisms responsible for the calcification of newly formed bone matrix are incompletely understood despite intensive investigation: precipitation of calcium phosphate in bone may depend on some critical calcium concentration and is associated with the activity of certain enzymes (e.g., alkaline phosphatase) and specific proteins capable of binding calcium.

With aging, the balance between rates of bone formation and rates of bone resorption is disturbed and the ensuing changes lead to a decrease in bone mass. After the age of 40 years, formation rates remain constant whereas resorption rates increase. Over several decades, the skeletal mass may be reduced to half the value it had at 30 years. Progression of this loss may be measured by counting the number of Haversian canals or osteons (i.e., cavities represented by cylinders of bone containing a central blood vessel and nerve fibers). With advancing age, there is an increase in osteons at the bone shaft region, beginning at the ends of long bones. Between the ages of 42 and 52 years, this medullary cavity extends to the neck of the femur (a long bone extending from the pelvis to knee); between 61 and 74 years, the cavity reaches the epiphyseal line close to the articular end, where the bone proper joins the epiphyseal cartilage. Changes that occur in the humerus (the arm bone extending from shoulder to elbow) may serve as example: after 61 years, the outer bone surface becomes rough and the cortex thinner; the medullary cavity reaches the epiphyseal line; after 75 years, there is little spongy tissue left and the cortex is very thin. Joint surfaces become very thin and may collapse. A major factor in these age-related changes is a loss of bone matrix that is confined primarily to the inner bone core. In the elderly, the periosteal tissue at the outer surface of the bones tends to remain constant or even increases in some bones (e.g., metacarpal bone of the hand) (1), but the endosteal tissue at the interior of bones is increasingly resorbed (2).

Long bones grow in length by endochondral apposition (i.e., contact with the cartilage) and, in width, by deposition of bone on the outer surface (periosteum). During bone growth, gender and racial differences are responsible for variations in endochondral apposition and cortical resorption (9). With aging, major changes involve a reduction in cortical thickness and, thus, induce differences in bone strength. Variations are also found in the vertebral bodies, characterized by a porous structure; bone strength is greater in men than in women and this difference is attributed, in part, to the men's larger vertebral cross-sectional area (10). *The structure and composition of bone may also vary with load. For example, adaptation of size and shape of the arms of tennis players is well documented* (11).

■ Cellular Elements Involved in Bone Remodeling

In the remodeling process of bone, the function of osteoblasts and osteoclasts are intimately linked: osteoblasts synthesize and

BOX 2 *Structure of the Joints*

The articular system is comprised of simple and complex joints associated with the skeletal system and blood and nerve supplies to the joints. They represent junctions between two or more bones or cartilages (Fig. 2).

In the skull, the joints (synarthroses) are immovable and the connected bones are separated by a very thin layer of connective tissue.

The articulations between the vertebrae (amphiarthroses) are somewhat more movable and the bones are united by dense fibrous tissue and intervening cartilage.

Most bones are freely movable (diarthroses) as the adjoining ends are coated with smooth cartilage separated by a short tube of strong fibrous tissue containing synovial fluid.

Joint cartilage is a special type of connective tissue with an extracellular matrix of proteoglycans and collagen, synthesized by the cartilage cells or chondrocytes.

secrete molecules (3) that, in turn, initiate and control osteoclast differentiation (4). Osteoclasts are specialized macrophages (12) (Chapter 14); their differentiation is regulated by growth factors such as granulocyte/macrophage colony-stimulating factor, a cytokine (Chapter 14), by a nuclear factor kappa B [receptor activator of nuclear factor kappa B (RANK)] and its ligand (RANKL), and by the protein osteoprotegerin (13,14). Binding of nuclear factor RANK to its ligand (RANKL) provides signals for survival and proliferation of osteoclasts whereas overexpression or administration of osteoprotegerin reduces osteoclast formation (13,14). RANK and RANKL are members of the tumor necrosis factor (TNF) and TNF receptor families, respectively (14,15). Other molecules important in the resorption process, and, hence, in bone remodeling, are the *integrins,* a superfamily of adhesion molecules involved in the adhesion of cells to the extracellular matrix; for the resorption process to take place, recognition and physical intimacy are essential between the osteoclasts and the surrounding matrix and these are controlled by integrins (16).

The realization that the osteoclast is part of the monocyte–macrophage family (Chapter 14) has prompted the development of techniques to stimulate bone formation in which macrophages may be made to differentiate into osteoblasts or, conversely, differentiation into osteoclasts may be inhibited or slowed (17,18). For example, osteoporosis, particularly frequent and severe in women after menopause (Chapter 10), is associated with a rise in osteoclast number, driven by increases in cytokines that induce osteoclast generation (19). Replacement therapy with estrogens or selective estrogen receptor modulators will block this increase and slow down the osteoporotic process and its consequences (falls and fractures) (Chapter 10). A better understanding of the biology of osteoblasts and osteoclasts provides new opportunities for developing therapies to act, singly, or in synergism with other substances (e.g., growth factors and hormones), to treat diseases of bones (18).

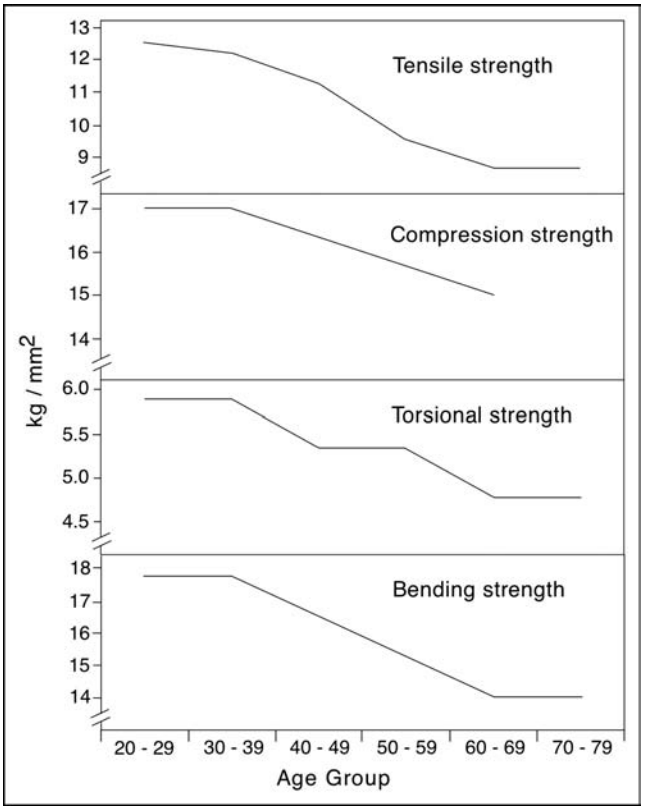

FIGURE 3 Changes in bone strength with aging. Bone strength provides the ability of the bone to undergo force applied in (*bottom to top*) bending, twisting (torsional strength), compressing, and stretching (tensile strength).

■ Bone Strength

Bone strength is an important property that allows bones to withstand the forces applied in the various movements of daily life. Several tests of bone strength have been studied: the resulting observations show life cycle trends from youth through maturity into old age (Fig. 3) and disclose a consistent decline of strength with aging (20). Comparison of the change in strength of several musculoskeletal components indicates that the fastest decrease in strength involves the cartilage followed by muscle, bone, and, finally, tendon. Also, the comparison of bone with other tissues shows a much slower rate of decline for bone than for intestine and muscle but faster than for kidney.

■ Factors Affecting Bone Aging

Role of Calcium in Bone Metabolism

Adequate circulating calcium is vital in maintaining bone mass. Indeed, the body regulates few parameters with greater fidelity than the concentration of extracellular and intracellular calcium. As mentioned above, *the constancy of extracellular calcium levels depends, in part, on its absorption in the intestinal mucosa and its excretion from the kidneys* (Fig. 4). The regulation of the relationship between calcium in blood and calcium in bone depends primarily on the PTH but also involves other factors acting on calcium in tissues and cells. Calcium has many vital functions and is known to be a "universal regulator" (21–23). In addition to its role in bone structure and function, calcium regulates many important functions that can be grouped into six categories:

- Cell movement (e.g., muscle contraction, ciliate and flagellate movement, and chemotaxis)
- Cell excitability (e.g., action potential at neuromuscular junction, photoreceptor, and response)
- Cell secretion [e.g., neural (neurotransmitters), endocrine (hormones)]
- Cell phagocytosis (e.g., uptake of particles)
- Intermediary metabolism and respiration (e.g., role in glucogenesis, lipolysis, and blood coagulation)
- Cell reproduction (e.g., regulation of various phases of reproduction)

Despite the universal role of calcium in cell function and metabolism, relatively few studies have investigated in depth the potential changes in calcium with aging. Some of the most prominent actions of calcium related to aging are listed in Table 2. Considering the importance of calcium in regulation of cell metabolism and function, the study of changes in calcium concentration and the consequent alterations in cell function should be pursued actively as these changes may have an important bearing on the causes and course of the aging process.

Calcium of the human body comprises 1.5% of body weight (1100 g, 99% of which is in the skeleton) (23). In healthy individuals, dietary calcium absorption is typically 25% of ingested calcium. The need for calcium increases with

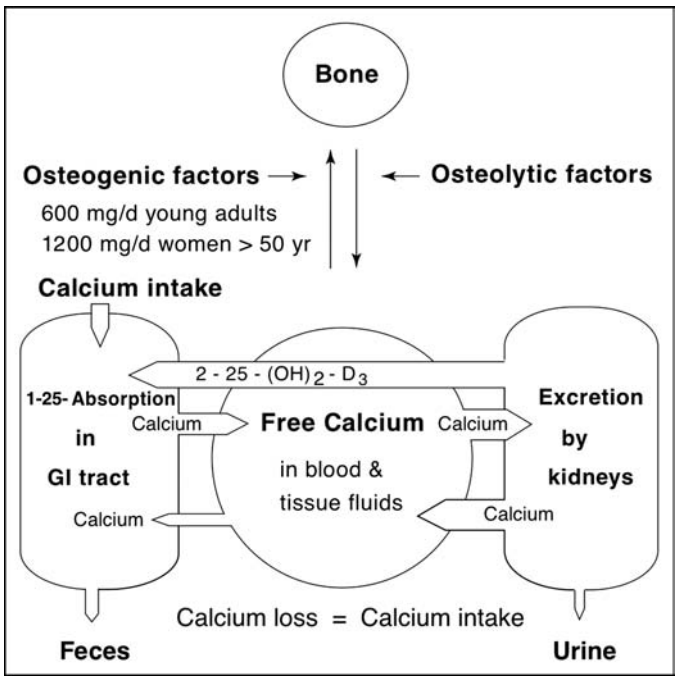

FIGURE 4 Calcium metabolism. Note the different daily requirements of calcium with age: almost double in women after 50, as compared to young adults. *Abbreviation*: GI, gastrointestinal, 1-25 dihydroxy cholecalciferol or calcitriol.

aging. For example, while for young adults the recommended daily allowance is 600 to 800 mg, this value is increased to 1200 mg and even more (in some special conditions) than 1200 mg for women over 50 years of age. This increased need for calcium may be explained by a progressively less efficient absorption from the upper intestinal tract (Chapter 19). Hence, in the elderly, more dietary calcium is needed to maintain an adequate calcium balance (Chapter 23). In addition to dietary calcium, a dose of 400 IU vitamin D3 is recommended to facilitate optimal intestinal absorption of calcium for individuals of 50 to 70 years, of 600 IU for those older than 70 years, and of 800 to 1000 IU for those infrequently exposed to sun and all postmenopausal women. Calcium absorption is not only a vitamin D–dependent process but it is also influenced by age, weight, menopausal status, body weight, and dieting (24).

Body weight is related to bone mass: thus, low body weight is usually associated with low bone mass (24) and increased risk of fractures (25). Conversely, obesity is associated with increased body mass and reduced bone turnover and loss (26,27). Although the additional bone mass in the obese is small, the risk of osteoporosis is less in the obese than in lean subjects. Severe chronic malnutrition may enhance calcium absorption to maintain normal calcium levels.

Epidemiologic studies in elderly men, older than 70 years of age, reported that weight loss (voluntary or involuntary) is an important predictor of bone loss and increased incidence of osteoporosis (28). Given the importance of sex hormones in promoting bone growth and maintenance, bone loss generally begins later in men than in women, perhaps due to persistently higher level of sex steroids in men until 65 to 70 years (29) (Chapters 10 and 11).

TABLE 2 Intracellular Calcium-Dependent Changes Relating to Aging

Actions	Relation to aging
Intermediary metabolism and respiration	Activation of oxygen radicals Obesity and diabetes Arthritis and other diseases
Tissue calcification	Loss of bone calcium (osteoporosis) Abnormal deposition on normal and injured tissues (e.g., atherosclerotic lesions)
Cell excitability	Changes in cell potentials Changes in response to drugs
Cell secretion	Alterations in neurotransmitter and hormone production
Phagocytosis	Alterations in immune responses

Note: The most prominent involvements of calcium related to aging appear to be (*i*) triggering the intracellular production of oxygen radicals, which, as discussed in Chapter 5, lead to the accumulation of potentially toxic substances; (*ii*) affecting normal and abnormal bone metabolism; a possible role of calcium in the pathogenesis of a number of diseases such as arthritis, obesity, diabetes, cystic fibrosis, etc.; calcification of injured and necrotic tissues such as occurs in atherosclerotic lesions (Chapter 15); (*iii*) inducing age-related changes in cell excitability and in the action of several classes of drugs such as anesthetics, analgesics, cardiovascular drugs, etc.; (*iv*) being involved in ion movement and affecting neurotransmitter and hormone production and secretion; and (*v*) participating in phagocytosis, with relation to aging of the immune system (Chapter 14).

Hormonal Regulation of Bone Metabolism

Several hormones that affect bone formation are briefly discussed here. Additional information is available in Chapters 9 to 12, where the corresponding endocrine gland is presented in more detail. *Hormones may affect bone function by stimulating resorption or formation.* Bone remodeling is accomplished by the interaction of several growth factors and locally acting cytokines operating on diverse cell populations in a highly coordinated manner. Three hormones, PTH, calcitonin, and calcitriol are primarily concerned with calcium metabolism (Fig. 5).

- *Parathyroid hormone* (PTH) directly increases bone resorption and mobilizes calcium from the bone into the blood, thereby elevating plasma calcium. It also depresses plasma phosphate by increasing its urinary excretion.
- *Calcitonin* lowers body calcium by inhibiting bone resorption.
- *Calcitriol* (dihydroxycholecalciferol, vitamin D3), a sterol derivative, increases calcium and phosphate absorption from the intestine and decreases their renal excretion; it also enhances bone resorption.

Bone changes with aging apparently occur without marked alterations in the parathyroid gland or in the levels of the PTH. However, in some old women, PTH levels increase with aging, but the contribution of this increase to bone loss may be minimal (31). Some racial and gender differences have been reported: Afro-American and Asian postmenopausal women have lower levels of PTH and higher levels of calcium into advanced age than Caucasian women; men maintain lower PTH levels coincident with a lower incidence of osteoporosis than women.

As stated in Chapter 12, PTH hormone levels increase with aging; this increase may reflect a compensatory response

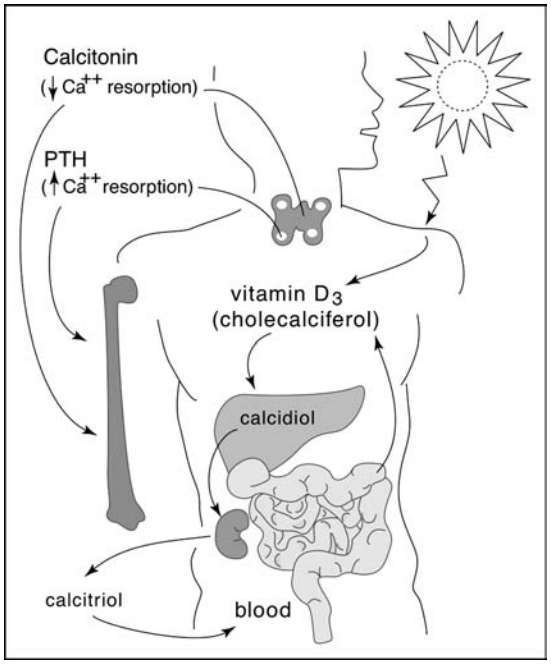

FIGURE 5 Major hormones that regulate calcium metabolism. The parathyroid gland secretes the PTH, which acts directly on bone to increase its resorption to mobilize calcium, thereby increasing (+) plasma calcium. Calcitonin from the thyroid gland inhibits bone resorption and lowers (–) plasma calcium. Calcitriol, a sterol derivative, increases calcium absorption from the intestine and decreases renal excretion. It also enhances bone resorption. Vitamin D3 derives from dietary sources and is absorbed in the intestine or is formed in the skin under the influence of ultraviolet radiation. In the liver, vitamin D3 is converted to calcidiol, which, in turn, is activated in the kidney to calcitriol, the most active form in calcium regulation. *Abbreviation*: PTH, parathyroid hormone. *Source*: From Ref. 30.

to the reduced intestinal absorption of calcium and the need for the organism to keep calcium levels consistently optimal. There is some evidence that intermittent PTH administration increases mechanical strength and mass of bone and that this action is mediated by the transformation of the precursor cells of bone into osteoblasts (32) and/or by preventing apoptosis of osteoblasts (33,34). Diseases of the parathyroid gland that may be relevant to bone structure and function are summarized in Box 3.

Calcitonin inhibits bone resorption by blocking osteoclastic activity (Chapter 12). It is secreted by the C-cells of the thyroid gland; it fine-tunes extracellular calcium regulation. Over time, a resistance to the hormone develops due to loss of receptors (35), therefore restricting the therapeutic use of the hormone (Chapter 12).

Calcitriol derives through a series of steps either from the diet through absorption in the intestine or through transformation of cholesterol in the skin by the action of sunlight. In the skin, the first step is the formation of previtamin D3 that is converted into vitamin D3 (cholecalciferol). Vitamin D3 from the skin and intestine is carried to the liver where it is hydroxylated to calcidiol, which, in turn, is carried to the kidney where it is again hydroxylated to form the active product calcitriol (Chapter 23).

Other hormones that affect bone formation include the following:

1. *Glucocorticoids* lower plasma calcium levels and, over long periods of time, may cause osteoporosis by decreasing bone formation (due to inhibition of cellular replication, protein synthesis, vitamin D production, intestinal absorption of calcium, and function of osteoblasts); they also increase bone resorption (due to stimulation of PTH secretion) (36) (Chapter 9).
2. *Growth hormone (GH)* increases calcium excretion in urine but, because it has a greater effect in increasing intestinal absorption of calcium, overall it produces a positive calcium balance. *Somatomedins* [insulin-like growth factors (IGFs-I and -II)], induced by GH also stimulate protein synthesis in bone. Risks and benefits of GH and IGF administration to increase bone mass in elderly men and women are discussed in Chapter 9 (37).
3. *Sex hormones* have stimulatory actions on bone growth and maturation during the periods of childhood and adolescence, which are well known; those occurring during aging have been considered in Chapters 10 and 11.
4. *Thyroid hormones* may induce hypercalcemia and hypercalciuria and in some cases may also produce osteoporosis, but the mechanisms of these actions are unclear (Chapter 12).
5. *Insulin* promotes bone formation whereas insulin deficiency (diabetes) is associated with significant bone loss (Chapter 13).

BOX 3 *Diseases of the Parathyroid Gland*

Structure, hormone, and hormone regulation of the parathyroid gland are presented in Chapter 12. Diseases of this gland are infrequent at all ages; however, they bear mentioning here for the symptoms associated with aging may mask parathyroid pathology (31–33). Indeed, if the disorder is recognized as a possible parathyroid dysfunction and is corrected, the symptoms described as aging changes may be ameliorated. Hyperparathyroidism presents with a variety of symptoms such as increased plasma calcium (hypercalcemia), renal calculi, peptic ulceration, and, in a few individuals, mental aberrations with psychotic components. The latter have been sometimes erroneously identified as senile dementia of the Alzheimer type (Chapter 7). In advanced stages, characteristic bone lesions are present. Hypoparathyroidism is quite rare at all ages and is easily recognizable for it generally follows ablation of the glands during thyroid gland surgery.

6. *Local-growth promoting or inhibitory factors* may also influence bone formation and resorption, thereby participating in continuing bone remodeling. Most of these factors promote bone growth (e.g., epidermal growth factor, fibroblast growth factor, platelet-derived growth factor, and transforming growth factor) whereas some inhibit growth [e.g., tumor necrosis factor (TNF)] (15). Prostaglandin E secreted by certain tumors increases plasma calcium levels. Osteoclast-activating factor, produced by lymphocytes, induces bone loss in tumors of the bone marrow. Cytokines may play a key role in bone metabolism by their ability to promote differentiation and maturation of either osteoblasts or osteoclasts.

The Role of Physical Activity in Bone Metabolism

Physical activity also affects bone in several ways; it increases stress and strain on the skeleton due to muscular contraction and gravity; it improves blood flow to exercising muscles and, indirectly, increases cardiac output; and it stimulates bone mineral growth. These effects are most marked in the young but are also present, although to a lesser degree, in the old (Chapter 24).

■ Aging-Related Fractures

Fatalities and injuries associated with falling and automobile crashes represent a significant public health problem in older populations. Falls represent the leading cause of nonfatal injury and automobile crashes of accidental death in populations aged 65 years and older (38). Fall injuries and crashes represent "geriatric syndromes," meaning that they are caused by a variety of concomitant factors, among which bone fractures predominate (34). Although not as well developed as that of falls, the study of automobile crashes is methodologically related to the study of falls: many of the same factors that elevate the risk of falls and injuries are also valid causes of injuries from automobiles.

Fracture patterns in the elderly differ markedly from those in the younger adult. Whereas in the younger adult (20–50 years), considerable violence is required to break the bones, in the elderly, fractures result from minimal or moderate trauma. This is due to the progressive loss, with aging, of absolute bone volume, both compact and spongy bone. Although the consequences of bone loss become manifest at 40 to 45 years of age in women and 50 to 60 years in men, bone loss may, in fact, begin much earlier, at the end of the growth period.

In younger adults, fracture incidence is higher in males than in females, perhaps due to the greater physical activity and exposure to accidental falls in the former. In the elderly, however, the reverse is true, especially in the case of fracture of the vertebral bodies, the lower end of the forearm, and the proximal femur. The relation between menopause and accelerated bone loss (osteoporosis) in females is discussed in Chapter 10.

Not only is the incidence of fractures higher in the elderly than in the young but also the sites of fracture are often different (39). In the elderly, fractures occur through cancellous bone, usually next to a joint, rarely through the shaft of the bones, the most frequent site of fracture in the young. Orthopedic interventions to prevent or repair fractures are more difficult and recovery is slower in the elderly than in the young, due to bone fragility and overall less robust condition of the elderly (39).

In addition to osteoporosis (Chapter 10), other age-related bone disorders are associated with fractures. One of the most frequent of these is Paget's disease or Osteitis Deformans characterized by pain, deformity, and fractures of the bones. The incidence is higher in men than in women. The early lesions are localized osteolytic lesions followed by accelerated bone turnover, that is, rapid remodeling leading to excessive and somewhat disorganized ("mosaic" or "woven" aspect) bone–tissue balance. The resulting bone, although more dense than normal bone, has lost some of its elastic properties and is more susceptible to fractures. Complications include neoplastic transformation, neurologic signs due to compression of central nervous system structures, and heart failure. Management is based on symptomatic administration of aspirin for pain relief and of calcitonin (see above) and biphosphonates for inactivation of osteoclasts. Biphosphonates are synthetic analogs of pyrophosphate that are not biodegradables, that have a skeletal half-life of years, and that adhere to mineralized surfaces. They are ingested selectively by osteoclasts where they disrupt energy metabolism or specific enzymatic pathways and thereby decrease bone resorption. Some side effects include gastrointestinal irritation and possibly gastroesophageal reflux, in which case, they should be contraindicated (40). Research is still needed to determine if prolonged suppression of bone remodeling may be eventually deleterious (41).

Ethnic Differences in Bone Mass and Consequences in Aging

The rates of fractures due to osteoporosis and other bones diseases associated with old age are substantially lower among blacks than among white persons (three times lower among black women and five times lower among black men) (42). These differences are generally attributed to the 10% to 20% greater mass and density of adult bone in blacks. This greater bone mass and density are apparent before birth (43,44). In blacks, bone remodeling also proceeds at a lower rate than in whites. Such observations suggest that hormonal, metabolic, and genetic factors play a role in these black/white differences. Age-matched bone density and mass among other ethnic groups in the U.S. population, although less studied than those among blacks and whites, show significant differences; for example, a survey of proximal femur fracture rates reveal that incidence rates are higher among Asian than among white women.

■ Aging of the Joints

Joints or articulations comprise the junction between two or more bones (Box 2). They contribute to the function of the skeleton in promoting movement of body parts and locomotion. The pattern and prevalence of joint changes in the elderly reflect that (*i*) certain disorders arise more frequently with increasing age and (*ii*) there is a steady cumulative effect with age inasmuch as many joint disorders are chronic (43–46). By age 65, 80% of the population has some articular disorder. Beyond the aging changes affecting the articulations themselves, the problems of added illness, frailty, diminished motivation, and social isolation affect the problems of management and outcome of the disorder (Chapter 3).

The list of musculoskeletal disorders of the elderly is quite long and only an abbreviated one is presented here (Table 3); one of these disorders, osteoarthritis, will be briefly compared with "normal" aging changes. Joint disorders are essentially divided into two major types (Table 3).

TABLE 3 Types of Musculoskeletal Disorders

Nonsystemic	Systemic
Noninflammatory	Nonautoimmune
Osteoarthritis	Polymyalgia rheumatica
Inflammatory	Autoimmune
Gout	Rheumatoid arthritis
	Systemic lupus erythematosus

- Those that affect joints without involvement of other organ systems
- Those that affect the skeleton as a manifestation of systemic disease involving several organ systems

Most of the systemic disorders are often referred to as "collagen-vascular" as they are frequently manifested by changes in connective and/or vascular tissues. Others are referred to as "autoimmune" because they are associated with the presence of antibodies that attack and damage the host's own tissue (Chapter 14). Among the nonsystemic diseases, osteoarthritis and gout are the most frequent.

Pathologic and Clinical Aspects of Osteoarthritis

Osteoarthritis (also known as degenerative arthritis or degenerative joint disease) and rheumatoid arthritis are examples of articular disorders occurring with increasing frequency from middle to old age. In fact, osteoarthritis from the Greek osteo, meaning bone, and arthro, meaning joint, is so common among the elderly that it is often assumed to be a normal accompaniment of aging rather than a disease. It affects currently about 21 million people in the United States, accounting for 25% of visits to primary-care physicians and half of the nonsteroidal anti-inflammatory drug (NSAID) prescriptions (see below).

Osteoarthritis is a condition in which low-grade inflammation results in pain in the joints, caused by wearing of the cartilage that covers and acts as a cushion inside the joints. Radiological signs of osteoarthritis are almost universal in the later decades of life: 80% of individuals 65 years of age and older are affected, but only 60% of these will be symptomatic. In a large proportion of the elderly, early osteoarthritic lesions are often discovered incidentally (for example, osteoarthritis of the spine at the occasion of a chest X ray).

It is particularly difficult to draw a dividing line between disease and normal aging with respect to degenerative changes of the cartilage (43). In fact, it has been suggested that these alterations of the cartilage may be extremely important in the etiology of osteoarthritis (44). With aging, decreased proteoglycan synthesis, loss of chondroitin sulfate and collagen glycation (i.e., covalent attachment of a carbohydrate to a polypeptide or polynucleotide) render the collagen network of the cartilage stiffer, more cross-linked, and more prone to fatigue (45).

With aging, bony excrescences or osseous outgrowths (called osteophytes or bone spurs) occur on the heads of long bones, for example, on the head of the femur in 33% of individuals beyond 50 years of age; they are viewed as a purely age-related phenomenon. *Areas where smooth cartilage has been replaced by a rough surface occur in all joints and are often seen as early as the second decade of life and spread to the periphery of the joint with age.*

The undulations and hollows, which can be seen on the cartilage surface by electron microscopy, increase both in depth and in diameter with increasing age, and the outer surface irregularities become more common. Whether these consistent surface changes may be related to the development of osteoarthritis is not known. Osteoarthritis is associated with cartilage thinning.

Such findings are contrary to the observation that the cartilage, in the absence of pathology, actually becomes thicker with increasing age (46) except in the case of the patella (kneecap) that becomes thinner, especially in women. As cartilage ages, it looses some of its elasticity and becomes more easily stretched. Such changes lead to easier fatigability and higher susceptibility to osteoarthritis. There is a progressive pigmentation of the cartilage cells due to deposition of amino acid derivatives (probably from the protein of the matrix) and a gradual reduction in collagen but no change in water content; this latter change is invariably associated with the early stages of osteoarthritis.

The major symptoms of osteoarthritis are pain, loss of mobility, and, often, stiffness. They affect usually hands, feet, spine, and the large weight-bearing joints such as the hips and knees. Normal and pathological changes of the skeleton in the elderly severely curtail the well-being of this population and contribute significantly to immobility. It is difficult at best to encourage an elderly individual to maintain a program of physical fitness or activity if mobility is limited by joint pain. Abnormalities of the joints appear more clearly in the elderly population during their movements and motions and manifest themselves in terms of reduction of speed (47–51).

Chronic pain and disability of whatever cause or nature induce anxiety and dampen the morale of any individual, especially the elderly already prone to depression. For the elderly person who may be suffering from other concomitant losses, the inability to participate in certain physical activities can be quite devastating (Chapter 3).

Risk factors include heredity as well as a history of trauma or injury to a certain part of the body or bone. Little is known of the pathogenesis of osteoarthritis. Why the process occurs at an accelerated pace in some individuals and why it affects certain joints and not others remain a mystery.

Loss of proteoglycans represents the cause of early changes that occur in the articular cartilage that lines the joint surfaces of the bones. Subsequent changes involve stiffening of collagen. Eventually the joint space narrows. The bone underlying the articular cartilage is itself undergoing aging changes and becomes more susceptible to damage and microfractures. In the attempt to repair the damage, cysts and osteophytes form, superimposed on sclerosis.

Osteoarthritis is not a systemic disease; it affects only individual joints. Symptoms usually begin with aches and pain in the involved joints. Initially, pain occurs with motion or weight bearing. In later stages, pain can occur at rest. Hand involvement occurs more frequently in women than in men and usually affects the distal interphalangeal joints. Small paired dorsal cysts of these affected joints are called Heberden's nodes. The first carpometacarpal joint at the base of the thumb is also commonly affected and gives the thumbs a squared-off appearance. Hip involvement occurs more frequently in men and can present with knee or groin pain.

Diagnosis is made from clinical presentation and imaging [i.e., X rays and magnetic resonance imaging (MRI)]. It is important to differentiate the disease from other much more serious and life-threatening illnesses. For example, an elderly person with back pain should be carefully evaluated to ensure that no serious pathology such as metastatic malignancy exists before attributing the symptoms to osteoarthritis.

Management of Osteoarthritis: Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) and Other Therapeutic Interventions

Notwithstanding the ubiquitous distribution of osteoarthritis among elderly populations and its deleterious outcomes for the well-being and life expectancy of old people, advances in its management are still insufficient and, in some cases, ineffectual or even dangerous. *There are no measures known that prevent or reverse the progressive joint damage.* Therefore, management involves symptomatic relief of pain (analgesia). In view of the low direct inflammatory activity in the affected joints, some argue that acetaminophen (Tylenol), which acts primarily as an analgesic (i.e., to reduce pain) is a good first-line medication, particularly in individuals susceptible to the gastrointestinal side effects of aspirin. Other medications include the intra-articular injection of glucocorticoid hormones for temporary pain relief, due to the analgesic and anti-inflammatory actions of these hormones. However, adverse systemic effects may occur if the hormones are administered repeatedly (Chapter 9). The nonsteroidal anti-inflammatory drugs (NSAIDs) (such as aspirin and ibuprofen) and the more recently discovered class of cyclooxygenase inhibitors are more potent than Tylenol but may have more adverse effects. NSAIDs applied locally (on the joint) or administered systemically are among the most widely prescribed drugs in the world (52). It is estimated that 15% to 25% of the ambulatory elderly use NSAIDs; this usage may be higher in view of the fact that these drugs are sold over the counter. The widespread use of NSAIDs among the elderly is due to their unique features.

- They provide rapid and prolonged relief of pain.
- They are without risk of addiction or tolerance.
- They reduce swelling, tenderness, and stiffness of joints.
- More than 20 marketed medications [with different chemical structure, potency, and pharmacokinetic properties (Chapter 22)] provide great flexibility of choice.

NSAIDs used in the treatment of osteoarthritis and other joint diseases inhibit the enzyme cyclooxygenase that catalyzes the first reaction in the conversion of arachidonic acid to prostaglandins. These are ubiquitous molecules with diverse and numerous actions (Table 4) (53) and, in the case of osteoarthritis, decreased prostaglandin production/release is manifested by elimination of pain (analgesic action), reduction of fever (antipyretic action), and reduction of inflammation that accompanies articular damage (anti-inflammatory action) (54). *Unfortunately, NSAIDs induce severe side effects, particularly in the gastrointestinal tract where they may be responsible for impaired digestion (dyspepsia), erosion of the gastric mucosa, and gastric and duodenal ulcer (55) (Chapter 19); they may also have serious toxic consequences for the liver where they are metabolized (Chapter 19) and the kidneys, from where they are excreted (Chapter 18).* The identification of two isoforms of the enzyme cyclooxygenase, COX-1 and COX-2 and of two inhibitors of COX-2, seemed to offer a safer alternative to standard NSAIDs: these drugs have analgesic and anti-inflammatory efficacy comparable to that of the established NSAID with present, but significantly reduced gastrointestinal toxicity. COX-1 was found in many tissues, including the stomach where it provides some cytoprotection. COX-2 is also found in many tissues; it increases substantially during inflammation induced by a variety of stimuli (e.g., endotoxins and cytokines). Regrettably, Vioxx® (rofecoxib) apparently revealed in those using it an increase in the risk of heart attacks and strokes (56) (this problem occurred in those who had taken the medication continuously up to 18 months or more). The manufacturer, Merck, recalled the drug from the U.S. market in 2004.

Other helpful interventions include (Table 7)

- avoidance of heavy weight bearing (especially in osteoarthritis of the knee),
- weight loss (less mechanical stress on the joints),
- use of a cane and orthotics (mechanical devices to support and brace weak joints and muscles),
- muscle strengthening by physical exercise,
- oral or topical administration of chondroitin sulfate, glucosamine, collagen hydrolysate involved in the biosynthesis of glycosaminoglycans, the main ingredient of the synovial fluid (a fluid that fills the space between joints) and the cartilage; glucosamine is not found in food sources but it is produced by the body, and if, for some reason, the body does not produce it, its absence in the joint may contribute to the development of osteoarthritis (57–60), and
- surgical interventions (arthroplasty) especially for hip or knee replacement (61).

Irrespective of the severity or the location of osteoarthritis, conservative measures such as weight control, use of mechanical support devices, a rigorous diet rich in vitamins and low in saturated fats and oxidants (62), and a regimen of physical exercise may be helpful alone or in combination with the above-suggested interventions.

TABLE 4 Some Characteristics and Actions of Prostaglandins

Bioactive lipids generated by the action of cyclooxygenase from arachidonic acid
Produced by virtually all tissues but effects depend on specific prostaglandin and tissue/organ where produced
Released when cells are damaged
Detected in increased concentrations in inflammatory exudates
Mediate increased body temperature (fever) due to infection, tissue damage, inflammation, others
Contribute to pain and inflammation
Sensitize pain receptors, lower threshold of pain responses, potentiate transmission of pain signals
Protect gastric mucosal barrier
Regulate intestinal motility
Regulate renal blood flow and sodium reabsorption
Stimulate HCO_3^- secretion from the duodenum (to neutralize acid stomach contents)
Potent vasodilators and synergistic to other vasodilators (e.g., histamine)
Promote platelet agglutination

■ AGING OF MUSCLE

The major function of muscle is the generation of force and performance of work through the conversion of chemical to mechanical

TABLE 5 Major Cardiac Structures

The myocardium, the cardiac muscle closely resembling the skeletal muscle with the difference that myocardial fibers act as a syncytium
The cardiac conduction system, the specialized pacemaker tissue that can initiate repetitive action potentials and regulates autonomous rhythmic contractions
The endocardium, the endothelial layer lining the internal surface of the cardiac cavities
The pericardium, the serous membranous sac that encloses the heart

TABLE 6 Some Molecular Changes in Cardiac Muscle Cells with Aging[a]

Contractile proteins		Cytoskeletal proteins		Ca[b] sensitive proteins	
Changes	**Consequence**	**Changes**	**Consequences**	**Changes**	**Consequences**
Mutant gene encoding heavy chain of myosin, with preponderance for β isoenzyme	↓ Velocity of contraction saves energy; longer period of contraction permits blood ejection to last longer	↑ Microtubule component of cytoskeleton of cardiac muscle cells, perhaps due to posttranslational modifications of tubulin or ↑ MAPs	Cellular contraction dysfunction	↑Ca^{++} enzyme, calcineurin, activates the NF-AT proteins[b] by removing phosphate group and allowing them entrance into muscles, linking to transcriptional factor, GATA-4, and turning on the genes for hypertrophy	NF-AT–GATA-4 system induces cardiac-cell hypertrophy and dysfunction in vitro; cyclosporin-A inhibits calcineurin and prevents cardiac hypertrophy; in transgenic mice similar effects are seen
Mutant genes encode six proteins that regulate muscle contraction (two myosin light chains, tropo-myosin, troponins T and I myosin-binding protein C)	↑ Hypertrophic cardiopathy in young and old people with stretched-out thin-walled heart that fills with excess blood without efficiently pumping it out May impede smooth sliding of myosin over actin slowing down contraction Conversely, troponin T mutations accelerate sliding of actin past myosin consuming more energy and producing local energy shortage				
Gene mutations that encode structural proteins such as dystrophin, LIM, others	Failure of linking muscle cytoskeleton to extracellular matrix and, therefore, failure to anchor myocytes to extracellular milieu				

Apoptosis	
Changes	**Consequences**
↑ Apoptosis of cardiocytes (cardiac muscle cells)	Cardiac atrophy, thin myocardial wall, decreased strength of contraction, cardiac failure or Compensatory responses to loss of cells induces hyperplasia of remaining cells with enlargement of cardiac muscle and abnormal cardiac function resulting in cardiac failure

[a] For more information refer to text and references in text.
[b] The calcineurin/NF-AT signaling pathway is a signaling system to bridge cell membranes to the nucleus and vice versa. Their overexpression could induce severe cardiac hypertrophy. There are indications that calcineurin/NF-AT complex may represent a new target for development of antihypertrophic cardiac agents.
Abbreviations: MAP, microtubule assembly protein; NF-AT, nuclear factor of activated T cells; LIM; gene for actin-binding protein.
Source: From Ref. 76.

energy. Muscle force and work are necessary for maintaining structural integrity, maintaining posture, locomotion, breathing, digestion, and almost all functions of the body. Muscle strength reaches a peak between 20 and 30 years of age. It declines beginning in middle age and continues to decline at an approximately constant rate with increasing age, irrespective of the muscle group considered. There is, however, great variability among muscle groups in the rate of decline with aging (63). For example, the diaphragm remains active throughout life and undergoes little change with aging (Chapter 17). In contrast, the soleus muscle of the leg, relatively inactive in the less mobile elderly, shows decreased strength with aging (64). Many of the age-related changes identified in skeletal muscle are similar to those of cardiac (see below) and smooth muscle. Those occurring in skeletal muscle are particularly prominent because of the proportionally high distribution of this type of muscle throughout the body and its relationship to lean body mass (Chapter 13).

The lifestyle, sedentary or active, may exacerbate or delay the effects of aging. Additionally, it is possible to increase skeletal muscle power (i.e., speed and force of contraction) by physical training, even in old age; older people readily adapt and respond to endurance and strength training. Strength training helps prevent loss of muscle mass. It also prevents bone loss and improves joint function, postural stability, and mobility, often present in the elderly.

Endurance training helps maintain cardiovascular function, enhances exercise capacity, and reduces risk factors associated with heart disease, diabetes, and some cancers (Chapter 24).

Therefore, it was deemed appropriate to avoid repetitions—and more useful for the reader's personal health interest—to discuss the aging of the skeletal muscle together with the benefits derived with physical exercise. Although older individuals cannot expect to reach the same absolute capacity as the young, they may improve, relatively about the same amount. Aging-related changes in skeletal muscle structure and function, in muscle energy sources and metabolism, and responsiveness of the aging muscle to various types of exercise are presented in Chapter 24 together with practical guidelines

TABLE 7 Effects of Aging on Venous Return

Venous competence depends on
Venous diameter
Intrathoracic pressure
Total blood volume
With aging
Altered venous smooth muscle and elastin (Varicose veins, hemorrhoids)
Thrombophlebitis (inflammation of the vein wall followed by thrombus formation)
Orthostatic hypotension (↓ Sympathetic tone, ↓ baroreceptor activity, ↑ polypharmacy)

on how best to utilize this unique opportunity for better health and greater longevity.

■ AGING OF CARDIAC MUSCLE

The major function of the heart is to pump blood through the systemic and pulmonary circulations and thereby transport respiratory gases, nutrients, and metabolic products to and from tissues. It is not surprising, therefore, that most alterations in cardiac function have life-endangering consequences. Structural changes with aging (Fig. 6) involve all components of the heart as listed in Table 5.

Heart disease, associated with advancing age and atherosclerosis, remains the most important single cause of death, worldwide, in individuals aged 65 years and older and in both sexes (Chapters 3, 15, and 16). Although the mortality from cardiac diseases due to atherosclerosis (e.g., coronary heart disease) has progressively declined since the 1970s (Chapters 2 and 3), two other types of cardiac pathology are emerging and reaching epidemic proportions: they are *heart failure* and *atrial fibrillation* (65,66). Both conditions, generated by intrinsic alterations of cardiac muscle (heart failure) or of cardiac rhythm (atrial fibrillation), result in the inability of the heart to maintain blood flow to body tissues.

Heart failure is a sudden fatal cessation of the heart pumping function due to inability of the ventricles to contract and maintain an adequate blood ejection and flow. It is usually associated with hypertension and has been related to a loss of cardiac muscle cells below a critical threshold required for myocardial contraction; attempts to compensate the loss of cells by hyperplasia of remaining cells will generate cardiac hypertrophy and dysfunction manifested in both cardiac failure and arrhythmias. Atrial fibrillation, the most common form of chronic arrhythmia, is responsible for abnormal cardiac rhythm generated by loss or damage of pacemaker cells that drive the cardiac rhythm. As the cardiac rhythm is disrupted, the rate of ventricular contraction becomes rapid and irregular and blood flow is interrupted.

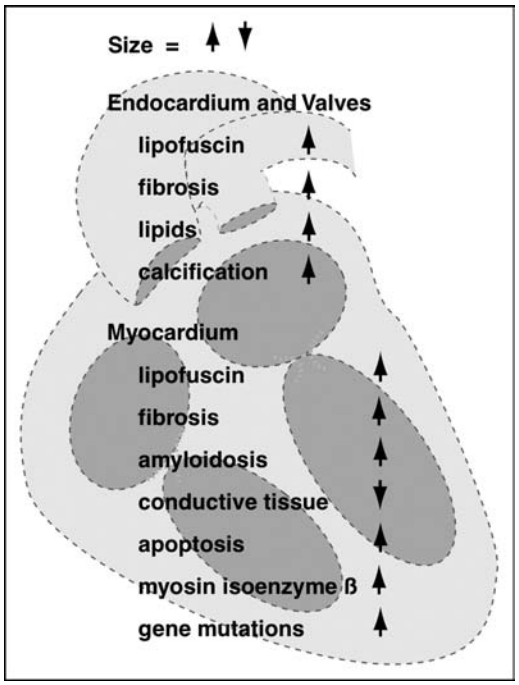

FIGURE 6 Some age-related changes in the heart.

■ Aging-Related Changes in Cardiac Structure

Cardiac structural changes may be caused by the aging process itself or may be secondary to disease. They may be

- primary to the heart,
- secondary to vascular lesions or to pulmonary disease,
- so severe as to be present at rest, or
- manifest only under conditions of increased demand, such as physical exercise.

They involve, in varying degrees, all cardiac elements:

- Muscle
- Connective tissue
- Conduction tissue
- Endocardium
- Valves
- Cardiac vasculature

Decline in cardiac function with aging is often associated with concomitant atherosclerosis of the coronary arteries, with consequent severe pathology such as coronary heart disease, the major cause of cardiovascular disease in the elderly (Chapter 15). Another consequence of age-related changes in heart and vasculature is *hypertension*, which today is usually amenable to successful treatment (67).

Cardiac size and weight remain essentially unchanged with aging although some studies suggest enlargement (particularly of the left ventricle) due to left cardiac muscle hypertrophy responding to increased contractile effort in hypertension and atherosclerosis. *This left ventricle hypertrophy* results in prolonged cardiac muscle relaxation. This lengthened relaxation may have some consequences in the presence of concomitant disease. However, in the absence of disease and in response to moderate physical exercise, ventricular performance remains normal.

Cardiac muscle atrophy may also occur due to loss of muscle mass. Normally, a steady dropout of cardiac cells occurs during life. With aging and, especially, when complicated by hypertension, apoptosis or cell-programmed death (Chapter 4) of myocardial cells becomes more rapid and extensive and the remaining cells are insufficient to maintain adequate contraction with consequent cardiac failure (68–71). In addition to loss of muscle cells by apoptosis, the surviving myocardial cells show an altered phenotype that has been linked to altered function and is associated with the secretion of molecules such as natriuretic and opioid peptides, usually released in response to stress. Another well-documented aging-related change is an increase in the pulse wave velocity within the arterial system. The pulse wave is due to the rise in arterial pressure in the

central aorta during the left ventricular ejection of blood. This pressure wave travels in the central arterial system toward the brain, arms, and feet and is much faster in old than in young individuals; the pressure increases linearly with advancing age. The increase in systolic blood pressure together with some aortic dilation have been associated with an increase in arterial stiffness and may be responsible for the mild left ventricular hypertrophy discussed above (72).

In rodents, molecular shifts with aging occur in the myosin heavy chain isoforms: these isoforms are present as α and β isoforms and, with aging, the β isoform becomes predominant. This predominance results in a contraction that exhibits reduced velocity and prolonged time course. Inasmuch as reduced velocity conserves energy and longer contraction extends blood ejection time, these changes may be interpreted as adaptive responses to the stiffness of the atherosclerotic vasculature (73–75). Other mutations that affect contractile proteins, cytoskeletal proteins, structural proteins, or calcium-sensitive proteins and their consequences for cardiac muscle contractility (76) are summarized in Table 6.

The normal contractile function of cardiac cells is further endangered by intracellular changes (accumulation of lipofuscin) and extracellular changes (increased connective tissue, accumulation of fat deposits both in the ventricles and in the interatrial septum, and inflammatory responses) (77). In the latter, such deposits may displace conduction tissue in the sinoatrial node and lead to disturbances of conduction in some severe cases (as in atrial fibrillation). Other extracellular deposits that may reduce the contractile efficiency of cardiac muscle include some degree of *amyloidosis* (Chapter 7) and *fibrosis* as occurs in myocardial infarction (Chapter 15). *Mitochondrial DNA damage,* perhaps due to free radical damage and lipid alterations, may play a role in decreased metabolic activity of human hearts, cell loss, and deterioration of function (Chapter 5).

Specialized conducting cells (forming the sinoatrial and atrioventricular nodes and the intraventricular bundle of His) may be lost with aging, although this loss is usually moderate and may be associated with an increase in connective tissue elements, particularly collagen and elastin. It is not clear, however, whether and to what extent these changes interfere with conduction.

Endocardial changes—lipofuscin deposition and varying degrees of fibrosis—are probably influenced by mechanical factors such as blood turbulence (Chapter 15). These mechanical factors may also be responsible for thickening of the atria and valves, which also show lipid deposition and calcification. The timetable of these valvular lesions varies with each valve.

Excitation-Contraction Coupling

In skeletal muscle, the nerve impulse triggers release of calcium from its stores in the sarcoplasmic reticulum and initiates contraction. Calcium binds to troponin C, uncovering several myosin-binding sites on actin. These molecular changes result in a decrease in the number of cross-linkages that bind myosin to actin and facilitate the sliding of the actin and myosin filaments along each other; they induce shortening of the muscle during contraction. In cardiac muscle during the action potential, calcium ions diffuse into the myofibrils from stores in the sarcoplasmic reticulum and also from the T system. Cardiac T tubules are much larger and contain many times more calcium than skeletal muscle. This extra supply of calcium from the T tubules is at least one factor responsible for prolonging the cardiac muscle action potential for as long as one-third of a second, 10 times longer than in skeletal muscle. Then, when calcium concentration is lowered, chemical interaction between myosin and actin ceases and the muscle relaxes. ATP provides the energy necessary for the active transport of calcium.

With aging, the excitation–contraction coupling in the heart appears to be markedly altered in some elderly. In experimental animals or with isolated heart models, excitation appears insufficient—or less sufficient than in the young—to trigger the release of calcium from its stores; the calcium stores themselves would be reduced and the accumulation of calcium in the sarcoplasmic reticulum would be slowed. *Mutations of the contractile proteins, of calcium-sensitive proteins, and of microtubule assembly proteins would inhibit active transport of calcium and prolong duration of contraction and relaxation (Table 6).* Alterations with aging in calcium stores and movements are also reflected in diminished cardiac responses to inotropic (promoting muscular contraction) agents such as catecholamines. These agents exert their stimulatory action on cardiac contraction by loading calcium stores and by facilitating calcium transport into the cells. With aging, both calcium storage and movements may be altered; hence, the reduced effectiveness of inotropic factors in activating cardiac contraction.

■ Aging-Related Changes in Cardiac Output

The overall expression of cardiac function is cardiac output, that is, the amount of blood pumped by the heart into the circulation per unit of time (in the healthy, resting, adult man, cardiac output is 5.5 L/min). Cardiac output depends on stroke volume and cardiac rate as well as on venous return (Fig. 7). Stroke volume depends on strength of muscle contractility, cardiac rate, autonomic innervation, venous return (amount of blood returning to the heart), and competence of the veins. Some aspects of intrinsic cardiac conduction and extrinsic autonomic innervation, in health and disease, are summarized in Box 4. Cardiac output may be reduced in a large number of elderly, although in others, it remains efficient well into old age.

Venous Return

Changes with aging in the venous compartment occur at all ages but are more frequent at older ages; as summarized in Table 7, they involve:

- Slower peripheral venous circulation due to impairment of elastic and smooth muscle components, as in varicose veins (i.e., tortuous and enlarged veins, most frequently found in the lower limbs) or in hemorrhoids (i.e., enlargement of the rectal veins)

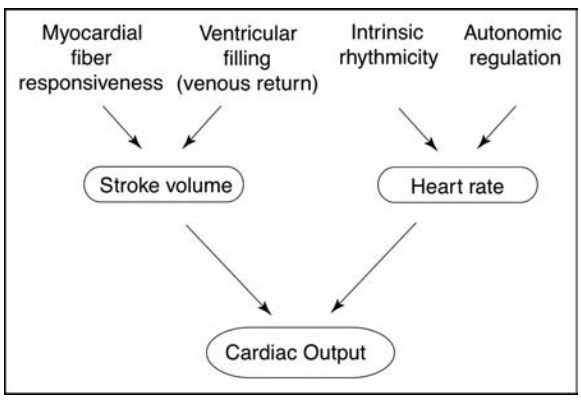

FIGURE 7 Factors regulating cardiac output.

BOX 4 *Cardiac Innervation and Some Disorders with Aging*

Intrinsic conduction tissue is characterized by an unstable potential related to an unstable permeability to potassium. With aging, intracellular accumulation of lipofuscin, extracellular amyloidosis, loss of conduction cells, and fatty and fibrotic infiltrates lead to a decline in function and an increase in instability. Thus, cardiac dysrhythmias or arrhythmias, (alterations of cardiac rhythmic contractions) are frequent in elderly subjects whose hearts are more vulnerable to such biochemical insults as hypoxia (decreased oxygen), hypercapnia (increased carbon dioxide), acidosis (decreased pH), and hypokalemia (decreased blood potassium). All these conditions increase cardiac irritability, particularly of the atria. This, coupled with reduced cardiac output and coronary blood flow, leads to cardiac failure and death in the elderly more than in young adults.

Another conduction defect in the elderly is the interruption or block of conduction, most often between atria and ventricles. Cardiac blocks reflect potential cardiac disorders but are themselves usually asymptomatic and non–life threatening.

The extrinsic innervation of the heart involves the two branches of the autonomic nervous system: the parasympathetic vagal innervation slows heart rate and the sympathetic increases cardiac rate and strength of contraction (Table 8). Both undergo changes with aging. Some consistent findings are (*i*) decreased inotropic (force of cardiac contraction) responses to catecholamines (primarily, epinephrine and norepinephrine), and (*ii*) decreased sensitivity to a variety of drugs (e.g., sympathetic agonists or antagonists) or hormones (e.g., thyroid hormones). Decreased sympathetic responses may be due to loss of neural cells, resulting in reduced catecholamine content. In the rat, for example, cardiac norepinephrine is halved between the age of one month (young) and 28 months (old). Another contributing factor may be the reduction in number and/or affinity of β-receptors. Although little is known in man, in experimental animals, the number of receptors appears to be markedly decreased.

- Inflammatory processes, as in thrombophlebitis, an inflammation of the veins due to the presence of a thrombus induced by slow blood flow in the unusually dilated veins, or
- Postural hypotension, common in the elderly, and its increasing incidence with aging. This condition, characterized by a fall in blood pressure rising from the supine to the standing position, has been ascribed to autonomic, particularly sympathetic, insufficiency, to low circulating levels of epinephrine, or to decreased activity of the baroreceptors that sense blood pressure.

Stroke Volume

Strength of ventricular contraction may be altered by a number of factors some of which are illustrated in Figure 8; some are related to changes in the muscle itself (loss of myocardial tissue, as mentioned above), others to alterations in autonomic nervous control particularly with respect to levels of catecholamines or to effects of hormones and selected drugs and alterations in blood oxygen, electrolytes, and pH.

Heart Rate

The decreasing cardiac output with age may not have significant impact on the circulation at rest but severely curtails the ability of the heart to respond to increased demand with acceleration of heart rate as in physical exercise. In the healthy young, exercise provokes a rise in cardiac rate and consequent increase in cardiac output, providing for augmented blood supply and oxygen consumption in muscle; this expected increase is considerably blunted in the elderly. During exercise, the expected rise in cardiac rate necessary to increase cardiac output in order to provide for the increased oxygen consumption by the exercising muscles is much lower in the elderly than in the young. Cardiac rate is regulated by the autonomic nervous system as summarized in Table 8. *The maximum achievable heart rate with exercise decreases linearly with age.* It may be calculated empirically taking 220 beats per minute as the maximum in the adult and subtracting the age of the individual from the 220 value; for example, in an 80-year-old, the maximal heart rate that can be achieved while exercising is 220 – 80 = 140 beats/min. This formula has been modified recently and replaced by a regression equation 200–(0.7 + Age) that is identical in men and women and not influenced by a wide variation in habitual physical activity levels (78). Although the aging-related decline in heart rate is progressive (Fig. 9), it may be more steep after 50 years of age.

During exercise in the young adult, an increase in stroke volume correspondingly increases cardiac output. The most important factor is the vasodilation in exercising muscles; vasodilation leads to a fall in vascular resistance, thereby (*i*) augmenting venous return to the heart and (*ii*) increasing ventricular blood filling in diastole, with a resulting stronger

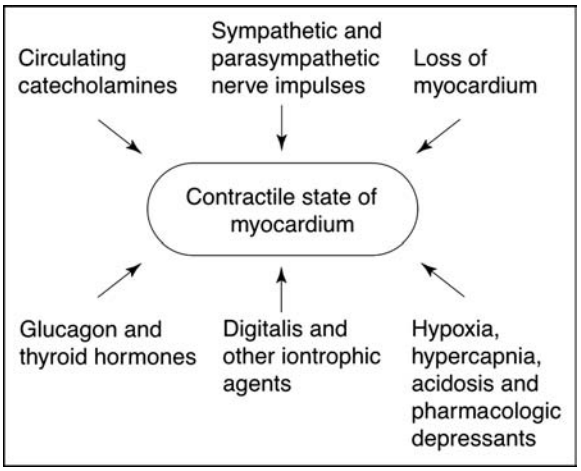

FIGURE 8 Factors that influence the contractile state of the myocardium.

TABLE 8 Autonomic Cardiac Regulation

Parasympathetic stimulation
Decreased heart rate
Decreased conduction
Increased refractory period
Sympathetic stimulation
Increased heart rate
Increased strength of contraction

stroke volume. With aging, peripheral vasodilation is less efficient or absent and muscle mass is decreased; vascular resistance is increased due to atherosclerosis and cannot be overcome by the strength of cardiac contraction.

The heart is highly dependent on aerobic processes, and, normally, less than 1% of the energy liberated in cardiac tissue is due to anaerobic metabolism. Under basal (at rest) conditions, carbohydrates supply 35% of the caloric needs of the heart, ketones and amino acids 5%, and fat 60%. The proportion of these substrates varies with the nutritional state. Oxygen consumption of the heart is primarily dependent, among other factors, on the heart rate and the contractile state of the myocardium. Increasing the heart rate increases oxygen consumption, at least temporarily until the onset of appropriate compensatory adjustments, and so, too, does increased peripheral resistance that causes the heart to work harder. This explains why in angina pectoris (Chapter 15) there is a relative deficiency of oxygen due to the greater oxygen demand to expel blood against increased peripheral pressure.

Irrespective of the changes that may affect the left ventricle, *the structures most affected by aging appear to be the cardiac valves, particularly the mitral and aortic valves;* aging-related changes include deposition of lipids and calcifications. In the myocardium, collagen and elastic increase to the detriment of the muscle fibers. The decrease in contractile cells may be responsible for the increased rigidity of the senile heart (79,80).

■ Adaptive Adjustments in the Aging Heart

Despite some of the cardiovascular alterations described with aging in this and previous chapters (Chapters 15 and 16), the heart is capable of mustering those compensatory responses necessary to maintain adequate function. For example, although the rate at which the left ventricle fills with blood during early diastole declines markedly between the ages of 20 and 80, the enhanced filling in later diastole keeps ventricular filling adequate in elderly individuals. The myocardium remains capable of adaptive hypertrophy to maintain normal heart volume and pump function in the presence of a moderate increase in systolic pressure. Such hypertrophy may be mediated through hyperplasia of myocardial cells and may be due to persistent activation of cardiac gene expression and its hormonal regulation (81,82).

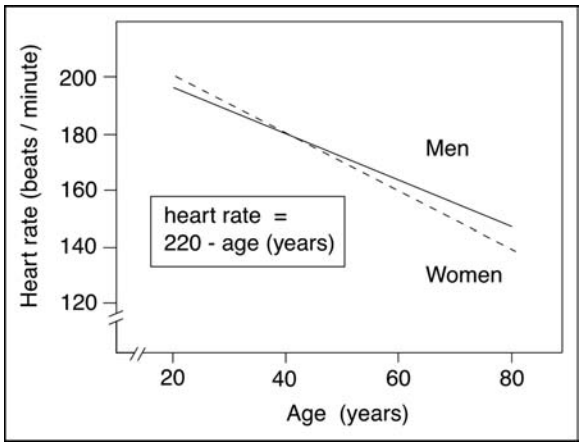

FIGURE 9 Changes in cardiac rate with aging in men and women.

■ Hormonal and Chemical Actions on the Aging Heart: The Emergence of New Therapies

Although several hormones may indirectly affect the heart through their generalized metabolic and physiologic actions, *glucagon* and *thyroid hormones* directly influence cardiac contractility. Glucagon (Chapter 13) increases the formation of cAMP through its binding to receptors other than the adrenergic β-receptors. Its inotropic action, therefore, can be beneficial to individuals suffering from toxicity due to the administration of adrenergic blockers. Thyroid hormones increase the number of β-receptors in the heart and their effects resemble those of sympathetic stimulation (Chapter 12).

Various drugs also modify myocardial contractility and rhythmicity. *Xanthines,* such as caffeine, exert their inotropic effect by inhibiting the breakdown of cAMP (Chapter 23). The inotropic effect of *digitalis* and related drugs is due to their inhibitory effects on Na^+K^+ATPase; this inhibition increases intracellular sodium, which, in turn, increases calcium availability to the cell and initiates contraction. Depressants such as barbiturates depress myocardial contractility. Evaluation of therapy should take into consideration that cardiac responses depend in large measure on the condition of the vascular tree and that the agents considered exert both cardiac and extracardiac effects and the latter may, indirectly, influence the myocardium.

Current molecular advances provide a better understanding of the pathogenesis of alterations of cardiac muscle contractility and excitability and open the way for better diagnosis and better strategies to cure the life-threatening disorders of the heart. By correlating clinical outcome with genetic susceptibility profiles, it will be possible to implement a more efficient therapy if the cardiopathy is diagnosed at young ages. By identifying genes that initiate or aggravate cardiac myopathy, it will be possible to target new drugs and gene therapies directly to the causative molecule(s). The improvements in the construction of artificial hearts and the availability of many models (e.g., transgenic animals, cultured cells, stem cells, cell tissue, and organ transplants) will make it possible to test new preventive and therapeutic interventions. In the current period, when scientists are eager for better drugs and technologies, the treatment of cardiac failure represents a worthy challenge that can save lives and improve the quality of life of many elderly persons.

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21

The Skin

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INTRODUCTION

Perhaps no manifestation of aging is as dramatic or readily obvious as that which occurs in the skin and its appendages. The development of either grey hair or facial and body wrinkles represents irrefutable evidence of the passage of time and of progressive aging. Intrinsic or genetically programmed factors that occur with time and extrinsic factors caused by environmental insults that accumulate with exposure are responsible for the complexity of skin aging processes (1). Yet, many descriptions of the aging skin fail to distinguish between intrinsic and extrinsic aging changes. For example, chronic solar damage changes are far more prevalent in the elderly than in young people, since they result from cumulative exposure over time (1–7). These changes involve cells (8–10), glands (11), and connective tissue (particularly, collagen) (12) of the skin. Intrinsic changes in the structure and function of skin that occur with aging also make the skin more vulnerable to external insults. It is not surprising then that dermatologic problems are very common in the elderly (13–17).

Several studies describe how almost one-half of persons over 65 years of age have at least one dermatologic disease requiring medical attention. About one-third of these have more than one skin problem. Multiple skin conditions are characteristic of the very old, and the common ones are different from those affecting the young (5–7). Given the current demographics and the rapid increase of the elderly population (Chapter 2), it is not surprising that much effort has been put in research to better understand the causes, consequences, and possible therapeutic and cosmetic interventions to improve the appearance, mechanics, and barrier function of the skin. An important psychosocial and economic impact of skin aging concerns the social anxiety and social isolation, and even sometimes the workplace discrimination, that prompt many older individuals to use a multitude of products and procedures purported to conceal or delay the signs of aging (18). Older people, therefore, in addition to their health needs, should also be offered treatments for the aging skin to promote their self-confidence, offer greater acceptance by society, and reinforce their treatment expectations.

After a brief introduction, this chapter will review some of the anatomic and physiologic changes that occur in the skin and collagen with normal senescence (see section entitled Aging of the Skin). Pressure sores will be discussed as a clinical example of skin dysfunction in the elderly (see section entitled Pressure Sores).

AGING OF THE SKIN

Functions of the Skin

The skin is one of the largest organs of the body and accounts for approximately 16% of the total body weight. It is part of the integument, a covering of the entire body, which, in addition to the skin, includes the nails, hair, and various types of glands, all accessory organs derived from the skin. The skin has several functions (1–9), listed below: of these, functions 1 through 7 are well established. Recent studies have added new important functions, which are listed in 8 and 9. Thus, the skin:

1. Provides a barrier to exclude harmful substances
2. Prevents water loss and regulates water and electrolyte balance
3. Plays a role in the control of body temperature
4. Repairs itself readily
5. Receives sensory stimuli: touch, pressure, temperature, and pain
6. Excretes waste products through sweat glands
7. Secretes special products such as milk from mammary glands
8. Operates as a huge and highly active biofactory for the synthesis, processing, and metabolism of a large number of substances (e.g., structural proteins. lipids, polysaccharides)
9. Represents an integral part of the immune, nervous, and endocrine systems, with numerous lines of cross-talk established intracutaneously between these systems

Conventional wisdom considers the skin as an organ that literally wraps around the body and has a primary protective (barrier) function against possible damage (physical, chemical, and microbiological) from the environment; it also transmits contacts (sensory, tactile, and painful sensations) with the external world and is crucial for the maintenance of temperature and electrolyte and fluid balance. In addition to these important functions, the skin also operates as a "huge and highly active biofactory" and may be viewed as an integral component of the immune, nervous, and endocrine systems (19).

Structural Characteristics of the Epidermis and Dermis

The skin consists of an epithelium, the epidermis, and the dermis, which is considered as part of the connective tissue (Fig. 1). The main layers of the skin include the surface layer known as the epidermis and a deeper connective tissue layer known as the dermis. The interface between the epidermis and the dermis is normally uneven and forms wavy interdigitations. The projections of the dermis into the epidermal layer are called dermal papillae.

The epidermal cells produce keratin, a complex of insoluble, protective, or structural, fibrous proteins that form the dead superficial layers of the skin (stratum corneum) and are essential for the protection of the outer body surface. There are two classes of keratins: α and β. The epidermis is locally specialized into

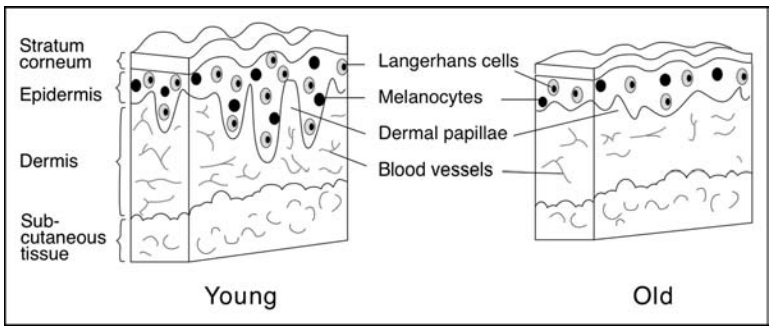

FIGURE 1 Histologic changes in normal aging skin. Changes from top to bottom include (*i*) rougher stratum corneum, (*ii*) fewer melanocytes and Langerhans cells, (*iii*) flattening of the dermoepidermal junction, (*iv*) fewer blood vessels in the dermis, and (*v*) less subcutaneous tissue.

various *skin appendages such as the hair, nails, sweat glands, and oil or sebaceous glands*. Nails are formed of α-keratin, a protein rich in cysteine; β-keratin contains little or no cysteine, is rich in amino acids, and forms the silk fibers spun by spiders.

The superficial keratinized skin cells are continuously exfoliated and are replaced by cells that arise from the basal layer of the epidermis. The sequence of changes of an epidermal cell from the basal layer of the epidermis to keratinization and exfoliation normally takes from 15 to 30 days, depending on the region of the body (8).

Other types of cells found in the epidermis include *"melanocytes" (8,20–22); they produce the pigment melanin that protects against ultraviolet (UV) radiation (UVR)*. Melanocytes have been proposed as a model for studying the complexity of cellular changes with aging (23). The *"Langerhans cells," also present in the epidermis, are part of the immune system*; they are responsible for recognition of foreign antigens and participate in the protective function of the skin (Chapter 14).

Since the epidermis has no blood vessels, the dermis, which has abundant blood vessels, plays an important role in providing the epidermis with nutrients and in contributing to thermoregulation. The amount of heat lost from the body is regulated to a large extent by blood flowing through the skin. The presence of direct connections between arterioles and venules bypassing the capillaries (arteriovenous anastomoses) in the skin of fingers, palms, toes, and earlobes coupled with a rich autonomic innervation supports a blood circulation that varies greatly (from 1 mL to as much as a blood flow of 150 mL/100 g of skin/min) in response to thermoregulatory stimuli (24,25).

The growth and turnover of blood vessels in the skin is fundamental in normal skin development as well as in wound repair, hair-follicle cycling, tumor-cell metastasis, and many other states of skin disease. While the mechanisms of angiogenesis (i.e., development of blood vessels) are the subjects of numerous investigations, much less is known about the influence of cellular aging. Cell aging and activation of telomerase in human dermal microvascular endothelial cells appear to affect their viability and survival both in vivo and in vitro (25,26). Shortening of telomeres occurs with cell proliferation and correlates well with aging in humans (Chapter 4). Thus, the telomere–telomerase system has been proposed to be an adaptation for organisms with prolonged life span to avoid cancer, at the cost of the cellular dysfunction associated with the aging phenotype (Chapter 4). Such dysfunctions may be operative not only in the vascular endothelial cells but also in other cellular cutaneous elements (26).

Although the epidermis does contain nerve endings, there is a richer nerve supply in the dermis because it also contains the nerves that lead to the sensory nerve endings. In addition, epidermal appendages such as the hair follicles and sebaceous and sweat glands extend down into the dermis and share the same nerve supply.

Below the dermis, there is looser connective tissue. This tissue usually consists of subcutaneous adipose tissue that plays a role in fat metabolism.

■ Aging-Related Changes in the Epidermis

Dryness and roughness of the skin are two of the most readily appreciated changes that occur with aging. This could be due to either a decrease in the moisture content of the stratum corneum or a decrease in vertical height and increase in overall surface area of epidermal cells. The increased dryness results in a surface likened to "shingles on an old roof" (Fig. 1) (27,28).

The exact pathogenesis of the drying process is still unclear but probably represents a combination of several contributing factors (e.g., lipid reduction, abnormalities of cholesterol synthesis) involved in the aging of collagen and matrix (28).

Another well-recognized change that occurs with aging is *the increased incidence of both benign and malignant epidermal neoplasms* (29). Although there is no question that chronic UV light exposure contributes to this problem, some intrinsic cellular alterations of basal cells may also contribute to the increased incidence of skin cancer in the elderly.

One of the histologic changes that occur most consistently in the elderly is the flattening of the dermoepidermal interface and effacement of the dermal papillae. This reduces the total area of dermoepidermal junction per area of external body surface area. This change predisposes older persons to blister formation and shear-type injuries and easy abrasions. Another observation is an aging-associated decrease of about 50% in epidermal turnover rate between the third and seventh decades of life. This means fewer basal cells are replaced and it takes longer for a basal cell to reach the stratum corneum and be exfoliated. The slower movement prolongs the exposure of epidermal cells to potential carcinogens and contributes to an increased incidence of skin cancer (29); in addition, it is responsible for the slowing of wound healing.

The number of melanocytes decreases with old age by approximately 8% to 20% per decade after the age of 30 in areas both exposed and unexposed to the sun. This reduction leads to irregular pigmentation especially in sun-exposed areas (hence the "age spots" frequently seen on the back of the hands) and the inability to tan as deeply as when younger (30,31). "Melanin," the dark pigment produced by the epidermal melanocytes, clearly protects the human skin by screening the harmful UVR. However, the value of hair pigmentation is less clear. Studies on epidermal melanocyte aging suggest that it is the reactive oxygen species damage of nuclear and

mitochondrial DNA that leads to mutation accumulation (30). Dysregulation of combined antioxidant mechanisms and proapoptotic factors may explain the reduced number of melanocytes (21,31). Other studies have implicated aging-related qualitative and quantitative changes in melanocyte stem cells as responsible for the proliferation of fewer stem/progenitor cells and, therefore, a smaller number of melanocytes (20–22).

The number of Langerhans cells decreases as well. This change contributes to a decline in cell-mediated immune responses in the skin. The reduction of melanin and its protective action, the reduced inflammatory warning signs, and the reduced immune capacity combine to increase the risk of tumorigenesis. Elderly patients require longer UV exposure to develop erythema and edema (sunburn) than younger patients. Thus, the body's warning system as well as its defense system in relation to skin cancer becomes blunted with age. In addition, UV light further reduces the quantity of Langerhans cells. These changes, together with the increase in cumulative irradiation exposure, possibly explain why skin cancers (nonmelanomatous) are prone to occur on sun-exposed skin.

Aging-Related Changes in the Dermis

The dermis in elderly individuals has a decreased density and fewer cells and blood vessels. The total amount of collagen decreases 1% per year in adulthood; therefore, *skin thickness decreases linearly with age after 20 years of age. With aging, collagen itself becomes thicker, less soluble, and more resistant to digestion by the enzyme collagenase*. Changes in the number and types of cohesive bonds make collagen stronger and more stable. However, since there is less "give" in the tissue, altered collagen predisposes the dermis to tear-type injury. Architectural rearrangements of collagen fibers may also be responsible for changes in dermal tissue properties (24).

The decrease with aging of the total amount of hyaluronic acid and dermatan sulfate, both mucopolysaccharides components of the extracellular matrix, affects the viscosity of the dermis, which, in turn, may alter the rate of dermal clearance of substances (32). Changes in the elastic fibers of the dermis also result in loss of stretch and resilience (32,33), a consequence being skin sagging and wrinkling (33) and predisposition to injury of the underlying tissues following trauma. *While even the very old (beyond 85 years) can effectively repair extensive wounds, elderly individuals, in general, lag behind younger controls at every stage of wound healing* (34).

Pale skin results from the decrease in dermal blood vessels. Skin surface temperature is also decreased due to the diminished vascularity (24,25). These changes, together with a decrease in the thickness of the subcutaneous tissue, make *thermoregulation more difficult in the elderly*. The vascular changes described above also result in a decrease in dermal clearance of foreign materials with consequent prolonging or exacerbating cases of contact dermatitis.

Aging-Related Changes of Skin Appendages

Older individuals produce less sweat because sweat glands decrease in number or in functional efficiency, a decrease that interferes further with thermoregulation. Although the number of sebaceous glands remains constant with age, their size increases while the sebum output as well as wax production declines with age (11). The diminished sweat and oil production no doubt contribute to skin dryness and roughness in the elderly. The rate of linear nail growth decreases with aging. Nail plates usually become thinner, more brittle, and fragile (35).

Hair graying occurs because of a progressive loss of functional melanocytes from hair bulbs (23). By the age of 50, it is said that 50% or more of the human population have at least 50% of their body hair gray regardless of sex or hair color. Heredity does play an important role in hair graying. With aging, the decreased number of hair follicles in the scalp leads to increased balding. Hair growth undergoes a cycle that includes periods of melanocyte proliferation, maturation, and melanocyte death (36). With aging, melanocytes are exposed to oxidative damage, with individual hair-follicle pigment dilution (or true hair grayness) due to a reduction in the enzyme tyrosinase activity and in keratinocyte interactions as well as in the defective migration of the melanocytes to the dermal papilla of the hair bulb (36). Hair graying and, in general, alterations in hair growth and appearance are a more complex process than hitherto suspected and represent also an index of health status (8,20–22,36).

Although free nerve endings do not usually change in the aged skin, the number of Pacini's and Meissner's corpuscles (end organs responsible for the sensation of pressure and light touch, respectively) decrease with age. This results in decreased sensation and predisposes the elderly to injury and decreased ability to perform fine maneuvers with the hands. A summary of structural and functional changes with aging in the main skin components, epidermis, dermis and appendages is presented in Tables 1 and 2, respectively; considerations on aging of connective tissue and collagen are presented in Box 1 (Figs. 2 and 3) (Table 3).

■ LOOKING YOUNG FOREVER!

In one of his tragedies, the seventeenth century French play writer Jean Racine wrote about the struggle of his heroine, Athalie, Queen of Judea, to "repair the irreparable outrage inflicted by the passing years" on her beauty. The enormous amount of money currently spent on cosmetic products and interventions throughout the world attests to the continuing human devotion to a beautiful appearance. Some cosmetics have a physiologic justification and some have well-proven therapeutic actions. This is the case of the *sunscreens* that protect the skin from cumulative environmental injury. They will be briefly considered here, first. *Estrogens and retinoids* will be considered subsequently followed by a brief listing of other types of interventions.

TABLE 1 Changes in Normal, Aged Skin

Decrease	Increase or other changes
Epidermis	
Epidermal turnover rate	Severe dryness and roughness
Number of melanocytes	Flattening of dermoepidermal junction
Number of Langerhans cells	
Dermis	
Density	Stiffer collagen
Cells	Stiffer elastic fibers
Blood vessels	
Clearance of foreign substances	
Appendages	
Sweat production	Gray hair
Sebaceous glands	Thinner nails
Hair follicles	
Rate of nail growth	
Sensory end organs	

TABLE 2 Functional Changes in Aging Skin

Decreased function	Increased pathology
Wound healing capability	Blister formation
Cell-mediated immune response	Incidence of infection
Thermoregulation	Incidence of cancer
Clearance of foreign substances	Dryness
Tanning	Roughness
Elasticity	Fragility
Sweat and oil production	Sensory deprivation
Thickness	

■ Prevention and Treatment of Cumulative Environmental Damage to the Skin

Several environment hazards cause injury to the skin, but, as indicated previously in this chapter, the most widespread and damaging is solar UVR with a wavelength of 200 to 400 nm (2–6,8–12). Of the three (A, B, and C) types of UVR, the UVB appears to be the most significant biologically. The degree of skin pigmentation is of critical importance in modulating the damaging effects of UVR: the darker the skin, the lesser the damage. *Chronic UVR exposure causes first hyperplasia (thickening) of the epidermis followed by atrophy.* The major alterations occur in the dermis, which undergoes degenerative changes affecting the connective tissue of upper and middle layers and causing wrinkling (characteristic of old age). Prevention of solar damage may be obtained—and in fact is strongly recommended—by reducing exposure to the sun and by using special compounds (applied topically to the exposed body parts) that may act as sunscreens (1,2,5,37–40).

■ Estrogens

Both male and female sex hormones change with age, but the female sex hormones, "estrogens," decrease at menopause more dramatically than any other hormone (Chapter 10). As the population of postmenopausal women continues to grow, interest on the effectiveness of replacement therapy with these hormones on various systems and functions is also increasing. Several of these effects have been discussed in Chapter 10. The relatively low levels of estrogens that accompany menopause exacerbate the deleterious effects of both intrinsic and environmental aging.

There are many ways by which both steroidal and nonsteroidal estrogens may influence skin aging (40–47). These mechanisms include:

- maintenance of skin thickness by preventing skin collagen decrease,
- maintenance of skin moisture by increasing mucopolysaccharides and hyaluronic acid in skin matrix,
- maintenance of the "barrier function" of stratum corneum,

BOX 1 *Aging of Connective Tissue and Collagen*

Aging of the connective tissue, a major constituent of many organs and systems, involves several functions, of which the most important include

1. mechanical support as provided by bones and joints,
2. exchange of metabolites between blood and tissues as provided by blood circulation,
3. storage of fuel in its adipose cells, and
4. protection against infection and repair of injury.

In the skin, *connective tissue* is represented by the ground substance (matrix) in which are embedded collagen and elastic fibers. Because it is ubiquitous in the body and undergoes identifiable changes with age, collagen has been considered a possible primary source for the onset of the aging processes. The striking changes that take place with age in the structure and chemistry of collagen fibers and the surrounding extracellular matrix have been ascribed to metabolic alterations in tissues; these alterations would lead to the formation of covalent bonds among polymeric chains or cross-linkages. Cross-linking, therefore, would represent a fundamental mechanism by which overall functional impairment is induced in the aged.

Collagen, produced by the fibrocytes present in the matrix, represents the major fibrous protein of connective tissue and forms a large portion (30–40%) of all proteins of the body. Collagen contains two specific amino acids—hydroxyproline and hydroxylysine—that do not occur in significant amounts in other animal proteins, and their content in a tissue can be taken as an index of its collagen content. The structure of collagen is illustrated in Figures 2 and 3 and major changes with aging are summarized in Table 3.

Changes with aging involve other cutaneous components of connective tissue including elastin and the extracellular matrix. With aging, matrix size is progressively reduced; this is associated with a corresponding decrease in water content—a water loss consistently observed throughout the life span in many organs and species. The loss of extracellular matrix has been ascribed to a slower turnover in aging compared to that of collagen. Extracellular matrix must be renewed within days or weeks, whereas the more inert collagen persists for a considerably longer period of time. Changes in the composition of the extracellular matrix with age have also been reported; they appear to differ from tissue to tissue, with a progressively increasing occurrence of cross-links. In addition, dysregulation of the matrix metalloproteinases with aging may result in excessive proteolytic activity and tissue damage. Changes with aging in elastic fibers have been discussed in Chapter 15 in relation to the arterial vascular wall and in Chapter 17 in relation to the lungs.

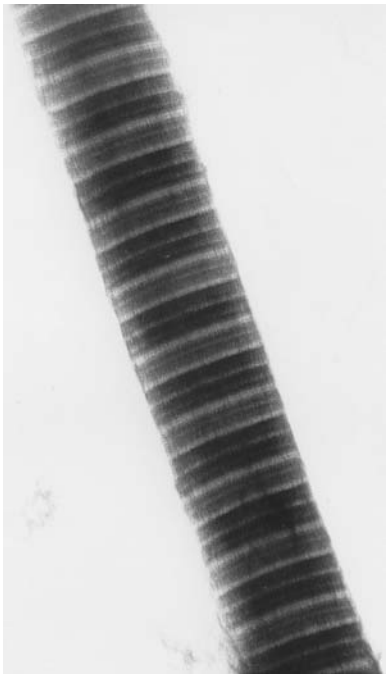

FIGURE 2 Electron micrograph of a negatively stained microfibril of collagen isolated from rat tendon. Magnification 96, 541x. One dark and one light segment represent a period produced by the arrangement of tropocollagen molecules that would come together in a parallel arrangement and overlap by about a quarter of their length to produce a staggered array, resulting in cross striation (refer to Fig. 3). This structural arrangement makes the collagen fibers flexible and highly resistant (e.g., capable of withstanding several hundred kg/cm^2 from a pulling force). These properties of collagen resemble those of a cable that ties a ship to shore: it is sufficiently flexible to be curled when not in use but strong enough not to allow movement of ship at anchor. *Source*: Courtesy of Dr. N. B. Gilula.

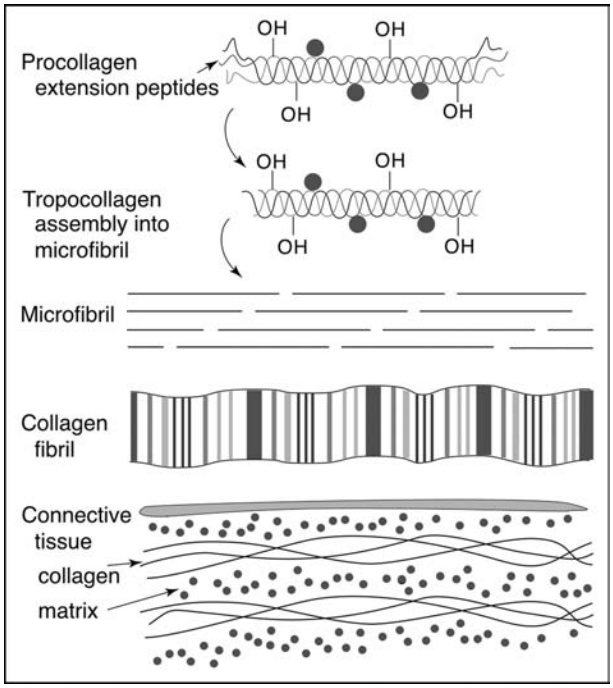

FIGURE 3 Schematic representation of the composition of a collagen fibril and the distribution of tropocollagen molecules in the connective tissue. Collagen fibrils derive from tropocollagen molecules formed from larger procollagen precursor molecules; the latter contain the amino acids that will give rise to the tropocollagen as well as extra amino acids called "extension peptides" that prevent the formation of large collagen fibrils damaging to the cell. Collagen fibers are embedded in a matrix (or ground substance) formed mainly of protoglycans (high molecular weight complexes of proteins and polysaccharides, serving as lubricants and support elements), some proteins, and interstitial fluid filtered from the capillaries.

- increase in the levels of sebum, a semifluid substance secereted by the sebaceous gland and composed of fat and epithelial debris,
- maintenance of skin elastic fibers and collagen, and, therefore,
- reduction/delayed appearance of wrinkles,
- improvement and acceleration of wound healing by regulating cytokine levels (both in elderly women and men)
- possible effects on skin scarring.

Although estrogens may improve skin in many ways (see above), they carry a carcinogenic risk (Chapter 10). Hence, their administration cannot be recommended routinely to treat skin aging (44–47). It is possible that the development of drugs such as the selective estrogen receptor modulators with mixed estrogenic and antiestrogenic effects, depending on the tissue and cell type, may offer a new way of targeting specifically the skin without systemic side effects (Chapter 10). It is also possible that other adrenal steroids (such as dehydroepiandrosterone) (48) capable of inducing the production of heat shock proteins (HSPs) (Chapter 9) may have a beneficial effect on the skin (48,49). HSPs are responsive to stress and may increase the resistance of the skin to UV and other environmental damage.

■ Retinoids

Retinoids, either naturally occurring or synthetic substances, include primarily, retinol, a synonym of vitamin A, an essential nutrient (Chapter 23) *and retinoid acid, a derivative of vitamin A (50)* (Chapter 23). Natural-occurring retinoids and their synthetic analogs are involved in the regulation of proliferation and differentiation of cutaneous cells as evidenced by the characteristic

TABLE 3 Major Changes with Aging in Collagen Structure and Function

↓ Chemical excitability
Fibers become tougher
Tensile strength is reduced
Plasticizing function is impaired or lost
↓ Effectiveness of enzymatic degradation
Turnover of fibers is slowed down due to
Amount of collagenase (the major degradating enzyme) and/or
↓ Increased resistance of fibers to degradation by collagenase
↓ Proportion of labile, (easily degraded) (to stable), (less degradable) collagen
↓ Ability of cortisol and other hormones to stimulate collagenase activity
↓ Possible Gluconeogenic action
Collagen would release amino acids that convert to glucose
Under stress, this release of energy conversion is decreased or prevented
↑ Cross-linking
↑ Intramolecular binding of ester bonds
↑ Rigidity of fibers
↑ Time of contraction and relaxation

hyperkeratosis (i.e., hypertrophy of the outer stratum corneum of the skin, Fig. 1) observed in vitamin A deficiency (51). Retinoids share the ability to bind nuclear receptors of the steroid/thyroid hormones/vitamin D superfamily (52,53) (Chapters 10 and 12). There are two subtypes of retinoid receptors: (*i*) retinoic acid receptors (RAR α,β,γ) and (*ii*) retinoid X receptors (RXR α,β,γ), both present in human skin. RAR-γ and RXR-α are predominant in the epidermal keratinocytes and RAR-β in the dermal fibroblasts (54).

Retinol receptors not only regulate skin cell proliferation and differentiation but also influence tissue homeostasis, lipid metabolism, and inflammation; they are essential in the treatment and prevention of photo aging, acne, and psoriasis (53–58). Retinol is a coenzyme necessary for the function of the retina, bone growth, and differentiation of epithelial tissue. Some retinoids, with topical action, like tretinoin, appear to prevent and repair skin photoaging damage by

- preventing loss of collagen or
- stimulating new collagen formation.

These actions are applicable to the aged skin which is, apparently, highly sensitive to retinoids, especially with respect to the wrinkles associated with photoaging (57,58). Studies of mechanisms of the beneficial effects of the retinoids on the aging skin indicate that some retinoids, with topical action, like tretinoin, have the ability to regulate complex programs of gene expression such as (*i*) inhibition of the metalloproteinases, enzymes involved in breakdown and loss of collagen and (*ii*) simultaneous enhancement of procollagen synthesis; the consequence of these two actions results in preservation of dermal collagen (54,58–60). *However, retinoids must be used with caution, especially when administered orally, because of their side effects*. Such untoward effects—local skin irritation (primarily, pruritus, burning, and erythema)—may occur even when applied topically (57).

The use of topical or oral retinoids continues to expand within and beyond the field of dermatology. According to a number of recent studies, cell-fate specification and terminal differentiation may be regulated by some extracellular factors, including retinoic acid. Thus, in cultured embryonic stem cells, retinoic acid converts undifferentiated embryonic stem cells to fully functioning neurons as well as glial cells (61,62).

■ Other Interventions

Other interventions for cosmetic use to flatten wrinkles and improve skin condition may be used alone or in combination with other treatments as the ones considered above. They include

- the administration of antioxidant vitamins, micronutrients to prevent free radical accumulation during skin aging (3,37,63–66) and
- the use of lasers (67–69), injections of the botulinum toxin (70), radiofrequency (71), and dermabrasion (72).

■ PRESSURE SORES

The pathophysiology of pressure sores illustrates several principles in geriatric management (73). Although not the most commonly seen dermatologic diagnosis in the elderly (skin cancer is), the problem of pressure sores is frequent enough and produces serious enough consequences to deserve discussion (73). *The prevalence of pressure ulcerations increases with age such that patients over 70 years of age account for 70% of those affected*. In this age group, 70% of patients develop pressure sores within two weeks of hospital admission. As with urinary incontinence (Chapter 18), the presence of pressure ulcers and their status frequently play a prominent role in the decisions made regarding the ultimate management of a patient.

Pressure sores represent a dreaded complication of immobility. The most commonly affected sites are the sacrum, ischial, trochanteric, and calcaneal tuberosities as well as the lateral malleolus. They arise from four different mechanisms:

- Pressure
- Shear
- Friction
- Moisture

leading to maceration (softening of tissues). *The role of pressure is the most critical in the development of the pressure ulcer* (74). The average period of time necessary to produce pressure-associated changes varies but can be as little as two hours. Hence, a patient who does not move himself/herself every two hours, for whatever reason, is at a risk of developing sores. Shearing forces are produced by an improper position, friction occurs with improper handling of the patients, and moisture due to perspiration or urinary and fecal incontinence. Due to all these factors, the skin changes, described above in this Chapter, increase the risk of developing pressure sores.

Several classification systems of pressure sores have been proposed and are routinely utilized in their management (74). Notwithstanding some individual variability, several stages have been identified.

1. Stage I occurs when there is redness not reversible with pressure (called "nonblanchable erythema") of intact skin.
2. Stage II occurs when there is partial thickness skin loss involving the epidermis and/or the dermis. The ulcer is superficial and presents clinically as an abrasion, blister, or shallow crater.
3. Stage III occurs when there is full-thickness skin loss involving damage or necrosis of subcutaneous tissue, which may extend down to, but not through, the underlying fascia.
4. Stage IV occurs when there is full-thickness skin loss with extensive destruction, tissue necrosis, or damage to muscle, bone, or supporting structures (i.e., tendon).

There is no cure for a pressure sore once it develops (74). Even if an ulcer heals, there is always a significant chance that it will recur. *Therefore, prevention of the development of pressure sores, as with many other geriatric problems, is the most important aspect of management (75,76)*. Risk factors for the development of pressure sores include incontinence, edema, obesity, diabetes with neuropathy, sepsis, vascular disease, immobility due to fractures, dementia or restraints, and, finally, systemic factors related to malnutrition such as hypoalbuminemia, anemia, and vitamin deficiency.

Once patients at risk are identified, specific measures need to be taken to avoid the causative factors mentioned earlier. This is best accomplished through the use of a multidisciplinary team approach. The medical primary-care provider optimizes physiologic function and treats any underlying illnesses. The nursing staff ensures feeding, turning, and positioning and initiates a bowel and bladder program. The nutritionist develops an aggressive nutritional strategy and, finally, physical therapy can institute a mobilization plan, encourage strengthening exercises, and provide special pressure-sparing equipment.

Relief from pressure is the most important factor in preventing as well as treating pressure sores. In patients at risk, this may

necessitate turning the patient in bed every two hours. Padding for the bed or chair and special air and water mattresses have also been recommended (76). Providing a clean and moist environment for tissue to heal is important for Stages II to IV. This is usually accomplished with sterile gauze moistened with normal saline or synthetic colloid dressings. In addition, for Stages III and IV, it is necessary to remove necrotic debris. This is accomplished through surgical or mechanical dressing change, debridement (i.e., removal of foreign material and dead tissue in or about a traumatic lesion) as well as the use of topically acting enzymatic products. Avoiding irritating substances such as betadine or hydrogen peroxide also helps with wound healing. Finally, treating local infection with frequent dressing changes or debridement and systemic infections with empirical antibiotics will also help wounds heal faster. If the wound is large enough, plastic surgery utilizing tissue flaps may be necessary to fill in large gaps. The management of pressure sores is much more successful and satisfying when underlying pathophysiologic principles are utilized in conjunction with a comprehensive and multidisciplinary approach.

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Part III

Prevention and Rehabilitation

22

Pharmacology and Drug Management in the Elderly

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INTRODUCTION

Advances in pharmacology throughout the past centuries and, particularly, the recent and rapid advances witnessed in the nineteenth and twentieth centuries, have contributed to the increase in the number and longevity of elderly persons (Chapter 2). In addition to decreasing mortality from infections and acute medical illnesses in youth and middle age, modern pharmacotherapy has begun to address the causes and treatment of disease and disability in old age, including hypertension, stroke, congestive heart failure, adult-onset diabetes, osteoporosis, and cancer.

Despite the evident benefits of pharmacologic medications, their use by elderly patients has always been a focus of concern for health professionals caring for the aging patient. The elderly make up a growing percentage, about 13%, of the U.S. population (Chapter 2), yet it is estimated that they consume 30% of prescribed medications and 40% to 50% of over-the-counter (OTC) medications (Fig. 1) (1,2). Physiologic changes, coupled with increased use of medications, place older patients at risk for adverse effects and drug interactions. *The concept of "polypharmacy," originally meaning "many drugs," has acquired a derogatory connotation of excessive and unnecessary use of medication* (Table 1). Studies have shown that 9% to 31% of hospital admissions in elderly patients may be medication related (3–5). In addition, the elderly seem to be two to three times as likely to experience adverse drug reactions as younger adults (6,7).

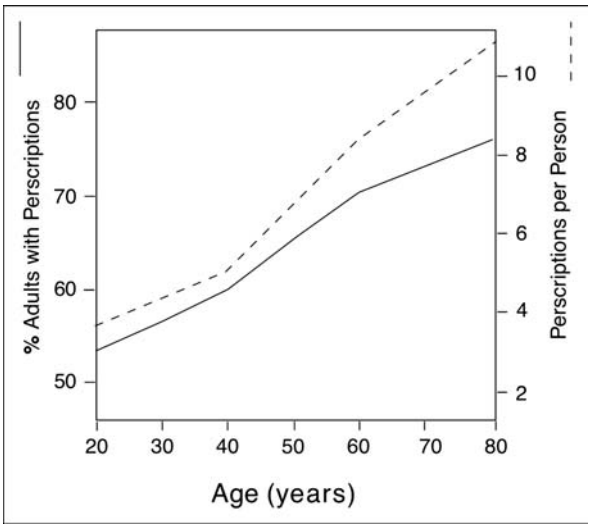

FIGURE 1 Increase with age in the percentage of adults receiving prescriptions and in the number of medications prescribed per person.

This chapter will focus on how best to evaluate the utility of a drug for use in the elderly. Ideally, one would consult available data, allowing for assessment of benefits and side effects in the appropriate age group and in persons with similar medical conditions. However, data for a complete evaluation of elderly patients are rarely available. *In general, risk for side effects increases with age*. Expectation of remaining years of life decreases with advancing age, and, therefore, the time for a potentially beneficial distant effect decreases.

Several diseases usually affect elderly persons, simultaneously [comorbidity (Chapter 3)]. Therefore, they are more likely to be taking multiple medications, so the *potential for drug–drug interactions increases with age, while the alterations in metabolism associated with age and chronic illness tend to increase the risk of adverse events*. Conversely, the potential benefit of preventative interventions may be higher in the elderly since the incidence of outcomes such as stroke, myocardial infarction, and many cancers increases with age. There is a large interindividual variation in drug metabolism that contributes to the susceptibility of the elderly to drug–drug interactions.

Advances in pharmacogenomics, that is, the relationship between functional genetics and rational therapy (based on genetic characteristics of each individual) have improved our ability to predict some of this interindividual variability (8–10). *Knowledge of the phenotype related to the metabolism of a particular drug can predict the risk of drug side effects and provide an individualized, so-to-speak "customized" treatment (10,11)*.

PHYSIOLOGIC CHANGES AFFECTING PHARMACOKINETICS

Pharmacokinetics is defined as the handling of a drug within the body, including its absorption, distribution, metabolism, and elimination. There are various physiologic changes that occur with aging that may affect drug disposition in the body (Table 2 and Figure 2).

TABLE 1 Features of Polypharmacy

Medication not indicated
Duplicate medications
Concurrent interacting medications
Contraindicated medications
Inappropriate dosage
Drug treatment of adverse drug reaction
Improvement following discontinuance

TABLE 2 Age-Related Physiologic Changes Affecting Pharmacokinetics

Gastrointestinal system—rarely clinically significant
Acid production generally unchanged
Drug–drug interactions may alter absorption
Splanchnic blood flow decreases with no effect on drug absorption
Liver
Decrease in hepatic blood flow often associated with decreased first pass metabolism
Phase I metabolism affected
Phase II metabolism generally preserved
Fluid and tissue compartments
Decrease in total body water
Increase in fat compartment
Decrease in muscle mass
Plasma drug-binding proteins—rarely clinically significant
Decrease in serum albumin (primarily disease-related)
Kidneys
Decrease in renal blood flow
Decline in creatinine clearance
Decline in tubular secretion

■ Absorption

The extent of gastrointestinal (GI) absorption of most orally administered drugs is not significantly changed in the elderly. However several changes in the GI tract may affect the pattern of absorption (Chapter 19). "Gastric emptying time" may be prolonged, causing a delay in GI absorption. This is only clinically significant for acutely administered medications such as analgesics. In the case of chronic administration, the slow absorption of the drug may cause its accumulation and consequent toxic effects.

GI motility may be decreased, thereby prolonging the absorption phase of a medication. Decreased mucosal cells have been noted in the aging GI tract, and intestinal blood flow may be diminished, particularly if the patient has congestive heart failure. Absorption by active transport is reportedly decreased, but, inasmuch as the majority of medications are absorbed by passive diffusion, changes in absorption are not usually clinically significant. Changes in absorption can be clinically significant in the presence of concomitant administration of other medications. *An example of this would be the marked decrease in absorption of fluoroquinolone antibiotics with calcium supplements (12).*

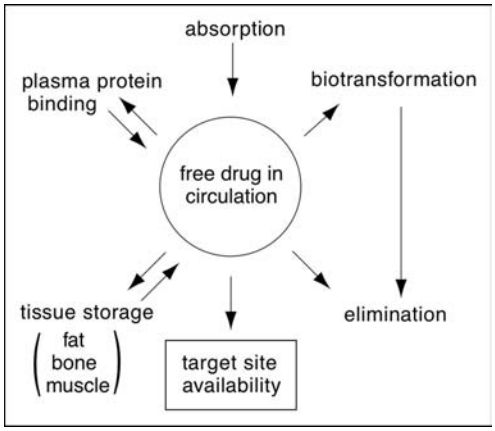

FIGURE 2 Pharmacokinetic: principles.

Absorption of medications via the intramuscular (i.m.) route has not been well studied in the elderly, but some predictions about absorption can be made. Absorption of i.m. injections may be impaired in the elderly because of decreased peripheral blood flow, particularly in patients with peripheral vascular disease. Increased connective tissue in aging muscle (Chapters 20 and 24) can impair tissue permeability and decrease systemic absorption of an i.m. injection. Intra-muscular injections may also be difficult to administer and painful to the patient, due to the decreased muscle mass in many elderly patients.

Systemic absorption must also be considered when using a transdermal product in older patients. Transdermal absorption of medications has not been extensively studied in the elderly, but there are changes in the aging skin that may affect transdermal absorption (Chapter 21). Older patients tend to have decreased skin hydration and decreased surface lipid content, factors important for transdermal penetration. They may also have increased keratinization, further impairing absorption. Decreased peripheral blood flow and compromised microcirculation may impair systemic absorption from transdermal products (13). Of the currently marketed transdermal products, most (nitroglycerine, estradiol, clonidine, and fentanyl) are commonly prescribed for older patients and should therefore be studied in this population.

■ Distribution

The distribution of medications within the body is dependent on whether the drug is lipid soluble or water-soluble and the extent of protein binding (Fig. 3). Age-related changes in body composition may significantly affect drug distribution. With aging, lean body mass and total body water decrease, whereas fat content increases (14) (Chapter 21). This can significantly increase the volume of distribution of fat-soluble drugs such as the benzodiazepines, a group of drugs with sedative-hypnotic actions (i.e., capable of inducing drowsiness and sleep). Clinically, it may take longer to reach steady-state levels and for the drug to be eliminated from the body.

The extent to which drugs bind to proteins can significantly alter the volume of distribution. Many elderly, particularly those living alone in the community, have inadequate protein intake, and

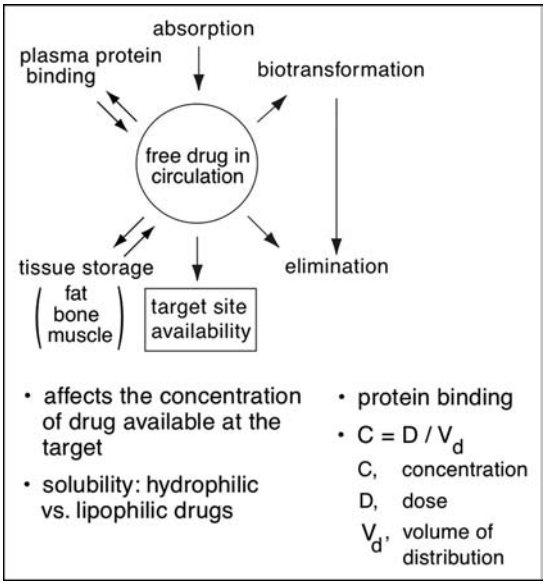

FIGURE 3 Pharmacokinetics: distribution.

therefore, are hypoproteinemic (Chapter 23). Because only the unbound portion of a drug is pharmacologically active, a reduction of plasma protein, specifically albumin, can result in higher free drug levels and increased effects. This is clinically significant for highly protein bound drugs such as phenytoin (an antiepileptic drug) and warfarin (an anticoagulant drug). Caution should be advised when prescribing two or more highly protein-bound drugs.

Cardiac output may be decreased in the elderly, altering regional blood flow. Decreased splanchnic, renal, and peripheral blood flow affects the distribution, and, therefore, the effectiveness of some medications. Tissue barriers may be altered with age, also affecting the outcome of the drug.

■ Metabolism

The liver is the main site of metabolism or biotransformation of drugs. Old age causes hepatic mass and blood flow to decrease, and consequently the relative bio-avalability of the drugs increases (2,15,16) (Chapter 19).

Hepatic metabolism of drugs occurs mainly through a number of chemical reactions that are classified as phase I and phase II reactions:

Phase I reactions usually convert the drug considered to a more easily excreted metabolite, primarily by oxidation but also by reduction and hydrolysis.

Phase II reactions, also called synthetic or conjugation reactions, intend to facilitate drug excretion by coupling of the drug or its metabolite with an endogenous substrate such as glucuronic acid, sulfuric acid, acetic acid, or an amino acid.

Various factors besides age, such as gender, genetics, smoking, alcohol, and medications, affect metabolism.

With age, phase I reactions are decreased, particularly in older men. Medications affected include theophylline [a compound found in tea with central nervous system (CNS) stimulatory, smooth-muscle relaxing, and diuretic actions (Chapter 23)], propranolol (a β-adrenergic blocker), and diazepam (an antianxiety drug), to name a few. Phase II reactions are minimally affected by age, but because polypharmacy occurs commonly, these reactions are affected by concomitant drugs that alter metabolism through these pathways. Examples here are lorazepam (an antianxiety drug) and acetaminophen (an analgesic/anti-inflammatory drug). The inducibility of hepatic enzymes by smoking, alcohol, and drugs appears to be diminished, although results from various studies are conflicting (16). Age-related changes in enzyme inhibition induced by cimetidine (a blocker of gastric acid secretion) have not been reported. It appears that enzyme activity is decreased by the same amount in elderly and younger subjects (16).

■ Elimination

Renal elimination is considered the most significant pharmacokinetic change in the elderly (Figs. 4 and 5) (Chapter 18). Between the fourth and eighth decades of life, renal mass decreases on an average by 20%, and renal blood flow decreases by 10 mL/min per decade after age 30 (17). Glomerular filtration rate, as expressed by the creatinine clearance (CrCl), also declines linearly with age beginning in the fourth decade. While measuring CrCl via a 24-hour urine collection is the best way to obtain an accurate level, these collections are often difficult, if not impossible, in the elderly patient, and usually entail inserting a catheter (a tubular device) into the bladder by intubation through the urethra (Chapter 18).

Alternatives to urine collections rely upon empirical estimations of CrCl. A common approach relates renal function

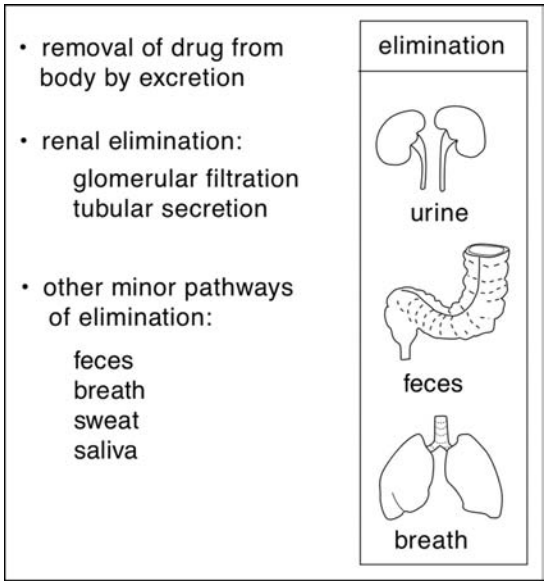

FIGURE 4 Pharmacokinetics: elimination.

to the serum creatinine (Scr) alone. However, in the elderly, Scr is an inadequate measure of renal function because creatinine production decreases as muscle mass decreases. Therefore, it is more appropriate to estimate CrCl using an equation (Cockcroft–Gault equation) that takes into account age, weight, Scr, and gender (18,19) (Chapter 18).

The Cockcroft–Gault equation is useful when calculating doses for drugs that are eliminated by glomerular filtration, such as aminoglycosides and vancomycin (antibiotics), digoxin (for the treatment of cardiac failure), lithium (a mood stabilizing drug), and histamine antagonists.

Drugs that are eliminated by tubular secretion also exhibit decreased excretion with age. This can occasionally be clinically significant for drugs that rely on tubular secretion for elimination.

■ PHYSIOLOGIC CHANGES AFFECTING PHARMACODYNAMICS

Pharmacodynamics refers to the processes involved in the interaction between a drug and an effector organ that results in a response, either therapeutic or adverse. Pharmacodynamics measures the intensity, peak, and duration of action of a medication. With aging,

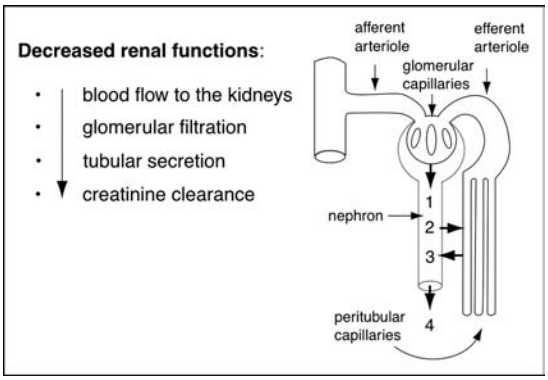

FIGURE 5 Age-related changes of drug elimination in the kidney.

some physiologic changes that may affect the body's response to medications include

- the increase of the older individual's sensitivity to a drug,
- the decrease of the sensitivity to of the medication, and
- the change of the susceptibility of the older person to the effects of the drug.

The predictability of a drug's response is decreased in the elderly, and the practitioner cannot only rely on the pharmacokinetic changes but must include pharmacodynamic factors as well.

Pharmacodynamic changes in the elderly can best be understood by reviewing examples of medications in which pharmacodynamic changes have been described. In the following section, several medications that illustrate pharmacodynamic changes are discussed.

Postural hypotension (i.e., lowered blood pressure when a person changes position, for example, from supine to erect position) appears to be more of a problem in older patients as compared to younger patients. This is due to a decrease in baroreceptor function (mediated through stretch receptors located in walls of the heart and blood vessels and modulate blood pressure) and decreased peripheral venous tone. When a younger person taking a vasodilator drug stands up abruptly, the body responds to the immediate hypotension with a reflex acceleration of cardiac rate (tachycardia), which helps to restore normal blood pressure. This tachycardia does not always occur in older patients, putting them at risk for dizziness, fainting, and falls. For this reason, *vasodilators should be used cautiously in the elderly.* Older patients who are treated with hypotensive or vasodilatory drugs should be instructed to change positions slowly when rising from a lying or sitting position. Increased postural (orthostatic) hypotension is seen with nitrates, nifedipine, tricyclic antidepressants, antipsychotics, and diuretics.

The hypnotic-sedative benzodiazepines are (unfortunately) widely used in the geriatric population. Based on pharmacokinetic factors, we can choose a benzodiazepine that would be relatively well tolerated in the elderly (short half-life and conjugated metabolism), but pharmacodynamic factors must also be considered (20,21). Older patients require a lower dose and plasma level of diazepam than younger patients to reach the same level of sedation. In general, the elderly will be more sensitive to the effects of a medication that depresses or excites the CNS. The elderly have less CNS reserve and, therefore, are more sensitive to an "insult" by a medication (Chapters 6–9).

Although the data are conflicting, most studies on adrenergic β-receptors have demonstrated that both the number and affinity of β-receptors are decreased in the elderly. The elderly have a decreased ability to form high-affinity binding complexes. The clinical implications of all of these changes are that the elderly may have a decreased response to β-blockers and agonists.

Elderly patients seem to be more susceptible to the side effects of antipsychotic medications. Extrapyramidal symptoms, orthostatic hypotension, and anticholinergic effects occur more frequently than in younger patients and are less well tolerated (22). Elderly patients on antipsychotics are more likely to experience parkinsonism, probably because of an already depleted dopamine reserve (Chapter 6). Adding a dopamine antagonist, such as a phenothiazine or haloperidol, can "tip" the older patient into parkinsonism or unmask latent Parkinson's Disease. Elderly patients experience tardive dyskinesia (i.e., involuntary stereotype movements) more frequently and earlier in treatment, than young adults even when on low doses of neuroleptics (23,24). This may be due to a "hypersensitivity" of the dopamine receptors in the substantia nigra/corpus striatum (Chapter 6). Tardive dyskinesia is more likely to be persistent and severe in the elderly, and women seem to be particularly at risk.

Anticholinergic agents (Chapters 6 and 7) are also commonly used in the elderly and are poorly tolerated due to the following:

- Slowed GI motility, which increases the risk of constipation (Chapter 19)
- Urinary retention, which is enhanced in patients with an outflow obstruction, such as an enlarged prostate (Chapter 18)
- Anticholinergic-induced CNS effects such as delirium and memory impairment that may be more pronounced in the elderly because of decreased CNS reserve
- Dry eyes and mouth (signs of parasympathetic block) (Chapter 6)

■ ADVERSE DRUG REACTIONS IN THE ELDERLY

As stated earlier, studies have demonstrated that the elderly are two to three times more at risk for adverse drug reactions as compared to younger adults (2). This is due to a number of factors summarized in Table 3, the most important of which include the following:

- Increased number of medications taken by the elderly
- Increased sensitivity to medications related to the pharmacokinetic and pharmacodynamic changes described above

Some adverse reactions are iatrogenic (medically induced) in nature. They may be due to the following:

- Choice of an inappropriate medication or dosage
- Inadequate monitoring of the patient
- Failure to recognize adverse effects or letting them go unnoticed by the patient and the prescriber
- Drug–drug interactions, drug–food interactions, or drug–disease interactions
- Noncompliance

Subtle effects such as GI complaints, dizziness, mental status changes, change in libido, instability and falls, and bowel or bladder habits may be attributed to "old age" or be treated as a new disease state. Health professionals should inquire about specific adverse effects and should encourage the patient to report any unusual occurrences while on medication.

Noncompliance is the failure of a patient to follow instructions regarding medications. It represents another factor often contributing to adverse drug reactions. Psychosocial complications such as poverty, dementia, and loneliness exacerbate this problem (25). Another barrier to patient compliance can be the "excessive availability" of drug information that patients and family members can easily access. The interpretation of this

TABLE 3 Factors Associated with Increased Incidence of Adverse Drug Reactions in the Elderly

Reduced (small) stature
Reduced renal and hepatic function
Cumulative insults to body
Disease
Faulty diet
Drug abuse
Medications, multiple and potent
Altered pharmacokinetics
Noncompliance

information is not always accurate and/or applicable to the individual patient and can lead to noncompliance (26). Effective communication between prescriber, patient, and caregiver can help eliminate this factor (27).

■ Some Specific Examples of Potential Drug Toxicity

Although a comprehensive review of all drug classes is impractical here, certain groups of medications are problematic enough in the elderly to warrant separate consideration. A list of undesirable medications has been formulated by a consensus panel (Table 4) (19).

Originally promulgated in 1997 (18), this list has been revised in 2003 (19). By such criteria, one-year prevalence of the use of inappropriate medication ranges from 21% to 48 %, depending on population (7). Among nursing home residents taking inappropriate drugs, there was an excess risk of hospitalization and death (5).

Drugs Acting on the Nervous System

Psychotropic medications are responsible for the most adverse drug reactions. Such reactions include worsening mental status, falls, dehydration, orthostatic hypotension, and disorders of movement (e.g., extrapyramidal signs), and tardive dyskinesia. An important disease state–drug interaction common to all psychotropics is the increased sensitivity to side effects exhibited by elderly persons with common brain diseases such as Alzheimer's disease, Parkinson's disease, and stroke. A side effect profile that minimizes anticholinergic effects should be chosen when prescribing psychotropics (28–30).

Analgesics (drugs relieving pain) are another group of medications frequently prescribed and therefore also warrant special consideration. Nonsteroidal anti-inflammatory drugs (NSAIDs) are currently ubiquitous, despite the plethora of adverse drug reactions that may arise from their use (31,32). The most common side effects are GI symptoms ranging from gastritis to life-threatening hemorrhage (33,34) (Chapter 19). Renal effects (35) are usually reversible (Chapter 18) as well as are photosensitivity and hives. Pulmonary edema arises from congestive heart failure due to fluid and sodium retention. NSAIDs also interfere with hypertension treatment. CNS effects range from headache, to altered mental status, to frank psychosis. Genitourinary effects include ejaculatory dysfunction. In view of the aforementioned problems, geriatricians are conservative in the use of NSAIDs (36). The nonpharmacologic management of osteoarthritis (i.e., physical therapy) is utilized whenever possible, and finally, acetaminophen is recommended for analgesia (37) (Chapter 20).

TABLE 4 Medications Best Avoided in the Elderly

Drugs with prominent anticholinergic properties
Amitriptyline (Elavil)
Belladonna alkaloids (Donnatal and others)
Chlorpheniramine (Chlor-Trimeton)
Dicyclomine (Bentyl)
Diphenhydramine (Benadryl)
Doxepin (Sinequan)
Hydroxyzine (Vistaril and Atarax)
Hyoscyamine (Levsin and Levsinex)
Oxybutynin (Ditropan)
Promethazine (Phenergan)
Long-acting benzodiazepines
Diazepam (Valium)
Analgesics
Indomethacin (Indocin)
Ketorolac (Toradol)
Meperidine (Demerol)
Propoxyphene (Darvon)
Long-term use of stimulant laxatives
Bisacodyl (Dulcolax)
Cascara sagrada
Cardiac medications
Amiodarone (Cordarone)
Clonidine (Catapres)
Doxazosin (Cardura)
Nifedipine (short-acting) (Procardia and Adalat)
Digoxin (Lanoxin) at doses that exceed 0.125 mg/day, except when treating atrial arrhythmias
Nitrofurantoin (Macrodantin)
Cimetidine (Tagamet)
Chlorpropamide (Diabinese)

Source: Adapted from Ref. 19

Anticoagulant Drugs

Elderly patients seem to be more sensitive to the effects of *anticoagulants* such as warfarin, and decreased doses are needed to adequately anticoagulate the older patient (38–39). Just why this sensitivity occurs is not entirely clear. There may be a relative deficiency of vitamin K or vitamin K-dependent clotting factors in the elderly (Chapter 23). There may also be increased concentrations of inhibitors of coagulation. In addition, warfarin is strongly bound to albumin, so alterations in binding must be considered. Whether age is an independent risk factor for complications of warfarin therapy has been debated (40,41). Either way, it is prudent for the practitioner to closely monitor all elderly patients on warfarin therapy.

Cardiovascular Drugs

Management of an elderly patient on *digoxin* illustrates all of the principles already mentioned. Digoxin is one of the most used preparations of digitalis from the leaf of the foxglove plant. *The main action of digoxin is its ability to increase the force of myocardial contraction*. Beneficial consequences of this action include increased cardiac output, decreased cardiac size, venous pressure, and blood volume, slowing down of cardiac rate, promotion of diuresis, and relief of edema. Many of these actions will benefit alterations in cardiac functions at all ages, including old age (Chapters 20 and 24). Administration of digoxin to an older individual with cardiac alterations depends on several considerations:

- First, the indications for the use of digoxin have been scrutinized, and digoxin is no longer utilized in an indiscriminate manner to everyone with congestive heart failure (42,43). Rather, the indications for its use have been narrowed to specific clinical situations (44–46).
- Second, because of its reduced elimination, it is usually given in lower doses in the elderly. Digoxin serum levels are monitored to prevent toxicity (47,48). Even at normal therapeutic levels, it can produce a range of adverse effects including anorexia and altered mental status.

Thus, this classic medication serves as a good example of a drug that undergoes altered pharmacokinetics as well as altered pharmacodynamics in the elderly (49).

■ GENERAL GUIDELINES

In the interest of avoiding adverse drug reactions, a number of principles, partly based on physiologic considerations, should be followed:

1. Nonpharmacologic management should be used whenever possible.
2. The number of drugs prescribed should be kept to a minimum.
3. The drug regimen should be simplified to aid in compliance.
4. Treatment should be prescribed only with clear goals or endpoints in mind.
5. Dosage should be adjusted to take into consideration altered physiologic parameters (i.e., start with low dose and increase slowly).
6. Laboratory monitoring should be utilized when indicated.
7. Medication regimen should be regularly scrutinized.

The drug regimen needs to be regularly reviewed and reassessed for possible changes (50). This includes an "obsessive" (i.e., as detailed as possible!) drug history that may be facilitated by the patient bringing in all medications as well as over the counter (OTC) drugs for scrutiny by a health professional.

Finally, one must suspect a drug reaction when any otherwise unexplained symptoms occur, such as a change in mental status.

One of the most common interventions a geriatrician makes is to discontinue medications. It has been shown that, when done judiciously, most patients benefit from this maneuver. Equipped with a basic knowledge of physiologic changes that occur with aging, pharmacologic principles, and common sense, healthcare professionals should be able to prescribe medications for elderly patients in a safe and effective manner.

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23

Healthful Aging: Nutrition and Experimental Strategies in Dietary Restriction

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INTRODUCTION

Epidemiologic studies in humans and experimental data reported in several animal species indicate that good nutrition and regular physical exercise not only contribute significantly to health and well-being but also may prolong average and maximum life span (1,2). With scientific validation, these two interventions are now accepted as "natural" ways to deal with a number of disabilities and diseases related to old age. Accordingly, a brief discussion of the impact of nutrition (Chapter 23) and of physical exercise (Chapter 24) on functional competence and on longevity will serve as an appropriate conclusion to the study of the physiologic changes that occur with advancing age.

Lengthening the "health span," that part of the life span enjoyed in good health and without disabilities, has become one of the major goals of those researchers and educators working in the field of gerontology and geriatrics. We know already that biomedical progress in the United States and in many other world countries have allowed us to live several years longer than our parents and ancestors (Chapter 2). Similarly, preventive and rehabilitative strategies, by preserving intellectual and functional competence into old age, are allowing elderly individuals to maintain an independent and active lifestyle. We are now witnessing rapid biotechnological advances that we must exploit, in full, to treat the diseases of old age and to improve the quality of life at all ages (Chapter 25). Some of the classic and new directions to come from this research are briefly presented in this and in the following two chapters.

In this chapter, after this short introduction, we will summarize, first, nutritional requirements in old age (see section entitled Nutritional Requirements in Old Age), followed by considerations on neuroendocrine control of food intake (see section entitled Neuroendocrine Control of Food Intake). The last section of the chapter (see section entitled Experimental Strategies in Dietary Restriction) will review calorie restriction as an experimental strategy to lengthen and improve the life span.

With respect to the preventive and rehabilitative potential of nutrition, physical exercise, and other interventions in an aging population, it must be kept in mind that

- the duration of the intervention, short term or long term, is likely to significantly influence the outcome;
- old age may result in or from differential gene expression, such that the requirements for nutrition and physical exercise of the elderly may differ from those of the young;
- genetic variation among individuals and ethnic groups may justify an individualized or "customized" approach to treatments involving specific dietary components such as vitamins and trace nutrients; and
- any preventive or therapeutic program for the elderly must take into consideration the possible presence of simultaneous multiple pathologies (comorbidity) (Chapter 3) and the possibility that polypharmaceutical interventions (Chapter 22) may interfere with or enhance the regimen chosen for a given individual.

Given the complexity of the aging processes, particularly in mammals, attempts have been made to develop experimental models in which the rate of aging can be manipulated in a predictable manner (Chapters 3 and 4). As briefly presented in this Chapter, one of the simplest but most effective methods of extending the life span and, by implication, of slowing down the rate of aging is dietary caloric restriction (CR) (3). This method consists of limiting access to food (in terms of calories) until the body weight of experimental animals is considerably lower than that of age-matched, fully fed, control animals. In addition to prolonging the life span, dietary restriction has the effect of maintaining a number of physiologic and metabolic processes in a youthful state, thus, delaying and reducing the incidence of aging-related pathology (see below). Indeed, this model is now being exploited as an important experimental tool in research in aging and longevity.

NUTRITIONAL REQUIREMENTS IN OLD AGE

Most old people retain dietary patterns similar to those acquired in their youth. The American Dietetic Association acknowledges this fact by its emphasis on nutrition and dietary changes as part of a continuum of one's health-care programs throughout life. Our intent here is to provide useful information about nutrition and diet among the elderly, drawing from selected physiologic and clinical observations.

Nutritional Risk Factors for Individuals Over 60 Years of Age

Although optimal nutritional status is critical to good health at any age, to achieve good health in the older age group demands special attention (4). For one thing, adults, 65 years of age and older, for numerous psychosocial reasons, often face obstacles in trying to prepare nutritious meals on a day-to-day basis. In addition, given the increasing number of prescriptions and over-the-counter medications many older people take (Chapter 22), they may suffer drug-associated nutritional deficiencies of which they might not be aware. Indeed, the detection and treatment of nutritional disorders in older persons has emerged as a major public health concern, nationally.

A variety of screening tests has been recommended by different agencies and organizations concerned with the aging population, but many of these tests are not easily performed or properly interpreted (4). The most important risk factor(s) associated with poor diet (in this age group) include low income, social isolation, and illness. Commonly used screening tests and assessments are listed in the following section.

Anthropometric and Biochemical Tests

One anthropometric measure frequently employed to assess overall nutritional status of elderly persons is the body mass index (BMI), which relates body weight (in kilograms) to height (in meters squared). Accordingly, "normal adult" individuals register BMI values ranging from ≥ 18.5 to ≤ 25.0 (Chapter 13).

Unfortunately, in the elderly, in whom intervertebral disc spaces are often narrowed and osteoporotic vertebral compressions are frequently encountered (Chapter 20), the BMI may give inaccurate information. Even alternative measures such as the height to the knee, the length of the arms (the so-called total arm length) and the arm span, are far from ideal measures (5).

From a practical as well as clinical point of view, an accurate serial recording of body weight may be considered the most used and useful screening method to ascertain nutritional status in all age groups: for example, significant signs of impending malnutrition are the (unintended) loss of 1 kg in one month or 3 kg in six months.

Poor nutritional status can be assessed according to the results of laboratory tests such as:

- Low ferritin levels (below 15 μg/dL)
- Low lymphocytic counts (<1200/dL)
- Abnormal level of thyroid-stimulating hormone (TSH) (Chapter 12)
- A total cholesterol (TC) level below 160 mg/dL (Chapter 16)
- Low albumin (below 4.0 g/dL) and prealbumin (below 1.3 g/dL) levels

Currently, the presence of hypoalbuminemia and, particularly, of hypo-pre-albuminemia is recognized as a strong indicator of undernourishment, a marker for the possible onset of medical complications related to malnutrition and a factor in the prognosis of mortality (6).

Clinical Assessment

In screening the nutritional status of the elderly, it is important, before any dietary recommendation is made, to assess the person's clinical status, particularly the functional status of the gastrointestinal tract (Chapter 19) and certainly to take a drug/medication history (Chapter 22).

In addition, it must be borne in mind that

- an elderly individual's ability to eat, specifically to chew and swallow, may be impaired by poor teeth and dry mouth (Chapter 19);
- the pleasure of eating may have declined as a result of decreased taste or smell (Chapter 19); and
- urinary frequency or incontinence (Chapter 18) may have led to restriction of fluids and, concomitantly, poor food intake.

Specific recommendations are in order for a number of conditions. For those elderly with gastroesophageal regurgitation disorder (Chapter 19) and with diaphragmatic hernia, sound advice is to maintain elevation of head and chest when sleeping and to avoid alcohol. The presence of diverticular disease of the colon (Chapter 19) may require initiating a low-fiber diet, certainly during flare-ups (associated with abdominal pains and diarrhea). Constipation may be corrected by increasing fluid and fiber intake, exercising (simple walking), review of medications for side effects of constipation, and reassurance that a bowel movement every two or three days is not abnormal. Should rectal bleeding be present, all possible causes (hemorrhoids, ischemic colitis, diverticular disease of the colon and colon cancer, etc.) should be explored.

Even though digestion in the elderly tends to be slower than in younger adults, actual intestinal absorption appears essentially unchanged. Absorption of some nutrients like vitamin B_{12} may be adversely affected by low gastric hydrochloric acid content (Chapter 19). Likewise, intestinal absorption of calcium may be reduced and this reduction may require an increased amount of dietary calcium to compensate for the calcium loss (Chapter 20). In the elderly, the quality of the nutrients ingested and the prolonged use of medications is what interferes most with digestion.

The Obese Elderly

As previously discussed in Chapter 13, total body fat increases until about 40 years of age in men and 50 years in women. It remains unchanged thereafter until 70 years, in both sexes, when it tends to decrease. Fats provide some benefits, especially for the elderly by:

1. providing storage for excess calories and protection in acute illnesses,
2. protecting vital organs and bones from injuries due to falls, and
3. maintaining core body temperature.

Body fat distribution has been categorized in the so-called "low-to-high waist-to-hip ratio." The low ratio (a "pear shape" distribution of fat) may protect from some of the complications associated with obesity whereas the high ratio (an "apple shape" distribution of fat) is associated with an increased risk of hypertension, late-onset diabetes mellitus, coronary heart disease, and premature death (7).

Elderly individuals with pathological obesity (BMI > 35) are few because they die before reaching advanced age. In a population of Korean men and women between the ages of 35 and 90 years, underweight, overweight, and abdominal obesity increased the risk of death compared to that of a population of normal weight (8). In addition, the risk of death from respiratory causes was greater in those with a lower BMI and the risk of atherosclerosis was greater in those with a higher BMI (8). These observations were supported by data from a large prospective cohort of subjects aged 50 to 71; in this group, the risk of death was highest for the categories with the highest and lowest BMI among men and women at all ages (9). Less-severe obesity is relatively more frequent and associated with less-severe complications.

Treating obesity is a less-important problem in the elderly than in younger individuals. As discussed in Chapter 24, an appropriate exercise program is often all that is needed to maintain a healthy weight. A one-mile walk burns about 100 kcal, and a walk (even in a mall) of two to three miles four times a week, if accompanied by a slight restriction in caloric intake, should result in a gradual weight loss. If osteoarthritis (Chapter 20) is a problem, an upper body exercise is recommended. Elderly patients on weight reduction diets are at a significant

risk of developing protein deficiencies (6) and, in these cases, blood albumin levels should be monitored frequently (11). A discussion of the relationship between obesity and diabetes is presented in Chapter 13.

■ Dietary Recommendations

The Dietary Reference Intakes (DRIs), recently updated in 2002/2005 by the Food and Nutrition Board of the National Academy of Science (10), are grouped by life stage, gender, and age, particularly the 50- to 70-years and above 70-years-of-age population. The age-associated physiologic and metabolic changes occurring between these two elderly groups show only minor variations in nutrient requirements (Table 1). Factors that might modify these guidelines, such as bioavailability of nutrients from different sources, nutrient–nutrient and nutrient–drug interactions, and intakes from food fortificants and supplements, are incorporated into the guidelines in much greater detail than previously.

In 1990, the Food Guide Pyramid was developed as a tool to achieve a balance in nutrient intake. The Food Guide Pyramid was recently updated in 2005 to display the messages of the dietary guidelines and support the awareness of vital health benefits associated with nutrition, physical activity, and lifestyle behavior (Figs. 1 and 2) (Table 1). The symbol was modified to demonstrate the proportion of foods from each food group, with exercise being a new addition (Fig. 1).

The Food Guide Pyramid has been adapted from other cultures in particular the "Mediterranean diet" due to Keys's (12) observations of dietary patterns in relationship to cardiovascular mortality in 16 southern European populations from countries bordering the Mediterranean Sea (Fig. 2) (Box 1). The low rates of cardiovascular deaths in these populations led Keys to conclude that their typical dietary pattern might explain the health benefits observed (13). Recent research supports Keys's theory. A large randomized trial of 772 subjects showed that a Mediterranean diet, supplemented with olive oil and nuts, when compared to a low-fat diet, had beneficial effects on cardiovascular risk factors (16). Another study found favorable effects of the Mediterranean diet on lipoprotein levels, endothelial vasodilatatory factors, insulin resistance, antioxidant capacity, cardiovascular mortality, and cancer incidence in obese patients and in those with previous myocardial infarction (17).

The great emphasis on cereals, fruits, and vegetables is reflected in the present nationwide campaign to promote the daily consumption of at least five servings of fruits and vegetables per day. Current recommendations provided by the revised 2005 Dietary Guidelines emphasize science-based advice on proper dietary habits and physical activity. These guidelines are designed to promote health and reduce the risk of major chronic diseases by encouraging fewer calories consumed, more physical activity, and smarter food choices. These guidelines do state a handful of specific recommendations for older adults including adequate vitamin D intake, regular physical activity, adequate fiber to reduce constipation,

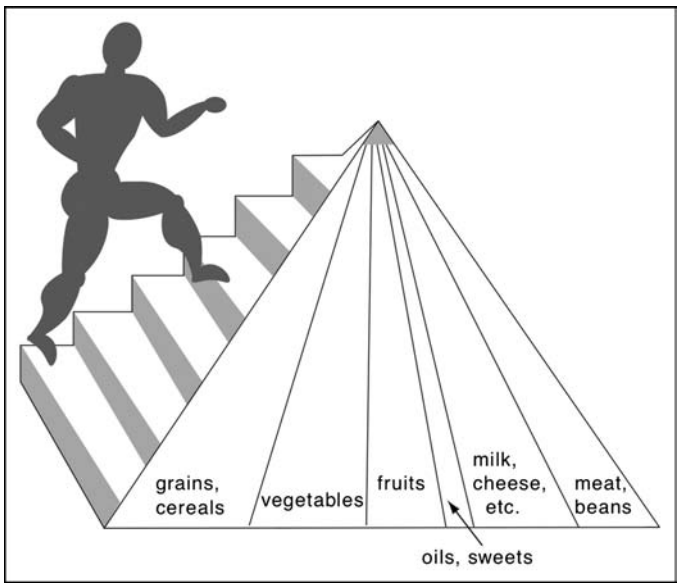

FIGURE 1 The Food Guide Pyramid, suggested by the Food and Nutrition Board of the National Academy of Science (10). Note the addition of the exercising man indicating the importance of physically exercising while maintaining a healthy diet.

TABLE 1 Dietary Guidelines[a]

Eat a variety of foods, keep foods safe to eat
Balance the food you eat with physical activity. Achieve and/or maintain optimal weight
Select foods low in fat (especially saturated fats) and cholesterol, moderate in total fat
Include plenty of grain products, vegetables, and fruits
Choose beverages and foods to moderate intake of sugars
Choose and prepare foods with less salt, lower salt intake to less than 5 mg/day
If you drink alcoholic beverages, do so in moderation

[a] Adapted from Dietary Guidelines Advisory Committee (10).

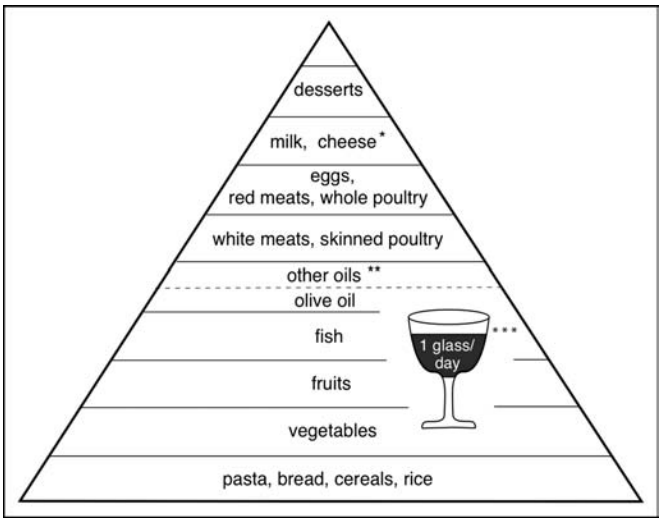

FIGURE 2 Adaptation of the Food Guide Pyramid for the Mediterranean diet. Note the two separate tiers for fruits and vegetables; the tier for fats consists primarily of olive oil and other oils. Note the presence of one glass of red wine per day. * Low priority due to presence of saturated fats, (esp. myristic acid) **Unsaturated fats ***May interact with a number of medications.

BOX 1 *Some Historical Notes*

The ancient Egyptians, in addition to the more common kinds of cereals and beers for the general population, used to prepare very special diets, set aside exclusively for the high priests and the Pharaohs in the temples of Thebes. These diets contained special ingredients including prepared extracts from leaves of lotus and papaverus in which modest amounts of opium were present; they were thought to ensure a much longer and happier life.

The word "cereal" is derived from the name of the Roman goddess Ceres, sister of Jupiter and patroness of grains in general. A temple to Ceres was built in Rome in the fourth century BC and its custody was given to the Ediles or elected individuals with the mandate to provide Rome with adequate quantities of cereals. In statues and frescos, Ceres is always represented with a crown of wheat on her head and a sheaf of wheat in her arms.

In the fifth century BC, Hippocrates of Coos proclaimed the benefits of barley in any form as conducive to a healthy and long life (14). Galenus of Pergamon, personal physician to the emperor Marcus Aurelius in imperial Rome (second century AD), was an advocate of the frequent use of onions and garlic, ingredients once again recommended for a longer and happier life (14). The benefits of garlic are touted even today, although, pharmacologically, no specific effect has ever been verified.

In the middle ages, a host of recipes were prepared by "witches" promising to provide humans with eternal youth—especially the rejuvenation of somnolent sexual organs to restore virility (15). Even as the Inquisition disposed of more than 1 million individuals, most of them old women, these special diets and potions for the nobility continued; witchery was apparently very well protected. Madame de Montespan, a favorite of King Louis XIV of France, "le roi soleil," used to prepare special dinners for her royal lover, dinners in which "cantaridin" was a prime ingredient (15). Cantaridin prepared from the drying of the insect cantaris induces congestion in the genitals. Although it facilitates penile erections, the cost in terms of renal toxicity and gastrointestinal problems is significant. Ingestion of this substance might well explain the chronic gastrointestinal upset of the poor king.

and sodium and potassium recommendations in association with hypertension.

The Dietary Reference Intakes (DRIs) for the Macronutrients: Energy, Proteins, Carbohydrates, Fibers, and Fats

Energy

In terms of energy, the DRI for Energy (10) has established the term estimated energy expenditure (EER) with a defined calculation for each gender incorporating age, weight, height, and physical activity (PA). The following equations define EER as the average dietary intake calculated to predict energy balance for normal-weight individuals with a BMR of 18.5 to 25. These equations, reflecting the average needs and incorporating specific characteristics (see above), represent the current and most accurate equations for estimating energy needs.

For males > 19 years:

$$EER = 662 - [9.53 \times \text{age (year)}] + PA \times [15.91 \times \text{weight (kg)} + 539.6 \times \text{height (m)}]$$

PA = 1.00 (sedentary)
PA = 1.11 (low activity)
PA = 1.25 (active)
PA = 1.48 (very active)

For females males >19 years:

$$EER = 354 - [6.91 \times \text{age (year)}] + PA \times [9.36 \times \text{weight (kg)} + 726 \times \text{height (m)}]$$

PA = 1.00 (sedentary)
PA = 1.12 (low activity)
PA = 1.27 (active)
PA = 1.45 (very active)

The previously recommended dietary allowance (RDA) in terms of energy listed was as follows:

- For individuals aged 51 to 70 years, a 2300 kcal diet for a 75 kg man, and 1900 kcal for a 56 kg woman
- For individuals aged 75 years and older, a 2300 kcal for men and 1700 kcal for women

These estimates were approximate and did not specify the kind of activity performed by the individuals over 24 hours. Accordingly, a higher calorie intake was recommended for older adults engaging in physical activity (18,19). Under the current DRIs, there is no RDA value for energy, because energy intakes above the EER would be expected to result in weight gain.

Protein

There are DRI values established for protein intake for each life stage. The estimated average requirement is 0.66 g/Kg/day for adults older than 50 years. It is similar to the previous protein RDA of 0.8 g/kg/day of body weight for the same, over 50 years of age, group. In the elderly, protein deficiency may be due to impaired utilization, lower metabolic demand, lower calorie intake, and, eventually, lower protein requirements. Protein deficiency is associated with lack of energy, weakness, decreased muscular strength, poor bone health, cognitive dysfunction, and depression (sometimes indicating the onset of anemia and hypothyroidism). These changes may be remedied by a diet with adequate protein intake and by the participation of the elderly in regular resistance-training exercise in the latter case; with both adequate diet and regular exercise (Chapter 24), protein synthesis rates are restored to a level similar to that found in younger individuals (18,19).

Not only too little but also too much protein may have deleterious side effects: for example, too much protein may increase the risk of renal glomerular sclerosis (Chapter 18) (20) and may contribute to the development of osteoporosis (21). Conversely, limiting protein intake may delay the development of age-related changes in renal function (Chapter 18).

The Third National Health and Nutrition Examination Survey, 1988 to 1991, evaluated sources of protein in the American diet (22). In terms of protein distribution for the age group 60 years and above, 65% were derived from animal protein and 35% from plant protein with the following breakdown: 40% from meat, fish, and poultry; 20% from dairy products, 19% from grains, 11% from fruits and vegetables, 5% from eggs, and 4% from legumes, soy, nuts, and seeds. The study also found that the age group of 60 years and above average 16% of their total calories from protein. An impressive 40 g of protein is present in each of the following:

- 200 g fish or poultry or red meat
- 400 g cooked rice
- 150 g tree nuts
- 470 g cooked beans
- 130 g peanuts
- 500 g tofu
- 200 g cheese
- 1 L milk
- 12 egg whites

Carbohydrates and fiber

Complex carbohydrate should represent 55% to 60% of the total caloric intake in a well-balanced diet. The RDA for carbohydrate for 50 year old individual is 130 g for males and females. The RDA for fiber for the same age group is 30 g for males and 21 g for females. In fact, the RDA for fiber decreases at age 50 and stays the same above 70 years.

Dietary fibers are of two classes, soluble and insoluble. The soluble fibers, e.g., pectin, mucillagen, and gums form a sort of gel matrix that slows down intestinal absorption but is later metabolized in the large intestine by bacteria, thereby giving rise to short-chain fatty acids. Fruits, vegetables, and legumes are an excellent source of soluble fiber (Table 2). The insoluble fibers, e.g., cellulose, hemicellulose, and lignin, have strong water-binding capacity and are found in wheat bran, whole-grain breads, and cereals and also in the skin of fruits and vegetables. Because of their water-binding characteristic, they tend to facilitate intestinal motility. In elderly individuals, high dietary fiber may interfere with the intestinal absorption of some drugs such as the cardiac medication, digitalis (Chapter 22).

Present advice from nutritionists is to limit the intake of refined sugars, but the consumption of starch is still recommended. The portion of starch that may escape complete digestion in the upper intestinal tract—whether as a consequence of insufficient mastication or rapid transit through the small intestine—is converted to butyric acid; this conversion, in fact, seems to improve colon health and may even add some protection against colon cancers (23).

Fats

The current recommendation regarding the DRI for fats in the diet is that they should be limited to 30% or less of the total energy requirements; the TC intake should be no more than 300 mg/day. A useful guideline is that a single egg yolk contains roughly 215 mg of cholesterol. Apart from cholesterol, fats should be essentially unsaturated, such as the monounsaturated fatty acids in olive oil and the polyunsaturated fatty acids in fish. There is no set RDA for total fat. However, there is an acceptable macronutrient distribution range (AMDR) representing a range associated with reduced risk of chronic disease while providing enough essential nutrients. The AMDR for total fat for those 50 years and older is 20 to 35 g/day.

Saturated fats should be limited to less than 10% of total calories, due to their association with increased risk for heart disease and cancer (24). Milk and milk products contain mostly saturated fat, in particular myristic acid; therefore, low-fat and nonfat milk and milk products are generally recommended. Neither milk nor cheese should be banned from the geriatric diet as they represent an invaluable source of calcium, proteins, and vitamins.

Definite benefits seem to be found in the oils of cold-water fish, the omega-3 polyunsaturated fatty acids. The RDA for omega-3 fatty acids for males above 50 years is 1.6 g/day and for females above 50 years is 1.1 g/day. Studies have indicated that in high amounts, omega-3 fats decrease triglyceride levels and perhaps increase high-density lipoprotein (HDL) levels (Chapter 16) (25). In addition, they may have antiarrhythmic properties and may reduce the risk of sudden death after myocardial infarction (24). High intake of omega-3, omega-6 monounsaturated fats, and weekly fish intake may reduce aging-related cognitive decline (11,26) although the current data are still inconclusive and ongoing studies are under way to decipher the true effect of the omega-3 fats (27).

Diets high in saturated fat and cholesterol have been linked to elevated cholesterol levels. The campaign against saturated fats and cholesterol, started 20 years ago, has been unquestionably effective. Reduced levels of serum cholesterol have coincided with a 43% decline in death rate from all cardiovascular diseases (Chapters 15 and 16). The Minnesota Heart Survey 1980–1982 to 2000–2002 found serum cholesterol levels have dropped over the past 20 years due to diet education and cholesterol-lowering drug use. Cholesterol levels were highest in the 45- to 54- and 55- to 64-year-old adults 20 years ago. Now the 35- to 44-year-olds show the highest cholesterol levels with a simultaneous doubling in cholesterol-lowering drug use during the same period (13).

A more recent campaign has been conducted against transfats, fats produced during the hydrogenation process of vegetable oils. These fats have also been linked to changes in cholesterol levels and increase the risk for heart disease (28). Therefore, the Food and Drug Administration has recently required that they be added to the food labels to help consumers make more informed decisions. The current goal of the National Cholesterol Education Program is to bring the mean blood cholesterol concentrations to well below 200 mg/dL and low-density lipoprotein (LDL) cholesterol levels below 110 mg/dL.

TABLE 2 Fiber Content in Common Foods

Food	Fiber (g)
Wheat bread, 1 slice	2
Broccoli, 1 cup	2
Raisin bran cereal, 1 cup	4
Apple, medium	6
Kidney beans, 1 cup	8

The Dietary Reference Intakes (DRIs) for the Micronutrients: Vitamins and Minerals

In the most recent recommendation, vitamin DRI does not differ between younger and older adults, with the exception of vitamin B_6 up from 1.3 to 1.7 mg/day in males and 1.3 to 1.5 mg/day in females. Usually, mild vitamin deficiency, with or without trace mineral deficiency, is common among the elderly, particularly in frail and institutionalized individuals, and is accompanied by cognitive impairment, poor wound healing, anemia, and increased propensity for developing infections (29–31).

The advisability of routine vitamin and mineral supplements in healthy older people remains controversial (32–34). A diet including

at least five to six servings of fruits and vegetables with at least two servings of dairy products, several servings of grains, and a few ounces of meat per day usually contains sufficient vitamins and minerals, including phytochemicals, to satisfy the necessary requirements. Supplemen-tation is often required in cases of less healthy diets, especially to maintain a normal immune status (32). A large and increasing body of literature advertises the possible benefits of vitamin and mineral supplements (34,35); however, more recently controlled clinical trials do not demonstrate any substantial benefit to added supplementation.

Fat-Soluble Vitamins

A list of fat-soluble vitamins, RDA for the aging population, selected major functions, and effects of deficiency or excess is presented in Table 3.

Water-Soluble Vitamins

The B-complex vitamins (so-called because they were originally identified in beer yeast) act as coenzymes for various metabolic reactions and are needed for cell replication and hematopoiesis (Chapter 17). Vitamin C is an important antioxidant to reduce disease (Chapter 5) and to support collagen synthesis (Chapter 21). A list of the RDAs for water-soluble vitamins for the elderly population, their selected major actions, and the effects of their deficiency or excess is presented in Table 4.

Minerals

A list of minerals, RDAs for the elderly population, their selected major functions, and effects of their deficiency or excess is presented in Table 5.

The DRIs: for Electrolytes and Water

Sodium and Potassium

Although *sodium* is present in a multitude of substances, unquestionably the main source of this important electrolyte is table salt (sodium chloride). A diet completely devoid of sodium would induce insufficiency of the adrenal glands (Chapter 9) and result in hyponatremia (i.e., low sodium levels or sodium insufficiency). Hyponatremia may induce brain swelling and heart failure. Hence, in areas of the world where salt is scarce, it is considered as valuable as money. Indeed, the term "salary" originates from the Latin *salarium*, that is, allowance given to the Roman soldiers for salt, hence any allowance or pay. Salt was frequently used by the soldiers of the Roman legions in Africa or Asia as barter in transactions with local people.

The current DRI, more specifically the adequate intake (AI), for 50 years of age and above for both males and females is 1500 mg/day with an upper limit (UL) of 2300 mg/day. The UL is equivalent to one teaspoon of salt. Table salt is sodium chloride and it is 40% sodium by weight. Therefore, 2300 mg of sodium is equivalent to 5.75 g of salt. The highest level considered acceptable by the National High Blood Pressure Education Program is 2300 mg. It is also the highest amount recommended for healthy Americans by the 2005 "U.S. Dietary Guidelines for Americans." In fact, the 2005 Dietary Guidelines state that specific populations such as those with hypertension and the older population should aim to consume no more than 1500 mg of sodium per day. The 1500 mg level, or 3.75 g of salt per day, can lower blood pressure further and, more recently, this is the amount recommended by the Institute of Medicine as an AI level and one that most people should try to achieve. Currently, American men eat about 4200 mg/day and American women 3300 mg/day; this amount is equivalent to 10 g of salt for men and 8 g of salt for women. Of this intake, 77% comes from food processing, while 11% comes from sodium added during cooking and that added at the table. In those affected by hypertension (especially in sodium-sensitive individuals), salt intake should be further reduced to 4 g/day. Fortunately, the taste for sodium diminishes after a few months of restriction, and such a goal is not difficult to attain.

Potassium is abundantly present in fruits and vegetables, especially those with bright colors. The AI for potassium for 50 years of age and above is 4700 mg/day. In the elderly, the frequent use of diuretics (prescribed for various medical conditions) may lead to a deficit; in these individuals, a laboratory test of serum potassium levels may suggest the need for supplementation. Hyperkalemia (high blood levels of potassium) may also occur in the elderly due to declining levels of aldosterone and impairment of renal function (Chapters 9 and 18).

TABLE 3 Fat Soluble Vitamins

Vitamin	DRI ≥ 50 yr	Major function	Deficiency	Toxicity
Vitamin A	≥50 yr Males: 900 μg; Females: 700 μg	Required for normal vision, gene expression, reproduction. and immune function	Night blindness	Teratological effects, liver and skin toxicity
Vitamin D	50–70 yr Males: 10 μg; Females: 10 μg >70 yr Males: 15 μg; Females: 15 μg	Maintains serum calcium and phosphorous concentrations, supports bone development	Osteopenia	Elevated plasma 25 (OH) D concentration causing hypercalcemia
Vitamin E	≥50 yr Males: 15 mg Females: 15 mg	A nonspecific chain-breaking antioxidant	Anemia	Excess supplementation may result in hemorrhagic shock and/or heart failure
Vitamin K	≥50 yr Males: 120 μg; Females: 90 μg	Coenzyme during the synthesis of many proteins involved in blood clotting and bone metabolism	Reduction in blood clotting; associated with high antibiotic use	Adverse effects associated with food or supplements have not been reported (potential adverse effects may be associated with excessive intake)

Abbreviation: DRI, dietary reference intake.

TABLE 4 Water-Soluble Vitamins

Vitamin	DRI ≥ 50 yr	Major function	Deficiency	Toxicity
Thiamin (B_1)	≥50 yr Males: 1.2 mg Females: 1.1 mg	Coenzyme in the metabolism of carbohydrates and branched-chain amino acids	Beriberi, often found in those with eating disorders	Adverse effects associated with food or supplements have not been reported (potential adverse effects may be associated with excessive intake)
Riboflavin (B_2)	≥50 yr Males: 1.3 mg Females: 1.1 mg	Coenzyme in numerous redox reactions		Adverse effects associated with food or supplements have not been reported (potential for adverse effects with excessive intake)
Niacin (B_3)	≥50 yr Males: 16 mg Females: 14 mg	Coenzyme or cosubstrate in many biological reduction and oxidation reactions—thus, required for energy metabolism	Inflammation of the coner of the lips	Excess supplementation may result in flushing and gastrointestinal distress
Pyridoxin (B_6)	≥50 yr Males: 1.7 mg Females: 1.5 mg	Coenzyme in the metabolism of amino acids, glycogen, and myelin lipids	Peripheral neuropathies, convulsions, impaired immune function	Adverse effects associated with food or supplements have not been reported (potential adverse effects may be associated with excessive intake)
Folate	≥50 yr Males: 400 μg Females: 400 μg	Coenzyme in the metabolism of nucleic acids and amino acids	Megaloblastic anemia	Masks neurological complications in people with vitamin B_{12} deficiency. Adverse effects associated with food or supplements have not been reported (potential adverse effects may be associated with excessive intake)
Vitamin B_{12}	≥50 yr Males: 2.4 μg Females: 2.4 μg	Coenzyme in the metabolism of nucleic acids and amino acids; myelin sheath production	Megaloblastic anemia, neurological abnormalities	Adverse effects associated with food or supplements have not been reported (potential adverse effects may be associated with excessive intake)
Vitamin C	≥50 yr Males: 90 mg Females: 75 mg	Cofactor for reactions requiring reduced copper or iron metalloenzyme and as protective antioxidants; supports formation of collagen	Scurvy	Gastrointestinal disturbances, kidney stones, excess iron absorption

Abbreviation: DRI, dietary reference intake.

Water

How much water should we drink daily? Advice continues to be six to eight glasses a day. The updated adequate intake (AI) for total fluid intake for 50 years of age and above is 3.7 L for males and 2.7 L for females per day. This fluid primarily comes from all beverages including water, roughly 80% of the fluid AI. The other 20% of the AI can come from moisture found in foods such as fruits, vegetables, soups, yogurts, and meats. Currently, there is not an established UL for water because normally functioning kidneys can handle more than 0.7 L (24 fl oz) of fluid per hour. However, water intoxication can occur, resulting in swelling of the brain and heart failure.

There are several ways of checking the hydration status:

- Measuring the concentration of sodium in the urine (more than 20 mEq/L is a sign of water depletion)
- Checking urine coloration looking for an almost colorless urine
- Checking frequency of urination, urinating every two to four hours
- Checking body weight especially after heavy bouts of sweating

A practical recommendation is to drink a full glass of water any time the urine color is yellow. If following this advice means the annoyance of taking frequent trips to the bathroom, it is an annoyance that must be borne; sufficient intake of water is vital in the young and the old. Elderly people need to pay special attention to their water intake, because, with age, there is a diminished sense of thirst and impaired renal concentrating ability (Chapter 18). Dehydration is one of the common causes of fluid and electrolyte imbalances in the elderly.

Addicting Substances

In 2002, 55% of the U.S. adults consumed alcohol (36). Those who choose to drink alcohol beverages should do so sensibly

TABLE 5 Minerals

Mineral	DRI ≥ 50 yr	Major function	Deficiency	Toxicity
Calcium	≥50 yr Males: 1200 mg Females: 1200 mg	Essential role in blood clotting, muscle contraction, nerve transmission, and bone and teeth formation	Osteoporosis; Urinary calcium excretion is increased by high intake of protein, sodium, food rich in phosphorus (meat)	Kidney stones, hypercalcemia, milk alkali syndrome, and renal insufficiency
Iron	≥50 yr Males: 8 mg Females: 8 mg	Component of hemoglobin to transport oxygen to cells and enzymes associated with energy production	Microcytic anemia	Gastrointestinal distress; excess correlated with coronary heart disease and oxidative damage
Selenium	≥50 yr Males: 55 mg Females: 55 mg	Defense against oxidative stress and regulation of thyroid hormone action, and the reduction and oxidation status of vitamin C and other molecules		Hair and nail brittleness and loss
Zinc	≥50 yr Males: 11 mg Females: 8 mg	Component of multiple enzymes and proteins; involved in the regulation of gene expression	Poor healing, compromised immune function, changes in taste	Reduced GI absorption of copper

Abbreviations: DRI, dietary reference intake; GI, gastrointestinal tract.

and in moderation (Box 2). Alcohol in moderation is defined by one drink per day for women and one to two drinks per day for men. A drink is equivalent to 12 oz of beer, 5 oz of wine, or 1.5 oz of 80-proof distilled spirits. Moderation is not meant to be averaged over several days but to be measured every single day. *In middle-aged and older adults, a daily intake of one to two alcoholic beverages per day is associated with the lowest all-cause mortality*. When compared to nondrinkers, adults who consume alcohol in moderation, one to two drinks per day, appear to have a lower risk of coronary heart disease (41). These same health benefits do not appear among the younger population. Consumption of one to six drinks weekly has been associated with a lower risk of dementia among adults 65 years and older when compared with abstention (42). However, encouraging alcohol intake for disease prevention is not recommended.

The beneficial effects of alcohol in red wine have been attributed to a class of molecules, the "sertuins" that target the SIR_2 family of longevity-promoting enzymes (43). Polyphenol such as resveratrol, found in grapes and red wine, and quercetin, found in apples and onions, activate these sertuin molecules during the time of stress in their respective plants to respond to infection, starvation, and dehydration (44). These polyphenols may behave similarly in protecting the brain of rats from the action of toxic substances and, possibly, the human body from stress (45). Benefits and dangers of other addicting substances are summarized in Box 2.

Dietary Supplementation

Despite a long history of reliance on various plant or animal compounds used to supplement the diet (Boxes 1 and 2), definitive proof is still lacking that dietary supplements of multivitamins, minerals, and other substances are appropriate or beneficial for the population, in general, and the elderly, in particular. A scientific review in 2002, evaluating vitamins for chronic disease prevention in adults found that elderly people are at high risk of AI or absorption of several vitamins, therefore placing this age group at high risk for vitamin deficiency and suboptimal vitamin status (29–32). In fact, these same authors followed the previous publication with a clinical application paper stating that all adults, especially the elderly, should take one multivitamin daily given the known or suspected benefits of supplementation on cardiovascular disease, cancer, and osteoporosis.

Recent research in the elderly population found that supplementation with multivitamins and minerals over six months and one year did not show significant infection reduction (31) or improvement in cognitive performance (33). Although scientific proof of supplement benefits may be lacking, the elderly are still "supplementing." In fact, the National Health and Nutrition Examination Survey in 1999 to 2000 found supplement use was higher in adults 60 years and older than in the 20- to 39-year-olds (34).

Mean number of supplements used increases with age, that is, older people take more supplements than younger people. Among individuals 60 years and above, 37% take one supplement, 22% take two supplements, 15% take three supplements, and 26% take four supplements and above per day (35). The Slone Survey found the highest prevalence of medication use over a one-week period in women aged 65 years and above. Nearly 60% have used some type of vitamin/mineral supplement and 14% use either a herbal supplement or some other type of dietary supplement. The survey found vitamins and minerals were frequently used for nonspecific reasons such as "health" by 35% of those supplementing. Herbals and other dietary supplements were also most commonly used for "health" by 16% of those supplementing (34).

Overall, the elderly are well advised in their use of supplements, especially, those individuals on a limited caloric diet in which key nutrients might be absent or deficient. The administration of a few

BOX 2 *Addicting Substances*

Alcohol

Alcohol has been with us since prehistory. Beer was the first alcoholic beverage ever reported in written documents. Since the year 3000 BC, the Nile River was literally lined with beer houses. Although alcohol in moderate amounts may indeed be beneficial on an individual basis, those benefits would seem to be outweighed by the statistics that alcohol contributes annually, in the United States alone, to more than 100,000 deaths (37). To be added are the many other social, economic, and health-related costs associated with alcohol use and abuse.

Given the socioeconomic conditions of many elderly individuals, it is understandable that they may be tempted to fight their loneliness and depression with a "drink or two." The impairment of judgment that accompanies alcohol use is reason enough to discourage the elderly from developing such a habit. On the other hand, if we look at the benefits of moderate drinking, we find evidence that alcohol may offer some protection against diseases of the cardiovascular system. Even patients dying of alcoholic liver cirrhosis may present, on postmortem examinations, surprisingly elastic aortas. High-density lipoprotein (HDL) (the "good cholesterol") is elevated in alcoholics and this high value may give patients a false sense of security and lead them to indiscriminate drinking. The debate continues, but recent studies conclude that a modest intake of alcoholic beverages—translated into "no more than one drink a day" and preferably wine—may be of some benefit (38). Still, the inappropriate interaction between alcohol and medications must always be kept in mind.

Coffee

Coffee and its component, caffeine, an alkaloid with a stimulatory action on the brain, make an extremely pleasant beverage but a remarkably mediocre foodstuff. In a cup of American coffee, 0.04 to 0.05 g caffeine is present; in the very small cup of Italian espresso, there is about 0.02 g of caffeine. Although coffee and caffeine may at times be toxic for children, they are usually safe for adults and elderly individuals. As most are aware, coffee is not recommended in individuals affected by insomnia, but it also should definitely not be drunk by elderly people with a tendency to disturbances of cardiac rhythm (tachycardia). In those elderly affected by orthostatic hypotension, i.e., a tendency to experience a drop in blood pressure upon moving from supine to erect position, one or, better, two cups of brewed coffee may be an effective aid that they can safely use (39).

Cocoa, like caffeine, provides a characteristic excitation, which is due to the presence of theobromine, a substance similar to, but less excitatory and weaker than caffeine. Chocolate or cocoa may induce cardiac arrhythmias, and its chronic use may cause loss of appetite.

Tea

A Chinese text, the Pent-S-Ag, dated 2700 BC, provides a description of tea as a tonic beverage. Its active ingredients are *teine*, similar to caffeine and teophylline, the latter in minimal amounts, fortunately; teophylline has powerful pharmacological effects on both the cardiovascular and the respiratory systems. The presence of tannin, a derivative of tannic acid, in tea is responsible for the astringent effect of the beverage. Coffee stimulates gastrointestinal motility, unlike tea, which, if it acts at all at this level, would have an inhibitory effect.

In summary, certainly caffeine and teine are both "drugs" capable of inducing dependency. Indeed, caffeine is currently the most widespread drug used the world over. However, genetic, social, and neurological factors render caffeine dependency far less onerous than that associated with its more feared cousins: alcohol, nicotine, cocaine, and opium derivatives (40).

specific vitamins and minerals, such as calcium and vitamin D, has been advocated in the elderly due to their possible effectiveness in reducing the risk of bone fractures and supporting bone density. The Women's Health Initiative evaluated calcium supplementation of 1000 mg/day with 400 IU of vitamin D per day for seven years in 36,282 postmenopausal women and found a small but significant improvement in hip bone density but no significant reduction in hip fracture (46,47). However, the risk of kidney stones was increased. High dietary calcium intake, hormone-therapy use, elevated BMI, and the few women in the study over the age of 70 seem to question these results.

Popular calcium salts include calcium citrate and calcium carbonate. Calcium citrate appears to be more bioavailable than calcium carbonate and can be taken any time. Calcium carbonate is optimally absorbed when consumed with food. Usually, absorption of calcium is greatest in doses of 500 mg or less and when taken with foods. Calcium intake can also become saturable at 1200 mg/day where much more than that does not confer added benefits. When evaluating the need for calcium supplementation, it is important to understand the medical history of the subject taking the calcium and the current calcium intake. Undoubtedly, vitamin D needs can be easily met with 30 minutes of sun exposure to forearms and face. It is also important to combine both of these with adequate ambulation to reduce fracture risk and improve bone density.

Recent recommendations for specific supplements such as *folate and* B_{12} in individuals with high serum homocysteine levels have become common due to the association of high serum homocysteine levels with heart disease (Chapters 15 and 20). In fact, there could be a cost-effectiveness in supplementing with 1 mg of folate and 0.5 mg of B_{12} as a regimen for

homocysteine-lowering therapy and reduction of coronary heart disease risk (48). There is some evidence that folate and Vitamin B_{12} supplementation may also reduce the risk of dementia although the effectiveness of this combined treatment is still up for debate (49). Vitamin B_{12} is often recommended, especially in individuals with low gastric hydrochloric acid content. It should also be noted that antitumor drugs block the effect of B_{12} and therefore may necessitate B_{12} supplementation.

Assuming a healthy and varied diet, most needed supplements are likely to be available in a single daily pill, which should not contain more than 100% of the recommended daily allowance of the respective vitamins and minerals. In general, for the elderly, the number of pills should be kept to the necessary minimum to avoid the confusion created by a multiplicity of pills on multiple schedules.

As difficult as it may be for the elderly, so many of whom live alone, it is highly recommended to eat with others for its obvious social benefit as well as for the fact that seeing others eat stimulates the appetite and may facilitate digestion. "Companatico e compagnia," meaning bread and whatever goes with it, including company, is an old Italian saying that remains valid everywhere.

■ NEUROENDOCRINE CONTROL OF FOOD INTAKE

Food intake is regulated not only by the availability of food but also by the factors that regulate appetite and satiety. Although the mechanisms of feeding behavior are not completely known, it is accepted that they involve nervous and endocrine controls. It is important to understand which of these factors contribute to food intake and satiety, especially in association with aging, to better understand their contribution to food intake in the elderly. A decline in food intake may predispose the elderly population to undernutrition.

■ Anorexia of Aging

According to several studies, people usually tend to eat less as they get older. In fact, a cross-sectional study conducted in the United States (10) found an average decline in energy intake between the ages of 20 and 80 years of 132 kcal/day in men and 629 kcal/day in women. On average, energy intake decreases by approximately 30% between 20 and 80 years of age. A seven-year longitudinal study of 156 subjects aged 64 to 91 years has reported a decrease of 25.1 kcal/day/yr in men and 19.3 kcal/day/yr in women. *Several other reports indicate that the elderly consume smaller meals and fewer snacks between meals, eat slower, and are less hungry than younger adults.* Thus, loss of appetite in the elderly has been referred to with the term "the anorexia of aging." Reduction in energy intake occurs in the healthy, older individuals as well as in those with medical issues associated with anorexia (loss of appetite).

A large part of the change in calorie intake is due to the reduction in calorie expenditure. However, the reduction in calorie intake is often greater than the reduction in calorie expenditure, thereby resulting in weight loss. The amount of weight loss is variable and it appears that lean individuals are more prone to lose weight. This loss of lean tissue after the age of 50, up to 3 kg/yr, is known as "sarcopenia" (Chapter 24). Recent evidence suggests that one of the major issues with muscle loss associated with aging is the tissue inability to respond to available nutrients (50). Sarcopenia can predispose the old person to muscle and bone weakness, resulting in falls, illnesses, poor recovery, and overall protein energy malnutrition.

There are many, potentially single or combined non-endocrine (Table 6) and endocrine (Table 7) causes for the gradual weight loss that characterizes the anorexia of aging (51,52).

■ Regulation of Appetite and Satiety

The major CNS regulatory appetite and satiety centers are in the hypothalamus, and the major hormones involved are those from the gastrointestinal tract, specialized adipose cells, and the pancreas. In the hypothalamus, the actual drive for food seems to be under the control of a powerful neuro-opioid peptide, *dynein* [also known as neuropeptide Y (NPY)] that stimulates appetite and decreases with age in some individuals. The sense of satiety is stimulated by the polypeptide hormone *cholecystokinin* secreted by the cells of the upper small intestinal mucosa. The neurotransmitter *nitric oxide* is secreted by the gastric mucosa cells and induces relaxation of the stomach fundus; in many elderly persons, its secretion may be deficient, and the resulting absence of stomach relaxation may induce a false sense of satiety and fullness even after minimal food intake.

Leptin, produced and secreted by fat cells, inhibits food intake and stimulates energy metabolism. High levels of leptin tend to decrease body fat. After age 70, leptin decreases in elderly women; in men, leptin tends to increase slightly in parallel with the decrease in testosterone levels.

The gut hormone fragment peptide *YY3-36 (PYY)* reduces appetite and food intake when infused in subjects of normal weight. In combination with the adipocyte hormone leptin, PYY reduces food intake by modulating appetite circuits in the hypothalamus. In obesity, the marked resistance to the action of leptin markedly limits the therapeutic effectiveness. However, a concomitant low level of PYY suggests that PYY deficiency may contribute as well to the pathogenesis of obesity. The infusion of PYY decreases fasting concentration of the appetite-stimulating peptide, *ghrelin*. Ghrelin, secreted by the HCl cells in the stomach, stimulates the release of growth hormone (GH) from the pituitary. A comprehensive review of ghrelin and food intake suggests that ghrelin stimulates NPY and the agouti-related protein in the hypothalamus; this stimulation results in increased food intake (53). Recently the putative roles of ghrelin in energy homeostasis (through regulation of leptin and insulin)

TABLE 6 Nonendocrine Causes of Anorexia of Aging

Social factors
Social isolation, minimal support
Lack of ethnic food preferences
Immobility hindering shopping, preparation, cooking and ability to feed self
Psychological factors
Dementia/Alzheimer's disease
Depression
Restricting diet, i.e., low-fat, low-cholesterol diet
Alcoholism
Medical factors
Poor sense of smell and taste
Poor dentition
Dysphagia, rheumatoid arthritis
Parkinson's disease
Malabsorption syndromes
Infections
Cancer
Gastrointestinal disorders
Chronic obstructive pulmonary disease
Heart failure
Medications

TABLE 7 Endocrine Factors Influencing Anorexia of Aging

Factors that inhibit appetite	Factors that stimulate appetite
CCK	Opioids
GLP-1	Dynein
Peptide YY	Galanin
Insulin	Orexins
Leptins	Ghrelin
Cytokines (TNF, IL-2, IL-3)	Testosterone
Activins	Resistin

Abbreviations: CCK, Cholecystokinin; GLP-1, glucagon-like peptide-1; IL, interleukin; TNF, tumor necrosis factor.

and in particular, premeal hunger and meal initiation have been identified. Ghrelin increases food intake through the stimulation of ghrelin receptors on hypothalamic cells. Despite the ghrelin signals for hunger and PYY signals for satiety, the eventual therapeutic effectiveness of these hormones is still in question.

The *cytokines*, produced by macrophages and lymphocytes, have important regulatory immune function (Chapter 14) and also tend to decrease food intake. In elderly individuals and in situations of stress, cytokines [especially tumor necrosis factor (TNF), interleukin (IL)-2, IL-3] (Chapter 14) are elevated. The peptide *activins* secreted by the testes and ovaries have receptors in many tissues, including the brain (specifically the midbrain) where they may act as neurotransmitters and modulators of appetite and satiety signals; in mice, they decrease food intake and produce a so-called "wasting syndrome." Activins increase in older men but not in women. A summary of signals affecting food intake is presented in Table 7.

Eating disorders are classically considered illnesses of adolescence and young adulthood. However, a small percentage of cases have a late onset, occurring between the ages of 40 and 75 years (average age at onset 56 years and clinical presentation at 60 years) (52). These cases comprise almost exclusively women of diverse ethnicity. They present all the characteristic symptoms of anorexia nervosa and bulimia nervosa: self-induction of starvation or binge eating, morbid fear of being fat, and the denial of the seriousness of their low body weight. As is common in eating disorders of younger individuals, many of the elderly cases have psychiatric components, especially affective disorders and perfectionism. As these elderly women are postmenopausal, alteration of the hypothalamo–pituitary–ovarian axis is not detectable: increased levels of follicle-stimulating hormone and luteinizing hormone characteristic of the postmenopausal profile are present and similar to those of unaffected women (Chapter 10). The treatment of these late-onset cases is similar to that of younger subjects, but increased morbidity and the presence of previous psychological disorders may complicate treatment (52).

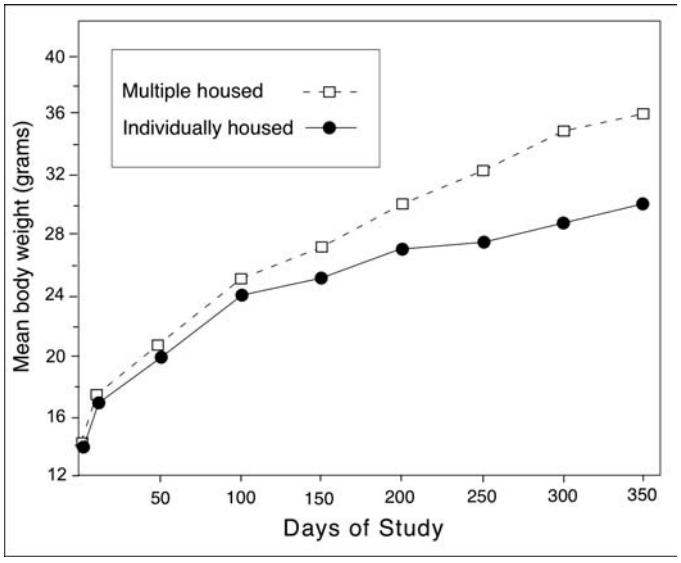

FIGURE 3 Comparison of body growth (body weight in grams) in individually housed and multiple-housed B6D2F1, female mice from weaning until one year of age. Multiple-housed mice showed a significantly heavier body weight than those housed individually, starting on day 150 and continuing until the end of the experiment.

■ EXPERIMENTAL STRATEGIES IN DIETARY RESTRICTION

The physiologic and pathologic changes associated with old age have been variously ascribed to (*i*) extrinsic or intrinsic factors that damage intra- or extracellular molecules, (*ii*) programmed changes in gene expression, or (*iii*) an interaction of the two (Chapters 1–5). Regardless of which hypothesis is the correct one, biogerontology research over the past 70 years has used calorie restriction as a key tool for testing causal theories about aging in animals (54).

As already mentioned in this chapter and in Chapters 3 to 5, *calorie restriction not only prolongs functional competence and postpones aging-related pathology, but it also reliably extends the mean and maximum life span in several species, including mammals*. Yet, despite years of intense research, the mechanism(s) of action underlying these effects remain elusive and the applicability of CR to humans remains improbable. A summary of the methods that have been employed in these studies and the results obtained to date are presented here to serve as a model for the effects of environmental manipulation on aging processes and its eventual implications for humans.

At least 10 different plausible theories have been formulated, over the past 70 years, to explain how CR works (54). Most of these theories have been abandoned due to uncertain or contradictory data. A comprehensive review of calorie restriction suggests possible mechanisms with emphasis on mitochondrial bioenergetics (55–57). CR would slow down metabolic rate, reduce production of free radicals and cell loss, particularly of those cells that cannot be easily replaced (e.g., stem cells and neurons); however, several other studies report that CR animals have equal or higher metabolic rates as ad libitum–fed (i.e., unlimited food available) animals. Other hypotheses emphasize the role of hormonal signaling, such as the "glucocorticoid cascade hypothesis" (58–60) (Chapter 9). According to this theory, the glucocorticoid hormones, through their key role in responses to stress, would selectively induce a number of pathologic lesions in the cardiovascular (e.g., atherosclerosis, hypertension) and nervous systems (e.g., neural cell death in the hippocampus, the hypothalamus, and the basal ganglia) (Chapter 9). Over the past few years, support has been steered toward the "Hormesis Hypothesis of Calorie Restriction" (54,61,62). As indicated in Chapter 9, moderate stress may induce an active, functional response to stress and stimulate a number of compensatory responses that would attenuate the metabolic alterations and strengthen the own defenses of the organism against the causes of aging. A permutation of this hormesis hypothesis shows how alterations in hormone signaling or the action of phytochemicals (e.g., polyphenols) may modulate stress responses in yeast, worms, and flies (Chapters 3 and 5).

The Calorie Restriction Model

With the exception of selective breeding, used especially in less complex animal species (Chapter 3), dietary restriction has been, so far, the only experimental approach for extending the life span that has been successfully reproduced in a wide range of invertebrate and vertebrate species and the only one in mammalian species (63). The majority of studies relying on dietary restriction have been conducted in rodents; indeed, it is in diet-restricted rats that life span extension was first reported in l935 by McCay and collaborators (64). A number of strategies have since been used to obtain robust and reproducible results (3,65,66). As summarized in Figure 4, these strategies take into account the following criteria:

Animal Species Selection

Species and strains most used include the rat (primarily Fisher, Brown Norway and a F1 hybrid of the two), the mouse (primarily, C57BL and DBA, or B_6), the hamster (Syrian), and some primate species (rhesus monkey, squirrel monkey, and others) although for the latter species, data are still incomplete (65–67). Also available are animals selectively bred as models of human pathology or short-lived strains carrying a harmful mutation (Chapter 4); a number of the resulting symptoms are ameliorated by calorie restriction (68,69).

Health Status of Animals

Animals may be kept under isolated, germ-free hysterectomy-derived, barrier-maintained environment or may be kept under conventional conditions without any of these protections. Under all conditions, if the animals remain in good health, calorie restriction will extend mean and maximum survival.

Age of Animals at CR onset

In early experiments using rodents, calorie restriction was initiated at weaning and continued for one year, at the end of which the animals were returned to full feeding. It is now accepted that the greatest effect (30–70% life extension) of calorie restriction regimens on subsequent survival occurs in animals underfed throughout most of their postweaning life. Successful calorie restriction, when started at adult ages, depends on gradual adaptation to the reduced rations; the expectation is of a lesser (10–20%) shortening of life span extension than that obtained (30–70%) when the restricted regimen is started at weaning (3).

Type of Diet

Restricted feeding regimens capable of successfully extending the life span need to provide all essential nutrients and vitamins while restricting calorie intake by 30% to 70% of the ad libitum intake. A detailed list of dietary components that assure success in a calorie-restricted (CR) diet is presented in the excellent review by Merry (70). Diets may be "nonpurified" (that is, composed mainly of unrefined plant and animal materials with added minerals and vitamins) or "purified" (i.e., manufactured from refined components). In general, commercially available diets are designed to maximize growth and fertility in young rodents rather than to promote longevity; further research is required to determine the optimal composition of longevity-promoting diets.

Another type of diet is one in which an essential amino acid has been significantly reduced such that the diet becomes extremely unpalatable and the rats spontaneously reduce their food intake. Rats on a tryptophan-deficient diet, for example, grow significantly less than controls (animals fed the same diet with normal tryptophan levels). When compared to calorie-restricted rats, they present a similar degree of life span extension, with prolonged functional competency in old age and delayed appearance of pathology (71). This approach obviates the need to measure the amount of food allotted daily to each animal. Changing the temporal pattern of food intake from "nibbling" for the ad libitum fed to "meal eating" for the calorie restricted does not appear to alter the beneficial effects of calorie restriction (72).

Animal Housing

Individual versus group housing may influence body weight of rats because group housing may impose an additional stress on the animals and, thereby affect their growth. On the other hand, some studies report lower body weights in singly housed animals as compared to those housed in groups where, presumably, the rats can huddle to conserve body heat.

Effect on Longevity

That calorie restriction significantly extends survival of rats and mice is shown by the example illustrated in Figure 5 (73). As already discussed, the extent of life prolongation depends on the species of animal, its strain and sex, the age at which the regimen is instituted, the animal health status, the type, severity, and duration of restriction, and the housing conditions Figure 4. Weindruch and Walford (3) and Merry (70) provide a useful comparison of these parameters in selected strains of rats and mice.

Enhancement of Functional Capacity and Delay of Disease

In addition to extending the mean and maximum life span, calorie restriction in rodents affects a number of functional changes and modifies the course of diseases associated with old age. Those most frequently reported are presented below.

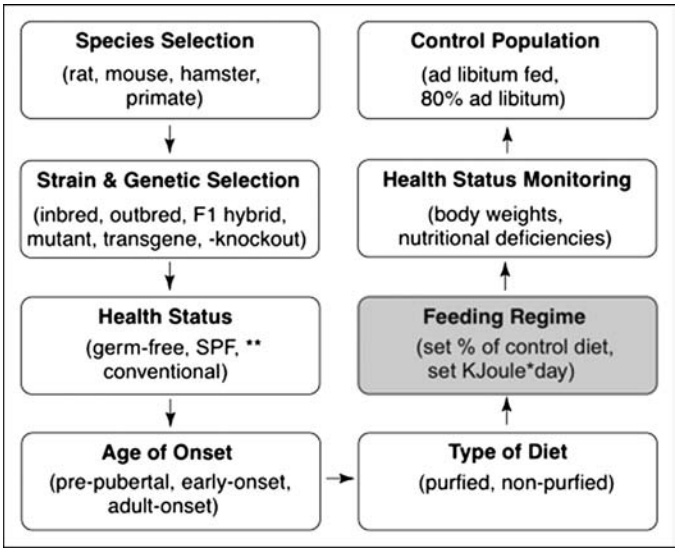

FIGURE 4 Schematic flowchart of the steps and selections needed to produce dietary extended survival. *, Adapted from Ref. 70; **, hysterectomy derived and barrier maintained with routine microbiological screening; ***, Joule, a unit of work or energy equal to 0.239 calories. *Abbreviation*: SPF, specific pathogen-free animals (specially bred for the production of antibodies).

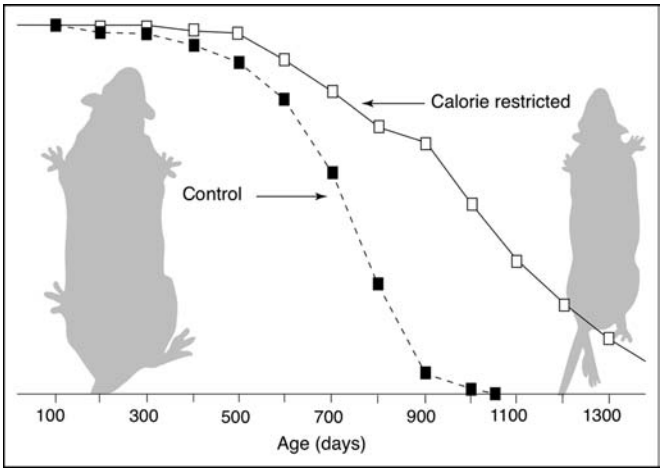

FIGURE 5 The effect of calorie-restricted feeding on lifetime survival for *CFY* male rats. Control rats were fed ad libitum, whereas food intake for experimental animals was maintained at approximately 50% that of age-matched control animals from weaning. *Source*: From Ref. 73.

Reproduction

Original studies in this research area linked arrested reproduction with enhanced survival; however, the dietary restriction was so severe and the duration so long (up to 2.5 years) that rats lived longer but remained in a prepubertal condition (75,76). However, refeeding the animals resulted in rapid sexual maturation and fertility as indicated by the production and survival of litters (77). When calorie restriction is moderate, puberty is slightly delayed in rats and markedly delayed in mice but there is no loss of fertility (75–77).

Endocrine and Neuroendocrine Responses

The delay in maturation of reproductive function in rats and mice kept on a calorie-restricted diet has been attributed to retarded maturation of the hypothalamo–pituitary–gonadal axis and its regulation by neural and hormonal feedbacks rather than to intrinsic alterations of the ovary or testis.

Other neuroendocrine axes appear to be similarly modified by calorie restriction. A defect produced at any level of the neuroendocrine pathways or in target tissues (Chapters 9–12) may be expected to produce secondary developmental and aging changes. Aging of the hypothalamus may proceed at a different pace in its various nuclei. Alteration of this differential timetable may disrupt or dysregulate normal hypothalamic function (78). Another view is that, with aging, the sensitivity of the hypothalamus to the peripheral hormones is decreased or lost, with consequent alteration in the secretory rate of several endocrines and ensuing alterations of aging processes (78). That this decrease in hormonal sensitivity may be the case is suggested by recent observations of the supraoptic and paraventricular hypothalamic nuclei of aged mice (79,80). With aging, the number of brain cells in these areas decreases in both control (i.e., fed ad libitum) and calorie-restricted mice. However, the number of insulin growth fator 1-receptor (IGF1-R) immunoreactive cells that carry the receptor (R) for binding to the hormone insulin-like growth factor-1 (IGF-1) is markedly higher in old calorie-restricted animals than in old controls (79,80). The greater proportion of IGF-1R–sensitive cells in the old calorie-restricted mice than in old ad libitum controls may be interpreted as providing a continuing IGF-1 role in these hypothalamic neuronal populations. IGF-1 regulates the integration of multiple neuroendocrine axes and its persistence in the hypothalamus of old mice may represent a continuation of its neuroprotecting influence (81).

The hypothalamo–pituitary–adrenocortical (HPA) represents another example of changes with aging in hormonal sensitivity. The HPA axis, for example, follows a triphasic pattern: on initiation of calorie restriction, it is stimulated (underfeeding may be considered a form of stress), but continued underfeeding desynchronizes the circadian corticoid rhythm and depresses responses to stress. When CR is further prolonged (and the animal has adapted to the stress of being underfed) and when normal feeding has been restored, the HPA ability to respond to stress is gradually recovered. In other words, the inability of calorie-restricted animals to increase glucocorticoids in response to the stress of underfeeding may originate at the hypothalamo–pituitary level rather than at the adrenal cortex (Chapter 9).

With aging, the ability of the anterior pituitary to secrete GH in response to several stimuli (e.g., hypoglycemia, arginine administration) usually capable of triggering such response in young animals is significantly diminished in rats and humans (Chapter 9). This decrease has been ascribed to (*i*) a reduced secretion from the hypothalamus of GH-releasing hormone (GHRH) or perhaps due to increased secretion of the inhibitory hormone, somatostatin, or (*ii*) the diminished responsiveness of the pituitary to the stimulatory GHRH (Chapter 9). The expected decline in GH levels with old age is associated with an aging-related loss of nocturnal surges of the hormone and a small decrease in plasma IGF-1. Replacement therapy of these hormones (GH and IGF-1) has shown some beneficial effects, suggesting that their absence may contribute to the aging phenotype. However, such potentially beneficial effects must be balanced against some real, untoward effects (Chapter 9).

Immunological Responses

A common response of mice, rats, guinea pigs, and monkeys to moderate but chronic calorie restriction is a depression of antibody production and natural killer-cell activity and an enhancement of cell-mediated immunity (Chapter 14) (82). Thymus development is delayed in CR animals, but, despite a reduction of cell numbers in immune organs and white cells in blood, resistance to pathology is enhanced and survival is extended. These responses, markedly influenced by genetic factors, may be mediated through changes in fatty acid composition of phospholipid fractions of cell membranes (83). Although the incidence of autoimmune diseases increases with normal aging (Chapter 14), it is significantly decreased in the presence of calorie restriction, especially in autoimmune-prone mouse strains. The mechanism(s) of this protective action remain(s) to be elucidated.

Metabolic Responses

Early studies in rodents had suggested that CR reduced metabolic rate. Current research, including work with monkeys (84,85), indicates that this is not the case; the animals on CR diets adapt by reducing fat-tissue deposition and increasing the efficiency of energy utilization, thereby preventing obesity and maintaining a metabolic rate similar to that of fully fed animals (86–88).

More controversial are the effects of dietary restriction on core body temperature: superimposed on the decrease in core temperature associated with aging in both control and restricted

animals are temperature increases that may be related to the timing and periodicity of feeding. Other effects of dietary restriction that have been extensively reported are reductions of mitochondrial damage, free radical accumulation, and lipid peroxidation (Chapter 5). This reduction would be mediated, at least in part, by the decrease in energy expenditure brought about by the increased activity of those enzyme systems responsible for detoxification of reactive oxygen species (ROS) (89).

In rodents, a consistent but still little understood metabolic response to dietary restriction is improved insulin sensitivity and permanently lowered glycemia. Improved insulin sensitivity may be due to tissue-specific effects on cell insulin receptor binding (perhaps related to effects on membrane lipid composition) that do not alter glucose transporter protein content (74,90–92). A similar action has been reported in monkeys; insulin sensitivity is increased in parallel with a decrease in fat deposits.

Because experiments in monkeys are still in progress, physiopathologic and metabolic changes with aging and their possible modification by calorie restriction cannot yet be ascertained (67). Focus on two metabolic outcomes of dietary restriction shows a reduction in oxidative stress and improved glucose regulation (91).

These actions can be imitated or "mimicked" by interventions other than calorie restriction, that is, actions capable of retarding the onset of aging-associated diseases, slowing down the rate of functional aging, and promoting longevity, without reducing food consumption compared to controls (91–93). So far, research on calorie restriction "mimetics" has dealt with improvement of mitochondrial function, use of antioxidants, administration of compounds known to lower blood glucose levels and increase insulin sensitivity, regular physical exercise, maintenance of body weight and composition over the life span, and others (74,91,92). The antidisease markers, currently observed, suggest that animals subjected to calorie restriction mimetics may escape the development of diabetes, cardiovascular disease, obesity, immune dysfunction, and possibly cancer more successfully than their counterparts fed ad libitum.

As discussed in Chapter 1, one major challenge in aging studies is the choice of biomarkers reflecting the rate of aging and concomitant functional capacity. This choice is especially critical when working with long-lived species such as monkeys and, eventually, humans. Interspecies comparisons as well as the use of several markers that can be measured routinely (and are minimally invasive) in biochemical and hematologic profiles show considerable promise not only for improving our understanding of the effects of CR in monkeys but also for their possible applicability to humans (94,95). So far, in monkeys, several blood chemistry measures (e.g., total protein, several enzymes, creatinine, dehydroepiandrosterone, and T-cell subsets) show a trend toward slower age-related change in CR animals compared to controls (70,95,96).

Collagen Responses

The rate of cross-linkage between the fibers of the structural protein collagen, a component of connective tissue, is usually taken as a biomarker for the rate of aging. In old animals, diminished gene expression for collagens and proteinases may slow collagen turnover and increase the cross-linking of collagen molecules (Chapter 21). Dietary restriction reduces the amount of total collagen, the amount and type of cross-links, and the accumulation of advanced glycosylation products (i.e., covalent attachments of a carbohydrate molecule to a polypeptide or a polynucleotide) in several tissues (e.g., kidneys, lung, liver). Although this reduction may have a beneficial effect on cataract development (Chapter 8), it may have adverse effects on wound healing despite a trend toward faster wound closure.

Tumor Incidence

With respect to tumors, the effects of calorie restriction vary depending on the type of tissue/organ and the animal species/strain. In both rats and mice, the frequency of tumors is

- reduced in pituitary gland, lung, pancreatic islet cells, liver, mammary gland, and skin,
- increased in epithelial tissue, adrenal and parathyroid glands, and reticulum cells of lymphoid glands, and
- unaffected in soft tissues, thyroid gland, and bladder.

In addition to its effects on spontaneous tumors, calorie restriction also confers a certain degree of protection against tumors (primarily skin and intestine) that are induced by highly mutagenic exogenous carcinogens. This protective action has been attributed to the animal's reduced body fat, which, in turn, limits the storage space for carcinogenic substances and increases their metabolism.

■ Effect on Gene Expression Profile

Reduction of oxidative stress within mitochondria remains a major focus of research associated to the mechanisms of calorie restriction. CR is hypothesized to decrease mitochondrial electron flow and proton leaks to attenuate damage caused by ROS. A recent in vivo and in vitro study with Hela (human cervical carcinoma cell lines) cells found that CR reduces oxidative stress as it stimulates the proliferation of mitochondria through the activation of enzyme-signaling pathways in the peroxisomes (intracellular organelles that contain enzymes involved in hydrogen peroxide metabolism); less oxygen consumption by the mitochondria is followed by a reduced membrane potential, which in turn generates less ROS (55–57). These CR cells were actually still able to maintain their critical ATP production even under the circumstances. Therefore, CR was capable of supporting efficient and balanced energetics within the mitochondria to promote the reduction of ROS and attenuate age-dependent endogenous oxidative damage.

Studies in skeletal muscle (which is composed of long-lived, high-oxygen-consuming postmitotic cells) of C57BL/6 mice have revealed that aging results in a differential gene expression pattern indicative of a marked stress response (characterized by cell energetic deficit, mitochondrial dysfunction, and damaged proteins) and lower expression of metabolic and biosynthetic genes (97). Of the 6347 genes surveyed, 58 (0.9%) displayed a greater than twofold increase in expression levels and 55 (0.9%), a greater than twofold decrease in gene expression; thus, the aging process does not appear to be due to a large, widespread alteration in gene expression. Of the 58 genes that increased in expression with age, 16% were mediators of stress responses, in particular, genes encoding enzymes involved in free radical inactivation, neuronal growth, and neurite extension. Of the genes showing decreased expression with age, the majority were involved in energy metabolism (especially mitochondrial enzymes), carbohydrate metabolism, and biosynthetic enzymes (for protein and lipid synthesis and turnover (97).

After chronic (28 months) reduction of calorie intake (76%), aging-related changes that occur in mice fed ad libitum were markedly (84%) attenuated: of those characterized by increased gene expression, 29% were completely prevented and 34% greatly diminished. CR may act through metabolic reprogramming with a transcriptional shift (in flies, perhaps triggered by

insulin) (97) toward reduced energy metabolism and increased biosynthesis and protein turnover (98). This modification of the metabolic profile of old mice may vary depending not only on calorie restriction but also on strain, sex, and age; as mentioned above, calorie restriction markedly influences the expression of pathologic genotypes in rodent species that have been selectively bred as models of human pathology (99,100).

■ Human Studies

Of the many CR studies conducted in humans, some use historical anecdotes or draw on social or religious customs for data, whereas others rely on more rigorously conducted experiments, which, however, have been hampered by the small population sizes and the short study periods. Hence, it has been difficult to obtain a definitive conclusion on the impact of calorie restriction. However, a few recent studies have helped identify a few ways in which CR supports longevity.

In the first category is a three-year study of Spanish nursing home residents (average age, 72 years) in whom morbidity was reduced by limiting food intake, although mortality rates remained unaffected (101). Another study was conducted among Islamic individuals who, during the month of Ramadan, fasted during the daylight and then consumed a meal after sundown; their HDL plasma levels were found to have increased by 30% (102). In a study of Japanese living in Okinawa, it was reported that Okinawans have a longer life span (81 years) and six times as many centenarians per 100,000 people than those living in the United States, and 97% of their lives were spent free from disabilities (103). Their longevity has been correlated with a diet relatively low in calories and high in fruits and vegetables. However, in addition to differences in the amount and composition of the diet, other factors (e.g., strong social, cultural, and religious ties, and insular isolation) distinguished the Okinawan group.

One of the early controlled studies was conducted by Keys in a small number of subjects placed on a diet that was not only restricted in quantity but also deficient in critical micronutrients (104). Two other examples are the Biosphere 2 (in Arizona) (105) and the Toxicology and Nutrition Institute study (in the Netherlands) (106). In Biosphere 2, although some changes in body weight and in blood chemistry (reduced levels of fasting glucose, cholesterol, triglyceride) were reported, the small number of subjects and the lack of appropriate controls made it impossible to draw definitive conclusions (105). Similarly, for the Toxicology and Nutrition Institute study, a reduction in body mass and an increase in HDL levels were reported, but again, definitive conclusion could not be drawn. A follow-up study to the Biosphere 2 published in 1999 utilized eight health subjects between the ages of 27 and 67 years. These subjects participated in a confined, high work output and severely restricted diet with high food quality to avoid malnutrition for two years. The study found total cholesterol and triglyceride levels decreased by 30% and 45%, respectively. The LDL levels decreased and the HDL levels decreased in some subjects while increasing in others (107). The authors felt that energy restriction may substantially reduce the risk for atherosclerosis. Yet another study initiated in the mid-1990s on a population of self-selected calorie restrictors, who were voluntarily consuming a calorie-restricted diet did not, unfortunately, yield credible data as it became apparent during the study that the self-selecting individuals were not really CR after all (108).

The theme of reduced atherosclerosis risk was again found in another study evaluating 18 calorie-restricted participants between the ages of 35 and 82 years for an average of six years compared to 18 age-matched individuals. The diet provided 100% of the RDI for all the essential nutrients, while minimizing calories to 1112 to 1958 kcal/day. Those on CR were lean and had lower BMIs, TC, LDL, ratio of TC:LDL, triglycerides, fasting glucose, fasting insulin, c-reactive protein (CRP). Platelet-derived growth factor-AB heterodimer (PDGF-AB), and systolic and diastolic blood pressures were markedly lower. In addition, those CR participants had higher HDL levels (Chapter 16). Therefore, it appears that CR protects against atherosclerosis (109).

More recent human studies have found additional health benefits with CR. A long-term CR, 3 to 15 years, averaging 6.5 years, with 25 subjects ranging from 35 to 82 years compared with 25 age- and gender-matched subjects found CR to have cardiac-specific effects that ameliorate aging-associated changes in diastolic function, reduction in CRP, blood pressure, TNF-α and transforming growth factor-β (110). These results suggest CR reduces blood pressure, inflammation, and myocardial fibrosis. CR has also been correlated with a sustained reduction in serum triiodothyronine (T3) concentration after 3 to 15 years of CR in 28 men and women with an average age of 53 years. It is postulated that this sustained T3 reduction is involved in slowing the rate of aging (111). Lastly, a six-month CR study involving overweight individuals found CR by 25% baseline proved to decrease two important markers of longevity, fasting insulin, and body temperature. This study supports the theory that metabolic rates are reduced beyond the levels expected from reduced metabolic body mass, thus suggesting CR attenuates the aging process in humans (112).

Despite the eventually positive side effects of CR including improving longevity and reducing the rate of aging as well as improving markers associated with longevity in humans, there are potential negative side effects that need to be acknowledged. These negative side effects include hypotension, loss of libido, menstrual irregularities, infertility, bone thinning and osteoporosis, cold sensitivity, loss of strength and stamina, slower wound healing, and psychological conditions such as depression, emotional instability, and irritability (113). Precautions should be taken before adopting to practice CR since long-term side effects are unknown.

Notwithstanding the ethical objections, the potential dangers and the methodological difficulties, there are now, three new pilot studies of CR being funded by the NIH National Institute of Aging. This initiative responds to the continuing demographic changes and to the public opinion (114,115) pressuring the legislature and governmental offices for promoting research in human aging and longevity. The planned centers include Tufts University, The Pannington Center of the Louisiana State University and Washington University, St Louis. The studies will last five years; subjects will be restricted by about 25% of normal intakes. As stated by Roth: *"It should be mentioned here that a newly emerging goal in the CR field is to reach stabilization of calorie intake and body weight, rather than follow life-long restriction. Certainly, less-severe CR is more feasible and may offer health benefits that will positively impact an individual's life" (116).*

As with other interventions for health, combining approaches that maximize efficacy while minimizing adverse effects will be most successful. In other words, with mild calorie restriction, regular physical exercise, abstaining from smoking, alcohol in moderation, a diet low in inflammation-inducing molecules and high in anti-inflammatory molecules, in particular antioxidants and polyphenols, is most likely effective in supporting optimal aging and disease reduction. This definitely leaves the door open for the possibility of developing CR mimetics, which may provide similar health benefits and slow the rate of aging without severe calorie restriction.

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Benefits of Physical Exercise

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INTRODUCTION

Research on physical activity and its relation to physiologic aging remains very active today. Exercise facilitates maintenance of sound cardiovascular function and lessens many risk factors associated with heart disease, diabetes, insulin resistance, and some cancers (1). In addition, other losses associated with aging and once thought to be inevitable (e.g., loss of muscle mass and strength, of bone density, and of postural stability) can be effectively counteracted through regular exercise (1). Concurrent psychological benefits of exercise include preservation of cognitive function and self-sufficiency as well as reduced episodes of depression. *A regular program of physical activity, therefore, is extremely beneficial, whether one begins early in life, in adulthood or after 60; indeed, the consequences of inactivity are being increasingly chronicled* (Chapter 3). A powerful endorsement of the role of physical exercise in promoting a healthy life span was provided by the 1996 Surgeon General's Report on Physical Activity and Health (1).

After a brief introduction, the present chapter focuses on the functions responsible for exercise performance [e.g., cardiovascular, respiratory, nervous, muscular, metabolic, and endocrine] and the physiopathologic changes that occur with aging in these functions (see section entitled Physiological Changes with Aging). The benefits of exercise programs will be considered in the section entitled Exercise Programs for the Aged together with a review of the major types of physical exercise recommended for the elderly, along with recommendations for healthy and safe exercise (see section entitled Recommendation for Healthy and Safe Exercise). Despite the overall benefits of exercise, some potential adverse effects may occur, and those will be reviewed in the section entitled Potential Adverse Effects of Exercise in the Elderly.

The benefits of exercise have been known for a long time. For example, in Greece, the physician Hippocrates, in his second book on hygiene (400 BC) wrote "Eating only, will not keep a man well: he must also take exercise." Hippocrates' influence extended to Rome, where Galen, a Roman physician, in his book on hygiene (second century) wrote, "The uses of exercise are twofold: one for the evacuation of the excrements, and the other for the production of good condition of the firm parts of the body" (2). The recognition that physical activity improves functional state and sense of well-being is acknowledged in the classic Chinese books of medicine as early as 3000 BC. The system of meditative movements "Tai chi chuan," well imbedded in the Taoism philosophy, teaches graceful movements widely practiced today (as Tai Chi) in this country and elsewhere, specifically to ward off the incidence of falls and fractures.

PHYSIOLOGICAL CHANGES WITH AGING

Cardiocirculatory and Respiratory Performance in Aging

Some degree of decrease in physical fitness is inevitable with aging. Most of the winners of the Olympic Games and World Championships in the majority of the sport disciplines are young athletes in their third or fourth decade of life. There are, however, some exceptions among sport disciplines such as swimming or gymnastics where athletes' performances usually peak at a younger age and endurance athletes such as marathoners or road cyclists who can extend high levels of performance into their fifth decade.

Not all physical qualities that determine sports performance and fitness follow the same timetable with aging, particularly in terms of magnitude and speed of changes. A simple way to explore the effects of aging on physical fitness and performance is to investigate the changes by studying

- the structure and function of muscle fibers (Box 1),
- the energetic mechanisms that sustain muscle contraction, and
- the neuromuscular function.

Each sport and physical activity may indeed be grouped according to the need for energy and the mechanisms for producing it. Major factors regulating oxygen transport in the circulation are listed in Table 1. For simplicity, consider the types of muscle performance and their reliance on oxygen.

- *Aerobic performance relies on oxygen availability to the muscles*, as needed in running, cycling, swimming, and cross-country skiing, especially on long distances. The limiting factor to performance in this case is the ability of transporting oxygen (O_2) to the working muscles.
- *Anaerobic performance relies on utilization of energy already "stored" into the muscle* as needed in the 100 m dash, resistance training (RT), and lifting heavy weights (for low number of repetitions). The limiting factor in this case is the amount of energy stored and the rapidity with which the stores can be replenished.
- *Neuromuscular performance depends on the ability to perform simple and complex movements with precision and quickness*. The limiting factors here are the functional integrity of the muscle, the nerve fibers innervating the muscle, and the myoneural junction.
- *Efficiency of cardiovascular and respiratory functions.*

BOX 1 *Skeletal Muscle Structure and Function*

The major function of muscle is to contract with utilization of energy and production of work and heat. Skeletal muscle is formed of individual muscle fibers containing fibrils that are divisible in filaments made up of contractile proteins, myosin, actin, tropomyosin, and troponin. Myosin forms the thick muscle filaments, and the three other proteins form the thin filaments. Cross-linkages are formed between myosin and actin molecules. During contraction, by breaking and re-forming of cross-linkages between myosin and actin, the thin filaments slide over the thick filaments, thereby shortening muscle length and utilizing adenosine triphosphate (ATP) for energy. Muscle fibrils are surrounded by the sarcotubular system formed of vesicles and tubules distinguished into a T system and a sarcoplasmic reticulum. The function of the T system is the rapid transmission of the action potential from the cell membrane to all myofibrils. The sarcoplasmic reticulum regulates calcium movement and muscle metabolism. Muscle fibers are distinguished into two major types: the slow-contracting twitch (or shorter, short twitch, ST), Type I fiber (e.g., long muscles of the back) and the fast-contracting twitch (or shorter, fast twitch, FT), Type II fiber (e.g., muscles of the hand). Of the two types, the most affected with aging are the Type II fibers.

Muscles are innervated by somatic nerves (to skeletal, voluntary muscles) or autonomic nerves (to smooth, visceral muscles) or have their own, specialized nervous conduction system (in cardiac muscle). In skeletal muscle, nerve endings rich in the neurotransmitter acetylcholine (Chapter 6) fit into a motor-end-plate, a thickened portion of muscle membrane, to form the neuromuscular or myoneural junction. In smooth and cardiac muscle, there are no recognizable end-plates and the neurotransmitter may be acetylcholine or norepinephrine. Muscle contraction is initiated by calcium release through depolarization at the neuromuscular junction; the action potential is transmitted to all fibrils via the T system and triggers the release of calcium from the sarcoplasmic reticulum. This process is called excitation–contraction coupling.

Movements of skeletal muscle are voluntary; they are regulated by inputs from the cerebral cortex (motor cortex, area 4), the basal ganglia, and the cerebellum, which are relayed to the spinal cord (by the corticospinal and corticobulbar tracts) and from there to the muscles.

Decline in Cardiovascular Performance as Expressed by a Decline in VO_2 Max

VO_2 max measures the maximal oxygen consumption of body tissues; it represents a reliable index of the aerobic potential of all individuals. With advancing age, VO_2 max undergoes a progressive and definite decline even in subjects who maintain an appropriate level of training. This decline is usually slow but inexorable and varies between 10% and 45% between 25 and 75 years of age (Table 2). This decline may be slowed remarkably with appropriate training (Fig. 1). The reasons for this decline with aging are multiple and involve respiratory, metabolic, and cardiovascular factors (Chapters 17 and 20): (*i*) The decrease of respiratory factors (maximal respiratory frequency, maximal ventilatory capacity, and maximal capacity of 02 diffusion); (*ii*) The increase of a metabolic factor (abnormal muscular metabolism; (*iii*) the decrease of cardiovascular factors (capability of maximally utilizing available 02, blood flow to skeletal muscle, maximal cardiac frequency, myocardial response to adrenergic stimuli vs. resistance to ventricular ejection).

The senile heart is capable of partially compensating for the combination of these unfavorable conditions by stretching the cardiac muscle so that the muscle tension increases to a maximum at the end of the diastole. The diastole is the period of cardiac muscle relaxation when the atria and ventricles fill up in preparation for the blood ejection into the systemic circulation during systole. For the heart, the length of the muscle fibers is proportional to the end-diastolic volume; therefore, diastolic maximal stretching of cardiac fibers strengthens ventricular stroke, increases cardiac output, and makes blood more readily available to the general circulation even during significant levels of exercise. Based on the Frank–Starling principle, this compensatory adjustment states, *"the energy of contraction is proportional to the length of the cardiac fiber."* Thus, healthy subjects, irrespective of whether they are 20 or 80 years old, present a similar and robust cardiovascular response to physical exercise, even if the mechanisms by which an efficient cardiac output may be reached vary [i.e., accelerated heart rate (HR) in the young vs. compensatory increase in diastolic cardiac volume in the old].

Heart Performance

Classic physiology always considered heart function as the limiting factor of the aerobic mechanism. Some recent studies, however, are questioning this assumption. Heart performance is measured as minute volume (Q), which is the amount of blood pumped into the vascular system in a minute. Q is the product of heart rate (HR) times the stroke volume (SV), i.e., the amount of blood ejected with each ventricular contraction in the general circulation. Under resting conditions, in a healthy 20- to 25-year-old man, Q is around 5 L/min. At peak exercise, Q is approximately 15 to 18 L/min in a healthy subject and up to approximately 35 to 38 L/min in a top-endurance athlete. With aging, however, Q and its two component factors, HR and SV, tend to decrease.

TABLE 1 Principal Components of the O_2 Delivery System

Central components
Heart (e.g., capacity of the blood to circulate)
Lungs (e.g., adequacy of pulmonary gas exchange)
Blood (e.g., adequacy of blood flow to a tissue)
Peripheral components
Arterial–venous pressure difference (e.g., vasoconstriction or vasodilation of vascular bed)
Muscle fibers (e.g., cardiac output)
Blood (e.g., binding of oxygen to hemoglobin)

TABLE 2 Changes in VO_2 Max Among Normally Active Men

Age (yr)	VO_2 max ($mL*kg^{-1*}min^{-1}$)	% change from 25 yr
25	47.7	–
35	43.1	−9.6
45	39.5	−17.2
52	38.4	−19.5
63	34.5	−27.7
75	25.5	−46.5

Source: Adapted from Wilmore and Costill.

Heart Rate (HR)

HR decreases with aging, primarily because of decreased responsiveness of the heart to levels of circulating catecholamines (epinephrine and norepinephrine, Chapter 9). The classical equation is

Peak heart rate = (220 – age in years).

This implies a maximum HR (HR Max) of about 155 beats/min at the age of 65 years. Incidentally, this formula has been recently modified and replaced by a regression equation 200 – (0.7 × age) (Chapter 20).

Yet, a well-motivated 65-year-old can attain a rate of 170 beats/min or more during an uphill treadmill running test, although muscle weakness may lead to somewhat lower maximal rate during cycle ergometry (3). If it is true that the general trend of HR Max is to decrease with age, it is important to remember that the standard deviation of the previously mentioned old formula of the "220 – age" is 14 beats/min, which expresses significant interindividual variability. As it will be described later, the loss of muscular mass will also add to limit the achievement of HR Max due to an overwhelming sense of fatigue.

Peak HR values are then further reduced if the subject experiences breathlessness (like in chronic pulmonary diseases) or develops myocardial ischemia (i.e., local blood deficiency).

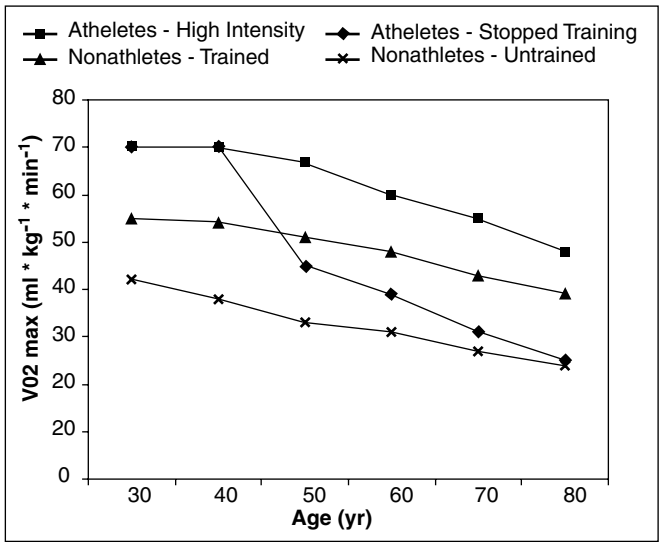

FIGURE 1 Changes in VO_2 max with age for trained and untrained men. Although training will improve VO_2 max in the elderly, it will not prevent the decline in functional capacity. *Source*: Adapted from Wilmore and Costill.

Stroke Volume (SV)

With the exclusion of subjects with myocardial ischemia, the heart of a typical 65-year-old subject compensates for the lower maximal cardiac rate by increasing the end-diastolic volume and thus the cardiac SV. During submaximal exercise, although the SV in an elderly man could be even greater than in a younger adult, the former will have more difficulty in sustaining the SV capacity as the maximal effort is approached (4).

In the elderly, there may be many additional constraints upon peak ventricular function such as the following:

- Venous filling may be impaired by poor peripheral venous tone, varicosities, and slow relaxation of the ventricular walls.
- Reduced sensitivity to catecholamine blunts the isotropic (i.e., general) increase of myocardial contractility during vigorous exercise.
- The after-load opposing resistance to the ventricular activity also rises more in older than in younger individuals, due to increased peripheral resistance secondary to loss of arterial elasticity (e.g., in atherosclerosis, Chapter 15).
- Ventricular contractility may be impaired by the eventual development of silent myocardial ischemia.

Lung Performance

Although the normal lung is capable of meeting ventilatory (i.e., the continually supplying of O_2 through the lungs) requirements even during maximal efforts, this reserve capacity begins to deteriorate gradually between 30 and 60 years of age and this decline accelerates after 60 years. The decline is faster if the individual is a smoker or is chronically exposed to significant amounts of airborne contaminants (Chapter 17). The three most important changes responsible for ventilatory decline are

- gradual increase in the size of the alveoli,
- disintegration of the elastic support structure of the lungs, and
- weakening of the respiratory muscles.

These changes can interfere with the ventilation–perfusion of the lung and, hence, impair the oxygen transport capacity.

The loss of pulmonary elasticity and the weakening of the respiratory muscles (Chapter 17) can exert a marked effect during exercise as both changes make expiration more difficult and increase the work of breathing, that is, the oxygen cost. Because of these airflow restrictions, there is an increased dependence on breathing frequently for ventilation during increased intensities of efforts. Despite some deterioration, changes in pulmonary ventilation, will remain adequate in the elderly even during exercise, unless the decline in pulmonary function is accentuated by disease.

In the old as in the young, endurance performance is indeed not very much limited by ventilation; rather the most important limiting factor is most likely the cardiac output. Incidentally, training increases maximum ventilation with a parallel improvement of cardiac output.

Peak ventilation during exercise as well as gas exchanges between alveoli and the circulating blood are extremely important factors in aerobic performance. Mechanical constraints on the pulmonary system progress with age to cause deterioration in both static and dynamic lung function measures. Thus, the pulmonary ventilation and gas exchange kinetics during the transition from rest to submaximal exercise slow substantially (Chapter 17). However, in elderly men, aerobic training increases the kinetics of gas exchange to levels that may approach the values of the young fit adults. Indeed, values for a number of parameters of

lung function (Chapter 17) such as vital capacity, total lung capacity, residual lung volume, and maximal voluntary ventilation in athletic individuals above age 60 remain higher than predicted from body size and definitely higher than sedentary individuals of the same age. Of course, even in these cases, individual and environmental factors play a major role in the changes (5).

A good way to assess the overall level of cardiovascular and respiratory fitness is the measure of the so-called VO_2 max or maximal oxygen uptake during an incremental stress test. The maximal oxygen intake tends to decline by about 5 mL/kg/min per each decade from 25 to 65 years of age, with some possible acceleration thereafter (6). It is difficult to assess how much of this progressive decrease is due to the habitual physical activity or actual anatomical–pathological changes. Fact is that the potential causes for this decrease may lie in the age-related loss in aerobic power, these including maximal HR, SV, and arteriovenous oxygen difference. It is interesting to note that in competitive endurance athletes, the decline of the VO_2 max is 4% for each decade, significantly less than that observed in sedentary elderly people. We have recently measured the VO_2 max of 76 mL/kg/min in a 51-year-old former Tour de France cyclist. In this individual, his best VO_2 max measured at age 30 was 82 mL/kg/min, hence a minimal decline.

Blood Changes

O_2 is carried into the blood bound to the hemoglobin (HgB) in a molecular complex of iron and protein within the red blood cells (RBCs) or erythrocytes (Chapter 17). The more HgB, the more O_2 will be delivered to the working muscles. Related hematologic changes derive mostly from changes in the bone marrow, but these are rarely of clinical significance. Truly, with aging, the average values of hemoglobin and hematocrit decrease slightly, but overall, they tend to remain within the normal adult range. The mean corpuscular volume increases slightly but the RBCs morphological characteristics do not change significantly. The RBC osmotic fragility increases while the content of 2,3 diphosphoglycerate decreases (Chapter 17). The erthrocyte sedimentation rate, the levels of fibrinogen and coagulation factors V, VII, and IX, increase significantly. Age does not change the RBC life span or the platelets' morphological characteristics. In fact, platelet count has been usually reported to be normal or slightly increased or decreased (7).

The slight increased prevalence of anemia (reduced level of HgB) noted with aging is more frequently related to chronic diseases or occult bleeding or deficiencies of iron, vitamin B_{12} and folic acid, and more (Chapter 17). This anemia could reduce, ultimately, the ability to carry O_2 to the muscles. Dehydration, also more common in the elderly, could reduce the plasma volume and the proper refilling of the right ventricle (preload) through the reduction of stroke volume (SV) and Q (see above).

The Arteriovenous Difference

The maximal arteriovenous flow difference decreases from around 15 mL/dL in a young adult to 12 to 13 mL/dL in an older individual. These changes reflect the direction of a larger fraction of the total cardiac output of the exerciser to vascular districts such as skin and viscera where oxygen extraction is quite limited (3).

In fact, in the elderly, the circulation to the skin is often poor, hence the frequency of "cold feelings" (Chapter 21). Still, in the elderly during efforts, a disproportionate amount of blood is directed to the skin, further hampering oxygen extraction and facilitating the episodes of "orthostatic intolerance."

Peripheral Circulatory Changes

Peripheral arterial problems increase in frequency with age and the most common cause is atherosclerosis (Chapters 15 and 16). Still, most patients, even if affected by atherosclerosis, have no symptoms. Indeed, almost 70% of a vessel lumen must be occluded before the disease could be clinically recognized. Risk factors for peripheral vascular disease include:

- Cigarette smoking
- Polycythemia
- Diabetes mellitus
- Hyperlipidemia
- Hypertension
- Increased levels of homocysteine (Chapter 15)
- Family history
- And, of course, age

It is appropriate to mention here, that, exactly in these situations, in addition to specific therapy, when available, a supervised progressive kind of exercise remains one of the most effective modality of treatment.

Even the incidence of deep vein thrombosis increases with age and, in this context, immobilization and/or prolonged sitting (as may occur during long drives or air travel or even a more simple relatively sedentary existence) can lead to venous stasis and predisposition to thrombosis. The emptying of the veins of the legs depends almost entirely on skeletal muscles pumping blood into and through the one-way venous valves that inhibit retrograde flow. Any condition that increases the hematocrit and blood viscosity will result in a higher incidence of clotting. In addition, a genetically determined "C-reactive protein" present in about 15% of the population will further increase the hypercoagulable state in the elderly (Chapter 15). These facts may lead to the increased phenomenon of deep vein thrombosis, which occurs after routine surgery in about 20% to 25% of patients older than 40 and in almost 50% after hip surgery when no prophylaxis is provided (8) (Chapter 17).

■ Aging, Muscle Performance, and Sarcopenia

With aging, a significant deterioration occurs in muscles due to loss of mass and strength. The loss of mass does occur for several reasons including:

- Malnutrition
- Inactivity
- Altered cytokines signaling (like in HIV, cancer, and burns)
- Sarcopenia (loss of muscle mass, also referred to as muscular wasting or cachexia)

The most significant reason is the process called sarcopenia (from the Greek "sarkos," meaning flesh). Sarcopenia appears to begin in the fourth decade of life and accelerates after the age of approximately 75 years (9–14). Some of the major causes of sarcopenia are listed in Table 3. In addition to muscle fiber loss, the spinal cord neurons regulating muscular activity are reduced in number and show increased variability in size (suggesting unsuccessful compensatory attempts to restore nervous control).

The consequences of sarcopenia have a severe impact upon the elderly, as progressively many essential tasks such as carrying household objects or lifting the body weight from the

bathtub, toilet, or bed may all become problems. Poor muscular support can lead to unstable joints and increased incidence of falls. The diminished muscle strength can also facilitate the onset or increase in the severity of osteoarthritis.

The most significant atrophy is seen in the fast twitch (FT), Type I fibers, or pale fibers, as compared to the slow twitch (ST), Type II fibers, or red fibers. FT fibers are mostly recruited during high-intensity anaerobic movements. Sarcopenia, while much more severe in inactive individuals, appears evident as well in those who maintain a physically active trend through their life. In fact, in addition to inactivity, a host of additional factors may contribute to the phenomenon, such as decreased hormone levels and decreased protein synthesis (Tables 3 and 4). A most substantial role, on the other hand, is played by a "remodeling of the motor units."

As mentioned above, the number of spinal cord motor neurons and functioning motor units decline in a sort of continuous process and irreversible pattern (11). It appears that the loss of muscle fibers begins with the loss of motor neurons and the timetable of this decline varies with the location in the body, age, and the presence of disease (12).

It is then the denervation process that will be responsible for atrophy and eventually death of the muscle fibers and the consequent decrease in muscle mass. However, when a motor neuron dies, an adjacent motor neuron, usually a ST motor neuron, may reinnervate the muscle fibers, preventing atrophy. *The process defined as "motor unit remodeling" is not functionally perfect as the ST motor units have a much slower firing rate than the FT cell.* Thus, the substitution of ST for FT cells leads to a slower speed of contraction and hence a less efficient motor unit. This may help explain the loss of balance and speed of movement with age. In addition, denervation rates of the FT fibers may exceed reinnervation rates by ST motor neurons, further explaining atrophy of FT fibers occurring in the elderly (13).

Additional structural and functional changes in the aging skeletal muscle include

- increased size, but decreased number of myocytes,
- increased connective tissue matrix,
- increased myocardial stiffness,
- prolongation of the contraction time,
- decreased contraction velocity, and
- diminished β-adrenergic contraction response.

Sarcopenia and the Satellite Cells

It is clear then that sarcopenia results mainly from a loss of fast fibers and this process induces on an average a loss of muscle mass at a rate of 0.5% to 2% per year beyond the age of 50 (14). Although the loss of fast fibers remains the major cause, another cause is an altered muscle protein metabolism. Observations on old frail individuals did demonstrate a decreased rate of basal muscle protein synthesis (15). Even more recently, an additional well-documented demonstration of lower rate of muscle protein synthesis in old (>70 years) than in young male subjects was clearly documented (16). These studies demonstrate that nutrients activate protein synthesis much less in old muscles than in the young ones (Table 3).

TABLE 3 Major Factors Responsible for Sarcopenia in the Elderly

Inactivity
Changes in central and peripheral nervous systems
Reduced protein synthesis
Reduced protein intake
Reduced blood supply to tissues
Reduced number and size of mitochondria in muscle

TABLE 4 Decrease of Hormonal and Metabolic Factors Contributing to Sarcopenia

Hormonal factors
↓ DHEA levels and other anabolic hormones
↓ Growth hormone
↓ Insulin-like growth factor
Metabolic rate
↓ BMR levels
↓ 10% between the age of 30 and 54 yr
↓ An additional 10% between 65 and 85 yr
Biochemical activity
↓ Decrease in glycolytic enzymes
↓ Myokinase
↓ LDH
↓ Triosephosphate dehydrogenase

Abbreviations: DHEA, dehydroepiandrosterone; BMR, basal metabolic rate; LDH, lactate dehydrogenase.

Muscle regeneration and growth of muscle tissue require also the assistance of the so-called satellite cells. Satellite cells are indeed highly specialized cells located in the basal membrane of muscle cells and are necessary for the formation and synthesis of new muscle tissue. As the individual ages, reduction in the number and/or activity of satellite cells in skeletal muscle decreases; this decrease may contribute to the progressive loss of muscle mass and hence strength (17). Nevertheless, adult skeletal muscle is a perfect example of a tissue that robustly regenerates throughout adult life but fails to do so in old age (18). The reason for such an age-related decline in the regenerative potential is not completely understood and the relative roles of the intrinsic changes in the muscle cells versus the alterations in their aged environment have not been well defined.

Satellite cells act as endogenous stem cells and, as such, constitute the physiological regenerative potential in muscle. Satellite cells reside in direct contact with the differentiated, multinucleated muscle cells (myofibers or myotubes) and underneath their basal lamina. About 2% to 5% of all muscle nuclei are in satellite cells in the adult muscle. Satellite cells express specific genetic signals such as Notch Delta-like ligand that plays an important role in the regulation of cell renewal and differentiation of various cells (18). In resting adult muscle, the vast majority, 99.9%, of satellite cells are mitotically quiescent. *In response to injury, these quiescent stem cells proliferate and then differentiate into the myogenic progenitor cells, which go on to become fusion competent myoblasts.* Myoblasts are the differentiated muscle cells that are still capable of division but can also fuse to form new, multinucleated myofibers. This coordinated cell-fate determination–cell expansion followed by differentiation serves to repair or replace the damaged muscle (18). The initiation of this process of muscle repair relies on the activity of the evolutionarily conserved Notch-signaling pathway that is necessary and sufficient for the activation of satellite cells (19). Among the first biochemical changes associated with the response of muscle to injury is the upregulation of the Notch Delta-like ligand, in both the satellite cells and the myofibers adjacent to the injury site (19). This provides a positional cue for Notch activation and promotes cell expansion near the injury site, which is important for efficient tissue repair.

Thus, consistent with its role in embryogenesis, Notch induces the expansion of the myogenic progenitor cells, whereas attenuation of Notch signalling promotes differentiation of these cells into fusion competent myoblasts and, ultimately, multinucleated myofibers (18). Although the inactive form of Notch continues to be expressed in aged satellite cells, the injury-specific induction of Delta and the subsequent activation of Notch become severely diminished with age, resulting in a lack of the satellite-cell proliferation and ultimately an inefficient generation of new muscle tissue (19). The activation of satellite cells and productive muscle repair can be restored to the old muscle by forced activation of Notch (19). Both Notch signaling and the ability of satellite cells to regenerate muscle can be rejuvenated by exposing old satellite cells to a young systemic milieu either in vivo, when young and old mice are connected in pairs that share blood circulation (parabiosis), or in vitro, when aged satellite cells are cultured in the presence of young serum (18,20). Therefore, systemic factors modulate the regeneration-specific molecular pathways and, importantly, the intrinsic regenerative capacity of the aged satellite cells remains largely intact but is not properly triggered in the aged systemic environment.

Hormonal and Activity Factors

Sarcopenia may also be linked to a decrease in concentration of anabolic hormones such as growth hormone (GH), testosterone (T), and insulin-like growth factor-1 (IGF-1). In fact, GH and T are required for protein maintenance whereas IGF-1 positively correlates with protein-synthesis rate, specifically actin and myosin filaments, and myosin heavy-chain synthesis (9,10). A decrease in muscle mass and an increase in body fat are actually linked to a sustained decrease in these and other hormones (Chapters 9–12) (Table 4). Studies examining effects of hormonal replacement therapy have mainly focused on GH. These studies have reported that GH replacement has not consistently been effective in increasing muscle mass and strength in older subjects (11).

Sarcopenia is severely accelerated by inactivity (Table 3). Inactive adults see a faster and much greater loss of muscle mass when compared with physically active subjects. Although sarcopenia cannot be completely prevented by exercise, a progressive program of resistance training (RT) may stimulate the rate of protein synthesis in muscle even in the elderly (Table 5). Thus, after only three months of supervised RT, an increased rate of circa 50% of muscle protein synthesis was observed in frail older men and women (15). These findings suggest that the elderly retain the ability to increase the rate of muscle protein synthesis as a response to acute and short-term progressive RT. In addition, RT also increases the number of satellite cells in the trained muscles, leading to faster muscle regeneration (13).

Myoplasticity as a Partial Defense Against Sarcopenia

Myoplasticity is a term that specifically refers to the capacity of the skeletal muscle for adaptive changes. Even in older individuals, there remains a capacity of the skeletal muscle for adaptations secondary to stimulations as those induced by exercise (20–23). For example, a particular muscle fiber (like a fast Type I fiber), instead of disappearing due to the sarcopenic effect, may actually become hypertrophic in response to training, especially RT. The fiber may actually tend to change its contractile phenotype becoming larger and slower. This occurs due to a protein turnover whereby muscle cells increase their rate of protein synthesis above the rate of degradation (Table 6) (15,16).

Thus, body composition prior to and during physical activity may influence the outcome on physical functioning (24,25).

TABLE 5 Selected Muscular Responses to Exercise

Increase of mitochondria (number and size)
Increase in muscle fiber myoglobin
Increase in capillary network and circulation
Increase in glycogen storage capacity
Increase in free fatty acid utilization (only in prolonged, steady exercise)

■ Aerobic Performance: Running and Swimming in Old Age

To investigate the effects of aging on muscle activity, an appropriate approach is to study the changes in performance with aging that occur in highly trained and competitive athletes (26–29). Indeed, high-level athletes represent an effective experimental model in as much as the changes they experience in old age are thought to reflect, primarily, a decline in physiologic competence.

Endurance in running performance decreases with age in a curvilinear fashion (26–29). Specifically, good performance is maintained until 35 years of age, followed by a modest decrease in performance, translated into an actual, though still modest, increase in running time until 50 to 60 years of age; from this age on, the increase becomes steeper. This degree of impairment appears to be up to threefold greater in women when compared to men, with the largest difference at above 60 years of age.

The physiological functional capacity during exercise declines with advancing age also in a curvilinear manner in both men and women similarly to the decline in running (26–29). There is nevertheless, in swimming, a difference from what is observed in long distance running: the magnitude of the *overall reduction in swimming performance with advancing age is as much as 30% lesser than that observed in running performances*. Moreover, the age at which exponential decline begins occurred later in swimming (at 70+ years) when compared with running (60+ years). This difference is probably due to the much lower incidence of orthopedic injuries during swimming compared to running.

■ Aging-Related Effects on Anaerobic Performance

Anaerobic performance is by definition a maximal exercise of short duration. While a classic example may be provided by a run for 100 m, it is nevertheless also a factor in many of the activities of an older adult. Climbing stairs, hurrying across a busy street,

TABLE 6 Clinical Significance of Myoplasticity

With resistance training:
↑ Amount of contractile proteins
↑ Muscles enlarge
With endurance training:
↑ Velocity of contraction
↑ Number of mitochondria
↑ Capacity to oxidize substrate
↑ The VO_2 max
↑ Velocity of contraction of the ST (slow) fibers
↓ Decrease the velocity of contraction of the FT (fast twitch) fibers

Abbreviations: ST, slow twitch; FT, fast twitch.

or carrying a heavy grocery bag may represent examples of short-term maximal efforts for older adults and the ability to perform these types of activities can have a major influence on the independence of older adults (Chapter 3).

The anaerobic work capacity still centers on the active muscle mass, which, at the beginning of an effort and for short periods thereafter, may derive its energy from anaerobic systems such as (i) resting levels of adenosine triphosphate (ATP), (ii) resting levels of creatine phosphate, (iii) and the concentration and activity of the related enzymes, namely ATPase and creatine kinase. The substrate is glycogen and the necessary enzymes for the anaerobic glycolysis are phosphorylase and phosphofructokinase (28–30). Despite decline in these functions, an older adult, after adequate training, may almost reach a very decent and appropriate performance, certainly superior to those older adults who remain sedentary. Hence, the older individual who exercises maintains a better ability to cope with the physiological demands of free living.

■ EXERCISE PROGRAMS FOR THE AGED

A large percentage (35–45%) of the U.S. elderly population practices some form of physical activity. However, only 20% to 25% undertake regular exercise, 30 minutes or more three times a week. Women are generally less active in this respect than men. Low income and poor education appear to be predictors of inactivity. It is well recognized that regular exercise not only enhances the quality of life but also decreases mortality rates by 20% to 50% and increases overall life expectancy by an average of two years (30). Recommended types of exercise are summarized in Box 2. Well-established and probable benefits brought about by endurance, resistance, balance, and flexibility exercises are summarized in Table 7.

The benefits of exercise by far exceed the risks. Regular physical activity

1. reduces mortality rates even in smokers and obese persons,
2. preserves muscle strength,
3. improves aerobic capacity,
4. slows bone density loss, and
5. may help with weight loss (when combined with reduced caloric intake).

Indirect benefits include those associated with social interaction, an enhanced sense of well-being, and improved quality of sleep (Table 7).

In addition to improved physical functioning, regular exercise in the elderly (i) increases insulin sensitivity and glucose tolerance (Chapter 13), (ii) reduces blood pressure, and (iii) normalizes blood lipid levels by reducing triglycerides and increasing high-density lipoprotein (HDL) levels (31,32) (Chapters 15 and 16). Exercise helps to prevent

BOX 2 *Selected Types of Exercise to be Followed by the Elderly*

Endurance exercise. Walking, cycling, dancing, swimming, and all low-impact aerobics provide the best-documented health benefits for the elderly. Walking is the most frequent exercise among the elderly in the United States and the most commonly recommended by physicians. According to some statistics, walking an average of 3.2 km/day (2 miles/day) may reduce mortality rate by about 50% (1). It also reduces the risk of heart disease and helps to prevent falls (1). Jogging is usually not recommended for the elderly unless they are already accustomed to this type of exercise.

The maximum heart rate to be achieved during the exercise period, the so-called "target heart rate," may be monitored during exercise through pulse checks or by wearing a monitor. For a moderate-intensity exercise, the target should reach 60% to 79% of the maximum heart rate, i.e., 220 beats/min minus age in years. However, this formula is less reliable for the elderly; a simpler way is to rely on one's actual "perception of exertion." If you can talk without discomfort while continuing to exercise, you can assume that you are not overexerting. In a clinical setting, the health-care worker can refer to a special table where you can identify your "rate of perceived exertion" during exercise. "Reconditioned" individuals, those who have interrupted their exercise programs with periods of inactivity, especially bed rest, should resume exercising at about half the intensity to which they were previously accustomed.

Resistance exercise. The consensus today is that elderly people can and should perform some kind of resistance exercise at least twice a week, such as lifting a weight equivalent to 60% to 70% of the individual's maximal capacity, and then executing two sets of 10 repetitions on different exercise machines. With this routine, in one year, the gain in strength can reach 100% or more and muscle hypertrophy may become apparent. Programs of this kind are highly recommended for individuals with clear evidence of sarcopenia. Even moderate-intensity programs using calisthenics or elastic tubing can increase strength by 10% to 20%. After initial training on free weights, these exercises can be performed at home after a period of warm-up. Weights can be gradually increased as strength improves. Starting with weights that are too high or using them without professional guidance can lead to injury, and the benefits to be achieved are lost.

Balance exercise. Tai chi (a sequence of movements originally used in the martial arts) is an example of balance exercise that is very effective in balance training (e.g., walking with outstretched arms, crossed arms over the chest, and crossed arms holding weights).

Flexibility exercise. Stretching exercises, recommended for all ages, are critical for older individuals who have not followed a regular fitness program. Many elderly report that stretching alone makes them feel appreciably better. These exercises, some standing and some lying on a floor mat, are recommended to precede and follow any endurance and resistance exercises. Most sequences of stretches recommended by trainers are posted in chart form in gyms and fitness facilities. Each stretch is held for 10 to 30 seconds and repeated three to five times per session.

TABLE 7 Major Benefits Obtained with Exercise

Definite benefits
Metabolic: increase in insulin sensitivity and glucose tolerance
Cardiovascular: stimulation of vasodilation and reduction of blood pressure and of occurrence of arrhythmias
Cerebral: increase of blood supply to the brain and reduction in episodes of thrombosis
Improvement of balance, hence, fewer falls
Lower mortality rate
Higher HDL/LDL ratio
Diminished severity of osteoporosis
Possible benefits
Prevention of osteoporosis; gender differences (better prevention in males than in females); better results with associated administration of calcium and vitamin D
Prevention of colon cancer (probably due to faster intestinal transit time)
Mood changes (variable; more significant in males than in females)
Improved sleep cycle (Chapter 7)

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein.

cardiovascular diseases, diabetes, osteoporosis, obesity-related diseases, colon and, possibly, pancreatic cancer. It also lowers the incidence and severity of falls, prevents or relieves depression, and may improve cognition (33–36).

■ POTENTIAL ADVERSE EFFECTS OF EXERCISE IN THE ELDERLY

■ Activity-Related Injuries

There is little doubt that the benefits of exercise in older people seem to strongly overweigh the potential hazards, provided there are no obvious contraindications such as acute illness, injury, or terminal illness (Table 8). Appropriate screening, training and, if needed, supervision will reduce significantly the potential occurrence of musculoskeletal injury. In fact, the most usual risks appear to be torn ligaments and pulled muscles. Similarly, falls and fall-related injuries (like hip fractures) can occur during exercise, but despite that, it is still extremely clear that physically active older adults appear to have a significantly lower rate of these occurrences (33). As a matter of fact, *the benefits of exercise by far exceed its risks regardless of the presence of other risk factors. A regular regimen of appropriate training can reduce mortality rates even in the elderly who smoke and/or are overweight*.

TABLE 8 Major Adverse Effects of Exercise in the Elderly

Musculoskeletal injuries:
Dislocation of shoulder
Achilles tendon tear
Intervertebral disk injury
Traumas due to repetitive motion (jogging)
Traumas due to falls or collisions
Metabolic abnormalities (consequent to severe exertion)
Hyperthermia
Electrolyte imbalance
Dehydration
Hypothermia (especially in water/winter sport)
More frequent pathologies occurring with exercise in the elderly
Manifestation of cardiovascular disease such as coronary heart disease
Atrial fibrillation
Stroke
Pathologies occurring with exercise (but seldom seen in the elderly)
Hemolytic anemia and/or hemoglobinurias (in distance runners)
Hematurias (blood in urine), secondary to urinary bladder traumas
Muscle degeneration (rhabdomyolysis) in very-heavy-weight lifters

■ Disease-Related Events

There is no question that patients affected by known coronary heart disease (Chapter 15) will need an appropriate screening (e.g., stress electrocardiogram test) before starting training so that a more precise set of indications may be provided together with some degree of supervision (Table 8) (32–36). Patients affected by diabetes mellitus (Chapter 13) should also receive appropriate instructions to prevent any potential hypoglycemia as induced by efforts (37). Those affected by hypertension should be aware that during any kind of exercise there is always an increase, especially in the systolic values and these increases are to be interpreted as a normal response and, importantly (Chapters 15 and 20), the prescribed medications to achieve a proper control should not be discontinued (Chapter 22). The dyslipidemic patients should be well aware that exercise is in itself an accepted modality for the correction of abnormal levels of lipoproteins (Chapter 16). Even a small amount of exercise at low or at moderate intensity (equivalent to walking or jogging on an average 12 miles per week) (22) is associated with beneficial changes of the lipoprotein profile in the plasma (31). There is actually a graded response of the plasma lipoprotein (Chapter 16) almost parallel with increasing levels of exercise. This fact explains the progressive decrease in cardiovascular risks associated with increasing levels of muscular activity (32).

Acute Cardiovascular Risks

A very important problem not infrequently encountered in exercising elderly individuals is the absence of the classic precordial pain even in presence of a definite coronary heart disease (Chapters 15 and 20). The classic precordial discomfort may be substituted by equivalent sets of symptoms of more difficult interpretation such as dyspnea or nonspecific sensations of thoracic constriction. In the very well-known Framingham study, a silent (symptom-less) myocardial infarction occurred in a group of individuals between 75 and 84 years of age (33). The silent myocardial infarction in the elderly can be explained by an increase in the number of receptors for endogenous opioids and their analgesic effect may mask the actual number of occurrences of ischemic pain and discomfort (34). In view of these observations, it may be appropriate to exert a degree of supervision for certain patients during exercise (35).

Exercise in the Presence of Atrial Fibrillation

With advancing age, the prevalence of atrial fibrillation (i.e., rapid uncoordinated contractions of the cardiac atria, resulting in a lack on synchronism of cardiac rhythm) increases from 0.5% among young adults to more than 6% among those 80 years of age or older (36). Although many cases of atrial fibrillation may be associated with valvular heart disease, hypertension, diabetes, alcoholism, thyrotoxicosis, and increased vagal tone, (this causing the interesting occurrence of postprandial atrial fibrillation), an increased sympathetic tone may cause a typical "exercise-related atrial fibrillation." There are also individuals with the so-called "lone atrial fibrillation" (17% circa), who have no clinical electro- or echocardiographic evidence of any

abnormality. There are as well very rare familial cases of atrial fibrillation due to abnormalities of chromosome 10.

It is of interest to know that, particularly in regard to exercise, cardiac rhythm control provides no advantage over ventricular rate control and survival. Indeed, it is control of heart rate frequency that should be considered the primary approach to the treatment of atrial fibrillation while rhythm control, if used, should be abandoned if not fully satisfactory (36).

Symptoms may be dyspnea, palpitations, and decreased exercise tolerance; the more serious complications are thromboembolisms and strokes. Obviously, in all cases, the anticoagulant therapy of choice should be implemented.

Exercise and the Obese Elderly

In the elderly obese individual, only relatively high-intensity exercise training seems to provide an improvement in the insulin-stimulated glucose disposal (ISGD). Moderate-intensity training instead does not initiate any change in ISGD nor glucose oxidation. Specifically, the recent Surgeon's general report on Physical activity and Health recommends 30 minutes of moderate-intensity aerobic exercise training on most days of the week for both health promotion and disease prevention. However, it is appropriate to underline that this amount of activity while providing healthy benefits for most people may not be sufficient for the obese/overweight individuals. *These cases may require a more aggressive therapeutic regimen that should include dietary modifications and increased duration of physical efforts*. Indeed, without dietary adjustments, neither high- nor moderate-intensity training (up to 1000 Kcal/wk) resulted in weight loss or change in the percentage of fat.

In the elderly obese, exercise programs that do not result in weight (or fat) loss only improve insulin action through short-term improvements in glycogen metabolism. *In conclusion, for the obese elderly individual, dietary manipulations should be added to the exercise programs to obtain a positive effect on the potential insulin resistance* (37) (Chapter 13).

"Highlander" Syndrome

In a series of recommendations for young and adult athletes, from several organizations for competitive sports–assessed cardiac disease and rehabilitation in these sports (38). The possible problems connected with excess exercise were debated with respect to older athletes forcing their cardiovascular efforts to a maximum. The conclusion apparently was that it is not true that the "old heart" can sustain excessively strong efforts as in a younger heart. The individuals attempting to force their cardiovascular capabilities to maximal efforts when not young any more or even when definitely old are themselves exposed to some risks (39). The group of older athletes was defined as affected by the "highlander syndrome" with all the risks involved (39). Indeed, in the highland of Scotland, some old shepherds persist in their activities of guiding their herd to pasture even at the very end of their life. *The conclusion of these observations is that older individuals should maintain a good level of exercise but probably should not attempt to reach maximal or excessive goals*.

Sudden Death

Sudden death may occur during physical exercise. An acceptable definition could be the one of "sudden death occurring within one hour from the beginning of acute symptoms in strict relationship with exercise but in the absence of any other external causative agent" (e.g., falling injuries).

Sudden death incidence in the general population is extremely low, circa 1 case per 100,000 people per year (39). According to the meticulous observations of Paffenbarger et al. (40) who followed groups of Harvard alumni involved in endurance efforts for years, the incidence of the event appears to be limited to 1 death per 15,000 joggers per year. The highest frequency seems to occur during or, better, immediately after the termination of the physical effort. Some attention should be devoted to the elderly individual involved in marathon races. Indeed, after years of no events of death, in 2006, two runners in the Los Angeles marathon, respectively 53 and 60 years of age, died of heart attacks. Three other episodes of cardiac events did occur in individuals in their 40s during marathons in Chicago, San Francisco, and Minneapolis. The number of race fields has grown tremendously and, at least in the United States, 328,000 people completed a marathon in 2005 (an increase of about 80,000 since the year 2000). *Despite the recent unfortunate episodes, the evidence today is still strongly in favor of the idea that endurance exercise is helpful in terms of cardiac health. The conclusion for the elderly then is: continue to walk or even "run" but only after a full medical screening*.

Some caution is necessary with regard to the distance and intensity of marathons; it requires selection of potential, as not everyone may not be fit to attempt the race. In patients younger than 35 years, the etiology is usually related to cardiomyopathies, and of these, the most frequent appears to be the right ventricular cardiomyopathy (Chapter 20). In people older than 35 years, the major cause of sudden death in relationship with exercise is still coronary artery disease. Occasionally, and still more frequently observed in the younger age range, sudden unexpected deaths may be related to various types of cardiac pathology (41).

Sudden deaths may also be related to noncardiovascular events, such as, for instance, the occasional anaphylactic shock occurring in joggers or even walkers when stung by a bee if and when allergic to the respective poison (42). Last but not least, the elderly must be alert to the potential "hyponatremic collapse" occurring in marathoners drinking an excessive amount of fluids without neglecting the need for sodium supplements (43).

■ RECOMMENDATIONS FOR HEALTHY AND SAFE EXERCISE

A most important initial step is to encourage the elderly to meet the population-wide recommendation of undertaking at least 30 minutes of moderate-intensity exercise on most or preferably all days of the week. This can be accomplished with a variety of activities such as a few minutes of brisk walking, climbing stairs, or with a variety of recreational endeavors. Indeed, it would be helpful to do the exercise with a friend and also to establish a schedule. It should be helpful, if not mandatory, to outline the intensity, duration, and frequency of the chosen task as indicated below.

Intensity

Exercise needs to be not too vigorous and continuous to be beneficial and in fact, as already mentioned, a daily accumulation of 30 minutes of workout already will provide definite benefits. Walking still remains an excellent mode of activity for many elderly, but, if available, aquatic exercise and/or stationary-cycle exercise may be especially advantageous for those with reduced ability to tolerate weight-bearing activity (31,44).

Duration

Exercise duration need not be continuous to produce benefits. Those who have difficulty sustaining exercise for 30 minutes

can be advised to exercise for 10-minute periods at different times through the day. In order to avoid potential injuries and ensure safety, older persons should initially increase the duration rather than the intensity of the efforts.

Frequency

Moderate-intensity exercises should be undertaken most days of the week. If exercise is performed at a substantially vigorous level, it should then be performed at least three times a week alternating, of course, with no exercise every other day.

Strength Training

Elderly persons should be encouraged to supplement cardiorespiratory endurance activities and an active lifestyle with some strength-developing exercises, namely resistance, balance and flexibility exercises.

How Much Exercise is Needed for Benefits?

As already stated, *exercise provides a significant number of benefits in elderly persons* (Table 6). Indeed, improvements in mood, blood pressure, insulin sensitivity, and plasma-lipoprotein profiles are well demonstrated but the actual amount of intensity of exercise is still poorly understood. Recent observations suggest that levels of plasma lipoproteins, as affected by different amounts of intensities of exercise in overweight men and women with dyslipidemia, seem to provide some very useful information. In fact, Kraus et al. (31) found that even low amounts of exercise at moderate or high intensity (an equivalent of walking or jogging 12 miles per week) are associated with significant beneficial changes in plasmatic-lipoproteins' profile. These studies clearly document the beneficial effects of exercise at whatever levels on the lipoprotein spectrum, even if incidentally accompanied by only minimal changes in body weight. These data provide a ray of hope for those who find it easier to exercise than to lose weight (45).

Mens Sana in Corpore Sano

At the conclusion of our chapter, Juvenal's dictum, by now 2000 years old, "a healthy mind (mens sana) in a healthy body (in corpore sano)," is taking a literal meaning. Ongoing research is accumulating evidence that physical activity not only improves overall body function but also may delay neurocognitive decline and Alzheimer's disease (Chapter 7) (46). It appears, in fact, that exercise in late life is inversely associated with risk for all-cause dementia and Alzheimer's disease. Recent studies by Larson et al. (47) reported a definite interaction between levels of physical activity and risk for dementia. We seem to be edging closer to placing prevention of cognitive deterioration and dementia on the long list of health benefits induced by physical activity.

Conclusion

Doctor Scannell (48) in a recently published "Perspective" article states that, too frequently, geriatric patients wonder why they are unable to ride a horse, hop on a plane, join the stock club, or dance the tango "as the old folks in the ads are doing". They painfully wonder what is wrong with them or with their enfeebled partners and why and how they have failed to thrive. Possibly, we should remind ourselves that getting old is not solely a "state of mind" as stated in the book "The Fountain of Age" by Friedan but also a state of the body (49). Very correctly, Dr. Scannell exhorts to limit the "struggle to differentiate aging from disease, healthy aging from unhealthy aging, and optional aging from obligatory aging." Acceptance of the reality of aging is a must and it is up to experts in the field to render this important part of the human life as pleasant and as productive as possible, including some leisure time (50).

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Regenerative Perspectives and Assistive Technologies

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INTRODUCTION

The major purpose of this book has been to present an overall, systematic view of physiologic aging in humans and for comparison and elucidation, in experimental animals and cultured cell models. Given the increased incidence of disease associated with aging, a few diseases have been selected for brief discussion to serve as examples of (*i*) how pathology influences physiologic adjustments with advancing age and (*ii*) reciprocally, how old age and declining physiologic competence augment the susceptibility to disease and disability.

Maintaining or restoring a state of "wellness" in old age demands that physiologic responses be strengthened and disease eliminated. We have seen some success in preventing and eliminating a number of diseases in recent years (primarily infectious diseases), in part due to progress in public health. However, much less has been achieved when it comes to strengthening basic functions and preventing or repairing functional decline in old age. As mentioned in previous chapters, attention to a number of conditions in combination, such as an appropriate diet (Chapter 23), regular physical activity (Chapter 24), a judicious use of therapeutic drugs (Chapter 22), a better education such as continuing learning through the life span (Chapter 7) are interventions that, in conjunction with strong economic conditions, social support, and continuing advances in technology to promote functional fitness at all ages.

After a brief introduction, this last chapter will present a short review of the potential role of mortal and immortal cells in regenerative medicine. This will be followed by some consideration on the interest and usefulness of engineering sciences in providing assistive technology to promote health and independence in the elderly. The last section, entitled "Life Extension Sciences" will list some new areas of future advances in the area of gerontology and geriatrics.

MORTAL AND IMMORTAL CELLS: GERONTOLOGY IN THE AGE OF REGENERATIVE MEDICINE

Mike D. West

Aging is a multifaceted degenerative process due, in part, to dysfunction at the cellular level (Chapter 4). The field of cellular gerontology has its roots in the work of the nineteenth century German naturalist Weismann. He proposed a fundamental distinction between germline cells that serve as an immortal vehicle for the transport of the molecules of heredity and all somatic cell types that periodically arise from these immortal cells, but upon their specialization, commit to dying within that generation. Weismann appears to be the first to fully appreciate the significance of this dichotomy, but beyond this, he attempted to provide deductions regarding the mechanisms of aging at the level of the organism. He wrote, "Death takes place because a worn-out tissue cannot forever renew itself and because a capacity for increase by means of cell division is not everlasting, but finite" (1). He was prescient enough to recognize that not all cells in a particular tissue would have to reach the end of their replicative life span in order for age-related pathology to emerge. As he put it, his theory "does not, however, imply that the immediate cause of death lies in the imperfect renewal of cells, for death would in all cases occur long before the reproductive power of the cells had been completely exhausted. Functional disturbances will appear as soon as the rate at which the worn-out cells are renewed becomes slow and insufficient" (1).

Weismann's theory that mortal somatic cells possess only a finite capacity for replication was initially challenged (2) and then finally vindicated by Hayflick (3). Hayflick's proposal regarding age-related dysfunction appearing earlier than a uniform exhaustion of replicative capacity is now well appreciated to be the result of the dominant effect of the senescent activation of numerous cell types (4,5). Hayflick's "replicometer" was soon shown to be counting cell divisions rather than metabolic time (6) but it required the clairvoyance of Olovnikov (7) to propose the unlikely hypothesis that this clock was the telomere (Chapter 4). His theory of marginotomy proposed that somatic cells lack a terminal polymerase activity that specifically adds telomeric sequences. As a result, immortal cells (such as germline and neoplastic cells) express this activity, resulting in telomere maintenance and replicative immortality. In contrast, he suggested that somatic cells do not express this terminal polymerase activity; therefore, with each round of cell division, and consequent DNA synthesis, somatic cells progressively lose a small number of telomeric repeats from the lagging strand, eventually leading to critically short telomeres and cell-cycle arrest (Chapter 4). The observation of telomere shortening in somatic cells during aging and the constancy of telomere length in the germline (8) was consistent with Olovnikov's hypothesis as was the report of telomerase activity in virtually all immortal human tumor cell lines but not normal human somatic cells (9). However, it required the cloning of the telomerase components human telomerase reverse transcriptase (hTERT) and human telomerase RNA (hTR) component (10,11) and, finally, the transfection of the differentially expressed catalytic component hTERT into somatic cells to convincingly demonstrate that telomerase regulates the mortal/immortal phenotype in cultured human cells (12).

To determine whether telomere maintenance plays a role in the aging of the mammalian organism, the RNA component of telomerase was knocked out in mice, resulting in no observable phenotype until the sixth generation, when telomere shortening began to become manifest as defects in cell proliferation. Similarly, mice null for the Werner syndrome (WS) gene *Wrn* appear normal (13) (Chapter 3). However, the mating of mice lacking the telomerase RNA component with those lacking *Wrn* resulted in mice displaying a constellation of age-related pathologies known to be common between aging and WS, including graying of hair, hair loss, osteoporosis, type II diabetes, and cataracts (14). Further supporting a role for telomere maintenance in the age-related disease observed in WS was the observation of accelerated onset of critically short telomeres in human WS fibroblasts (15–17) and the observation that the transfection of hTERT into WS fibroblasts rescues the premature senescence of the cells in vitro (18). These data suggest that telomere damage resulting from either replicative senescence or spontaneous fragmentation not repairable by *Wrn*-mediated recombination may be a common feature of WS and normal aging. The data also suggest that means to reactivate telomerase and restore telomere length may be useful in providing a therapeutic effect in those particular age-related diseases that overlap in *Wrn* and normal aging [i.e., atherosclerosis, cataracts, osteoporosis, dermal atrophy, and type II diabetes (19)].

Three strategies come to mind in repairing age-related telomere damage in the intact organism:

1. There may exist small molecule drug candidates that could reactivate telomerase activity in vivo (at least in some cell types).
2. It is conceivable that hTERT could be incorporated into a gene therapy vector to infect tissue and cause a transient expression of telomerase activity, resetting telomere length and cell life span. This approach has demonstrated some feasibility in animal models (20) despite the inefficiency or immunogenicity of current gene therapy.
3. It will likely be possible to utilize telomerase-positive totipotent germline cells to produce any particular cell type that would be expected to repress telomerase upon differentiation and then possess a long proliferative life span. This strategy is termed "regenerative medicine."

The isolation of human embryonic stem (hES) cells began with the anticipation that such primitive cells may display both a totipotency and a replicative immortality in vitro. Since the original publication of the propagation of these cells (21), their totipotency is well documented, though their replicative immortality must be inferred based on the presence of telomerase activity and long-term proliferation potential.

The cultivation of hES cells makes it feasible

1. to perform genetic modifications (including gene targeting) and
2. to generate virtually any somatic cell type early in their developmental and replicative life span, on a scale practical for use in transplantation.

One remaining issue, however, is to resolve the problem of histocompatibility between the hES-derived cells and the patient. The demonstration of the feasibility of somatic cell nuclear transfer (SCNT) in animal cloning (22) leads to the question of the feasibility of the use of SCNT to produce autologous hES cells (23). It is worth noting that most SCNT-derived "clones" are not strictly clones in that their mitochondrial genome is generally derived from the oocyte donor rather than the somatic cell (24). However, SCNT-derived tissues transplanted in large animal models show no evidence of transplant rejection despite the presence of allogenic mitochondrial DNA (25).

The initial report that SCNT led to cloned animals with abnormally short telomeres (26) led to the conclusion that oocyte cytoplasm could reprogram somatic cell chromatin to totipotency but could not reset cell life span. However, closer examination demonstrated that SCNT actually can reactivate telomerase and reset the telomere length of cells near the end of their replicative life span in bovine species (27) and this observation has been extended to numerous other mammalian species (28,29).

In summary, the combination of cellular reprogramming by SCNT or other means and embryonic stem (ES)-based technologies has the potential to generate autologous embryonic tissues of any kind for transplantation in a number of tissue types. In as much as these ES cell lines can be easily cryopreserved, such generation of young cell and tissue types is not temporally limited (i.e., young cells and tissues can be generated indefinitely). The limitations to the application of this technology in the treatment of age-related disease are currently the practical issues of:

1. Generating pure populations of the desired cell types and
2. Finding efficient means of replacing those cell types into tissue

Near-term practical applications may include both mitotic and postmitotic cell types. Examples of studies currently under way include:

1. The transplantation of hES-derived retinal pigment epithelium into the subretinal space to ameliorate the progression of age-related macular degeneration into the advanced form of the disease (30) (Chapter 8)
2. More provocatively, the hemangioblasts and myocardial progenitors that show promise in the ability to restore contractility to the heart following myocardial dysfunction (31,32) (Chapters 17 and 20)
3. The ability to generate neurons such as dopaminergic neurons that may prove beneficial in the treatment of age-related neurodegenerative diseases including Parkinson's disease (33) (Chapters 6 and 7)
4. circulating lymphocytes that show telomere shortening that correlates with increased risk of heart and infectious disease (34)

The generation of hES-derived vascular hemangioblasts may provide a cell type deliverable by intravenous infusion, with the final benefit of providing young lymphoid, myeloid, and erythroid and even vascular endothelial progenitors to aged patients (35) (Chapters 14 and 17).

It is unlikely that hematopoietic stem cells are the only cells capable of homing to their natural niche. The continued study of all the potential embryonic progenitors that can be propagated in vitro from hES cells may provide novel cell types capable of effective engraftment in vivo, useful in treating age-related degenerative disease.

■ ASSISTIVE TECHNOLOGY

Ruzena Bajcsy

It has been suggested that overall health and wellness of the elderly sector of the population can greatly benefit from the use of information and communication technology. Some of these

uses have been successfully adopted for the treatment of sensory impairment. Various procedures useful to improve vision and hearing have been listed in Chapter 8. Other technologies that have been also useful in diagnosing various diseases and physiologic impairment (e.g., memory loss) are discussed in Chapters 6 and 7. Indeed, several large technology corporations have already organized specialized research sections to investigate the possible use of devices to monitor and assist the elderly, primarily to remain independently at home. One of such researches will be briefly described here.

For example, new technology allows the creation of new small sensor "Motes," which combine a variety of micromachined transducers, a microcontroller to reduce data into information, and a wireless link to the outside world (36). Such devices improve the independence of people needing living assistance. In this particular research, methodology has been created to distinguish the occurrence of falls from other human movements. As already discussed in Chapters 6, 7, and 20, falls are some of the major causes of death in the elderly. The propensity to fall is also one of the major reasons why the elderly are placed in nursing homes where they can be given immediate assistance when needed.

The availability of specific movement sensors might allow older individuals to remain in their own home and environment rather than being relegated to a nursing home. In case of falls, the information will be relayed rapidly to responsible supervision for assistance. Sensor platforms are integrated into the clothing (therefore easily wearable) to monitor physical activity and to detect body postures (such as sitting, standing, and lying) as well as periods of exercise such as walking. The objectives in this type of experiment were

- to identify key features that can be used in the detection of movement recognition and
- to differentiate normal movement from a movement related to a fall.

Throughout these experiments, a sensor device with motion sensors (accelerometers), was placed on the waist. In case of falls, the sensor device is capable of sending triggering signals to a phone or a PC, and consequently, these devices can relay the alarms to emergency first-responders or family and friends.

Data reading from a motion sensor for several movements show that falls can be clearly identified by observing sharp changes in the data reading. The results indicate that it was possible to separate falls from regular movements with 85% accuracy. In fact, the accuracy of this distinction was even clearer in the older people than in the young, perhaps due to the more rapid movements of the young (36).

While the fall monitoring is pivotal in allowing the elderly person to remain at home it also provides the benefit of immediate assistance in case of emergency. Many other similar devices are being studied. The most successful so far include AMON—advanced care and alert a portable telemedical monitor (37), a device suggested by Najafi, which detects body posture (38), another triaxial waist-mounted device (39), and finally the sensors suggested by Aminian (40).

A number of other projects have been investigating the use of several apparatus to improve the physical activities of the elderly and patients. CodeBlue group at Harvard has developed a platform called SHIMMER to monitor patients with various motor dysfunctions (41). MIT Professor Neville Hogan's research has been building robots to help stroke patients regain movement faster. Anklebot is one of the devices that assist patients with walking on a treadmill (42). Several other groups have been working on similar projects. To name a few, Yoki Matsuoka of CMU has developed a domestic rehabilitation and learing scheme of task-specific movements (43). Edward Grant and Carey Reid Merritt of North Carolina State University have built artificial muscle technology for stroke patients and other people with muscular rehabilitation need (44).

■ Life Extension Sciences

Paola S. Timiras

Research in this area purports to extend as well as improve life in old age. Several types of strategies for combating disease and prolonging "health span" and "life span" come from daily biomedical advances and their technologic applications. Some of them have been discussed throughout the book. In this twenty-first century, bioscience is benefiting from the previous century's discoveries, especially those involving the role of antibiotics in reducing infectious diseases, as mentioned above and discussed in Chapter 2, and the identification of DNA as the carrier molecule of genetic information in cells. At the beginning of this century, the sequencing of the genome in several animal species, including humans, has opened exciting avenues for basic and applied research.

The recognition that cells such as neuron and muscle/cardiac cells, previously considered incapable of proliferating in old age, retain the potential for plasticity and regeneration has opened the way to the study of intrinsic and extrinsic factors that may facilitate tissue repair in neurodegenerative diseases and in gene therapy. Likewise, the identification of pluripotent cells, such as stem cells, potentially capable of acquiring the structure and function of a variety of specialized cells promises to let us effectively replace lost tissues and functions.

As suggested in Chapter 1, continuing advances in human genetics will lead to the identification not only of the genetic etiology of several diseases but also of the genetic variations that, combined with environmental risks, make people vulnerable to numerous diseases. Progress in this area will open the way for genetic therapy to restore the normal genome and will provide the basis for individualizing therapeutic treatments.

Some of the areas being explored, under the rubric of "life extension sciences," are still controversial. They include, among many others,

- to use dietary calorie restriction, or mimetics of calorie restriction to prolong life (Chapter 23)
- to elucidation of the mechanisms of action of calorie restriction,
- to identify conditions and substances that can mime the effects of calorie restriction (calorie restriction mimetics),
- to identify technology to preserve tissues and organs for transplants (e.g., cryopreservation) and to prevent tissue and organ rejection,
- to extend the uses of deep hypothermia (for special surgical procedures) and cryonics (for subsequent resuscitation),
- to foster bioengineered improvements for artificial internal organs (e.g., artificial kidney and heart) and prostheses for replacing malfunctioning body parts,
- to continue to progress in medical visualization (imaging) technology to improve our understanding of the function and the diagnosis and treatment of diseases,
- to continue to improve gene therapy and develop research in pharmacogenetics for the individualization of drug treatment according to each individual's genetic contribution, and
- to investigate cloning techniques to produce "human cloned bodily parts" to be used as a source of young

organs capable of being back-transplanted to replace failing organs. Nuclear transfer techniques, proven successful for cloning goats, cattle, mice and pigs, may be applied to human clones, despite the potential risks to the clone and strong ethical opposition.

- to clarify how inherited genetic variation plays an important role in susceptibility to disease and treatment response (Chapter 3 and 22–25) to better understand how genes harbor risk alleles, how common these genes are in the population, and how these genes and their protein products act in response to environmental stimuli.
- to continue to press for loosening legislation for human embryonic stem cell uses, given the therapeutic potential of these cells, research should continue to explore the possibility of re-activating adult somatic cells in order to remove the necessity of using embryonic stem cells.

Although some of the predictions for life extension may sound like science fiction, given the monumental changes taking place every day, these predictions are not that outrageous. Seeking immortality in the arduous old-fashioned way, doing good deeds, and taking care of (one's) children remains a goal worthy of pursuit, but it need not halt efforts aimed at improving and lengthening life. Aging and death remain, indeed, the last sacred enemies, a fact that is particularly frustrating to humans who have now harnessed nuclear energy, circled the moon, decoded the human genome, artificially reproduced DNA, and significantly extended life expectancy (Chapter 1). Such intrepid individuals can be expected to continue striving to improve the quality of life at all stages as well as to extend the duration of life.

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Geriatric Medicine

about the book...

Extensively revised and updated to reflect the current state of knowledge in the study of aging, this ***Fourth Edition*** offers a complete profile of the aging process at all levels, from molecules and cells to demography and evolution. Written by international experts in current basic and clinical aging research, this text includes aspects of individual, comparative, and differential aging, and discussions of theories and mechanisms of aging. This invaluable reference illustrates how bodily systems, organs, and functions are affected with aging, describes how genetic and environmental factors influence age-related changes, and addresses some of the clinical consequences of these changes for health and longevity. Well illustrated, with numerous tables and graphs, this book presents up-to-date information from internationally renowned experts in various biomedical fields.

New updated information includes emphasis on emerging technologies and recent advances in biology and bioengineering that allow greater and longer independence of the elderly…recent therapeutic developments in key diseases such as Alzheimer's, osteopenia, osteoporosis, high cholesterol, diabetes, gastritis, sexual impotence, and using transgenic animals as models of aging…evaluation of the latest knowledge on the roles of oxidants and antioxidants, and correlates them with other aging-based theories…expanded discussion of nutrition and physical exercise…gene and stem cell interventions related to the aging process…updated tables to reflect current trends, new research data, and up-to-date statistical information.

about the editor...

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