ANTIAGING AGENTS

1. General Considerations

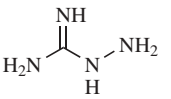
Aging in humans is associated with a decline in physical vigor and function, with progressive deterioration in most major organ systems including central nervous system and immune system functions. The current view is that molecular damage and disorder that occur with age in macromolecules are largely responsible for the age-related changes observed at the organism level. Such damage to macromolecules may be caused by free radicals and by the formation of advanced glycation end-products, to cite two examples. It is still unclear, however, whether AGEs, which accumulate to high levels in many age-related chronic diseases, are the cause or the consequences of the diseases. Some inhibitors of glycation and free radical formation are showing promise against chronic conditions, particularly diabetes and its related complications. The most impressive results in extending the life span of animals and preventing the incidence of age-related conditions are observed with the regimen of reduced caloric intake or caloric restriction. Since this regimen has not yet been verified to work in humans and since it is a very difficult diet to maintain, the search for pharmacological mimics of calorie restriction is the focus of much effort in drug discovery. Aging is associated with an increase in oxidative damage to cellular macromolecules probably arising from the electron transport system as part of the day-to-day metabolic process. Agents that can lower oxidative stress and damage are beginning to show potential as calorie restriction mimics. Several hormones have been shown to improve certain changes associated with human aging, such as body mass composition. Agents that have been studied in specific organ systems as they relate to prevention and treatment of age-related changes are discussed for each system. For skin aging, basic cell functions are reduced with advancing age. Therefore, an effective antiaging agent should provide acceleration of mitochondrial activity, enhanced cell proliferation, and increased matrix component synthesis in dermal fibroblasts. Several agents are discussed which show activity in one or more of these areas. Much progress has been made in understanding the molecular basis of hair loss. Two drugs are now approved and marketed in the US for prevention and treatment of hair loss. The central nervous system suffers from a gradual decline in cognition, behavior, and function with advancing age. Alzheimer's disease (AD) is the most common form of dementia in the elderly. Currently, available treatments for AD only diminish certain symptoms but cannot halt the dementing process. New therapies currently being developed for AD include agents that target amyloid β peptide and downstream pathological changes as well as agents that increase the activity of the cholinergic transmitter system. Newer inhibitors of cholinesterase are more selective and show fewer side effects than the first generation series of inhibitors.

Antiinflammatory and immunotherapeutic approaches to the treatment and prevention of AD are also being investigated. Age is a major risk factor for the development of chronic diseases of the cardiovascular system and such disease are a direct result of atherosclerosis. Since atherosclerosis has both an autoimmune and an inflammatory component, new approaches for the treatment and

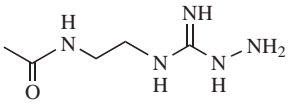
prevention of heart disease are beginning to focus on these areas as well as the traditional risk factors such as dyslipidemia. The human immune system gradually declines with age and the changes that occur result in increased susceptibility to infectious disease, cancer and autoimmune disorders in the elderly. It is believed that pharmacologic intervention to restore immune function in the elderly will provide widespread benefits in helping to maintain health in advanced age. Age-related conditions that involve the musculoskeletal system include osteroarthritis and rheumatoid arthritis as well as osteoporosis. Several biologicals are now in use for treatment of rheumatoid arthritis. Future treatments will likely involve the inhibition of proinflammatory cytokines by small molecules. Finally, age is a major risk factor for developing cancer in humans. Many agents are being investigated for their ability to inhibit the formation and progression of various types of cancer, including breast, prostate, and colorectal cancers. It is important to distinguish between the intrinsic process of aging and the diseases and conditions associated with aging. Important also is the distinction between aging, longevity, and maximum life span. Theoretically, if one slows the body's rate of aging, one can increase the maximum life span. Last year however, researchers on aging noted in a position statement that no treatment on the market today has been proven to slow human aging (1). Medical interventions for age-related diseases do result in an increase in life expectancy, but none have been proven to modify the underlying processes of aging. This article discusses agents relative to the prevention and treatment of age-related changes and diseases.

The current view of scientists is that random damage that occurs within cells and among extracellular molecules is responsible for many of the age-related changes that are observed in organisms. Molecular disorder occurs and accumulates within cells and their products because this occurrence outpaces the cell's repair mechanisms.

1.1. Advanced Glycation End-Products. There is considerable evidence for molecular disorder and damage to macromolecules such as proteins and cell membrane lipids being caused by so-called advanced glycation end-products (AGEs) and by free radicals. The AGEs are complex components formed nonenzymatically during the Maillard reaction involving monosaccharides and the amino groups of proteins, which alter protein structure and functions. The AGEs affect the biochemistry and physical properties of proteins and the extracellular matrix. The AGEs have been implicated in the pathogenesis of diabetic complications, atherosclerosis, arthritis, Alzheimer's disease and in the process of normal aging. AGEs accumulate to high levels in these age-related chronic diseases (2). However, it is not clear whether AGEs are the cause or the consequences of the age-related complications (3). A large number of compounds have been reported as inhibitors of glycation and AGE-protein crosslink formation (4). Among those most studied is aminoguanidine [79-17-4], also known as pimagedine as the HCl salt.



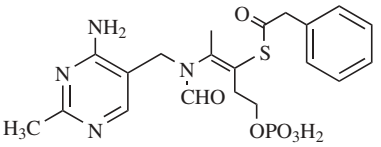
Aminoguanidine has shown promise at slowing or preventing the cross-linking of collagen molecules to each other in vascular wall and myocardium tissue in aging and diabetic animals (5). The acetamidoethyl derivative of aminoguanidine, ALT-946 [192511-71-0], is about fivefold more potent than aminoguanidine as an inhibitor of cross-linking of a glycated protein to rat collagen (6).



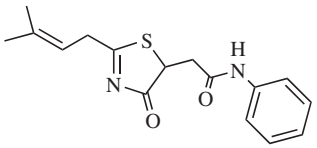
ALT-946

In addition, aminoguanidine inhibits the development of retinopathy in diabetic animals by a mechanism that probably involves, at least in part, the inhibition of retinal nitric oxide production (7,8). Other effective compounds studied for inhibition of AGEs include benfotiamine [22457-89-2], the thiazolidine OPB-9195 [163107-50-4], and pyridoxamine [85-87-0].

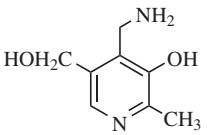
benfotiamine



OPB-9195



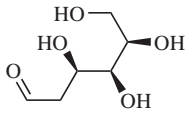
pyridoxamine



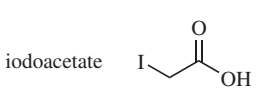
Benfotiamine has been shown to block three of the major biochemical pathways implicated in the pathogenesis of hyperglycemia induced vascular damage (the hexoseamine pathway, the AGE formation pathway and the diacylglycerol–protein kinase C pathway) and to prevent experimental diabetic retinopathy in rats (9). The compound OPB-9195 has been reported to reduce blood pressure and oxidative damage in the genetic hypertensive rat (10) and to be beneficial for the reduction of serum AGE and prevention of diabetic neuropathy (11). Pyridoxamine likewise is able to inhibit the development of retinopathy in experimental diabetes (12) and inhibits the formation of both AGEs and advanced lipoxidation end–products (ALEs) (13). Data suggests that the AGE/ALE inhibitory activity and therapeutic effect of pyridoxamine depend, at least in part, on its ability to trap reactive carbonyl intermediates that are formed during autooxidation of carbohydrates and peroxidation of lipids that lead to AGE/ALE formation, thereby inhibiting chemical modification of tissue proteins (14).

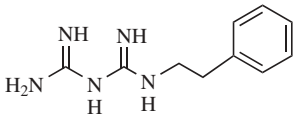
1.2. Caloric Restriction. Scientists first recognized >60 years ago the value of a low-calorie yet nutritionally balanced diet not only in prolonging the mean and maximum life span in a variety of animal species compared to

free-feeding animals, but also in reducing the incidence of age-related conditions (15). This regimen of reduced caloric intake has produced the most impressive results when the regimen was begun early in the life of the animal and involved a reduction of ~30–50% in total calories. Although caloric restriction might extend the longevity of humans because it does so in many other animal species, there is no study in humans that has proven that it will work, although there are long-term nonhuman primate studies currently being conducted (16–18). Indeed, a calorically restricted diet, to be effective, must approach levels that most people would find intolerable. For this reason, the search for pharmacological mimetics of caloric restriction is the subject of many investigations. In this regard, there is considerable current interest in the search for the biological mechanisms underlying the observed retardation of aging and diseases by caloric restriction since identification of such should help in the discovery of suitable mimetics. Two areas receiving considerable attention are oxidative stress and regulation of glucose metabolism (19). These mechanisms are not mutually exclusive. For example, AGEs may be formed by oxidative and nonoxidative reactions (2). Caloric restriction, resulting in lower levels of blood glucose and insulin over long periods of time, may be the best prevention of AGE formation since hyperglycemia is the major cause of AGEs. Agents that can effectively lower the levels of circulating glucose and insulin or increase insulin sensitivity are under investigation. One of the most extensively studied agents as a caloric restriction mimetic is 2-deoxy-D-glucose [154-17-6] (2DG) (15).



2DG works by interfering with the way cells process glucose. Cells use glucose from the diet to generate ATP. By limiting food intake, caloric restriction minimizes the amount of glucose entering cells and decreases ATP generation. When 2DG is administered to animals that eat normally, glucose reaches cells in abundance, but the agent prevents most of the glucose from being processed and thus reduces ATP synthesis. Studies in animals have shown that although 2DG can mimic many of the effects seen in caloric restriction, the range between biologically effective dose and toxicity for 2DG is very narrow, which precludes its use in humans (20). Two additional agents that have shown some promise as potential mimetics are iodoacetate [64-69-7], an inhibitor of glyceraldehydes-3-phosphate dehydrogenase, and phenformin [114-86-3], a down-regulator of *N*-methyl-D-aspartate receptor expression (21). However, studies are still preliminary for these agents.

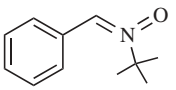




1.3. Reactive Oxygen Species. Recent work supports the notion that free radicals such as reactive oxygen species (ROS) play a central role in both the

formation of AGEs and in AGE-induced pathological alterations in gene expression (22). In general, the free-radical theory of aging postulates that free radical reactions are responsible for the progressive accumulation of changes with time resulting in the ever-increasing likelihood of disease and death that accompanies advancing age (23). At the same time, free radicals play an important role in normal physiological processes (eg, the immune response and cell communication). At present, there is relatively little evidence from human studies that supplements containing antioxidants lead to a reduction in either the risk of age-related conditions or the rate of aging (1).

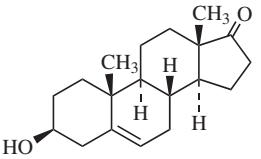
The role of oxidative stress of mitochondrial origin in aging is being studied extensively. Many studies have shown that aging is associated with an increase in oxidative damage to cellular macromolecules probably arising from the electron transport system as a normal consequence of energy metabolism (24). Likewise, there is much interest in the possibility that caloric restriction may act to retard aging by lowering oxidative stress–damage (25). It is possible, as preliminary data suggests, that α-phenyl-*tert*-butyl nitrone [3376-24-7] and other nitrone-based free-radical trapping agents (also known as spin-traps), as well as other antioxidants, will act as caloric restriction mimetics to prolong both average and maximum life span (19).



1.4. Telomeres. Telomeres, the repeated hexameric sequence of nucleotides TTAGGG at the ends of chromosomes, shortens in many normal human cells with increased cell divisions. Thus, normal cells undergo a finite number of cell divisions and ultimately enter a nondividing state called replicative senescence. Solid scientific evidence has shown that telomere length plays a role in determining cellular life span in several normal human cell types (26,27). It was found that telomeres are synthesized de novo by the terminal transferase enzyme telomerase (28) and this is the only known reverse transcriptase that is necessary for normal cell activity (29). Telomerase has been found to occur in extracts of most immortal cell lines and ∼ 90% of all human tumors studied, unlike normal cultured cell strains (30). Moreover, the level of telomerase activity found in normal cell populations is, per cell, significantly less than that found in cancer cells (30). In 1998 it was reported that normal, human cell strains could be immortalized with apparent retention of their normal properties by transfecting them with vectors encoding the human telomerase catalytic subunit, providing direct evidence for the role of telomerase shortening in cell senescence and telomerase expression in cell immortality (31). However, increasing the number of times a cell can divide may predispose cells to tumor formation (32,33).

1.5. Hormones. A number of hormones, most notably human growth hormone, have been shown to improve some of the physiological changes associated with human aging. A study in older men showed that administration of growth hormone resulted in an increase in lean body mass and a decrease in adipose mass, but there was no assessment of muscle strength or quality of life (34). A more recent study, however, showed that administration of physiological doses

of human growth hormone administration to healthy older men for 6 months also resulted in the same observations of body mass composition improvement, but functional ability did not improve and side effects occurred frequently (35). The steroid hormone dehydro-3-epiandrosterone [53-43-0] (DHEA) is normally synthesized in large quantities by the adrenal gland but serum concentrations decline with advancing age (36).



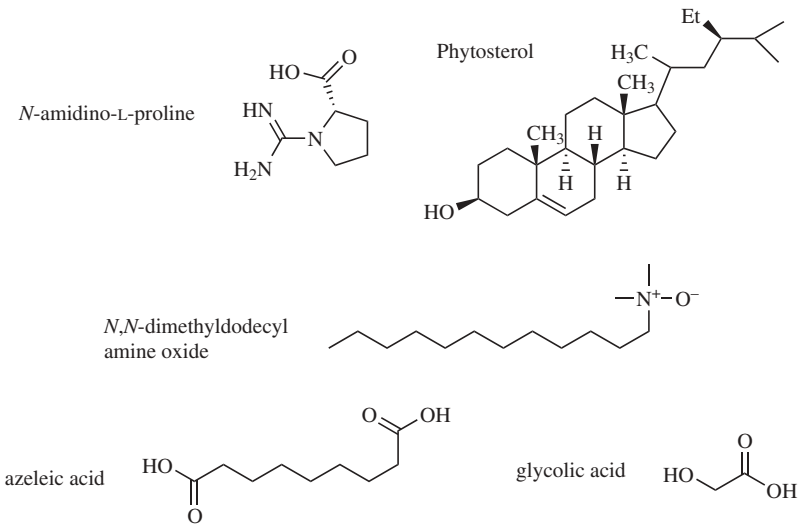
DHEA supplementation in elderly people has been advertised as an antiaging medication. The DHEA might be useful for improving psychological well being in the elderly, reducing disease activity in people with mild to moderate systemic lupus erythematosus, improving mood in the clinically depressed and improving various parameters in women with adrenal insufficiency (36). However, subjects with a physiological, age-related decline in DHEA secretion show little benefit from DHEA administration (37). Although many other claims have been made for DHEA in diverse conditions, such as aging, dementia, and AIDS, no well-designed clinical trials have clearly substantiated the utility and safety of long-term DHEA supplementation and there is currently not enough evidence to recommend it in advanced age.

2. Organ Systems

In connection with the general considerations discussed above regarding age-related changes and diseases, there are also organ-specific considerations of note. Following are discussions of agents that have been studied in specific organ systems as they relate to prevention and treatment of age-related changes and diseases.

2.1. Skin and Hair. Skin aging, which includes photoaging and intrinsic aging, causes the formation of wrinkles and sagging. Dermal matrix components change qualitatively and quantitatively over time in aging skin (38). In addition, basic cell functions such as proliferation, mitochondrial respiration, and production of matrix components in dermal fibroblasts are reduced with aging. Thus, an effective antiaging agent for skin should provide acceleration of mitochondrial activity, enhanced cell proliferation, increased matrix component synthesis and improvement of collagen bundle fiber (38). Many cosmetic formulations have been marketed, with claims of preventing or reducing wrinkles and lines, containing a variety of agents. For example, *N*-amidino-L-proline [35404-57-0] is claimed to improve skin elasticity (39), phytosterol [83-46-5] for enhancing collagen I in human fibroblast cells (40), *N,N*-dimethyldodecyl amine oxide [1643-20-5] containing composition for the activation of corneum protease activity resulting in higher turnover and repair rates of the stratum corneum (41), and azelaic acid [123-99-9] for inhibition of collagenase activity (42). The effects of

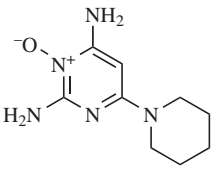
glycolic acid [79-14-1] on metabolic activity of human fibroblasts was also studied and results showed that cell growth was stimulated, dermal thickness was enhanced, and wrinkle-depth was repaired (43).



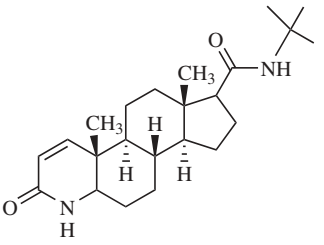
Skin wrinkles can also be the result of repetitive muscle activity. Clostridium botulinum toxin (Botox) type A [93384-43-1] has been widely used aesthetically for the past 15 years for facial skin rejuvenation (44). Botox works by clinically paralyzing the facial muscles underlying the lines and wrinkles on the surface with restoration of muscle activity usually commencing between 3 and 4 months after injection (45). A review of the correct use and complications of Botox has appeared (46).

Hair loss (androgenetic alopecia) occurs in men and women, and is characterized by the loss of hair from the scalp in a defined pattern (47). In men it is often referred to as male-pattern baldness and affects up to 80% of men by age 80 (48). The involvement of androgens in androgenetic alopecia has been established for some time, and is well accepted. Eunuchs, who lack androgens, do not bald (49). Individuals who lack a functional androgen receptor are androgen insensitive and develop as females; again, these individuals do not bald (50). Likewise, no baldness is seen in individuals who lack 5α-reductase, the enzyme that converts testosterone to the potent androgen dihydrotestosterone (DHT) (51). The exact mechanism(s) through which androgens act to cause baldness remain unclear; however, given that the complex formed between the androgen receptor (AR) and androgen acts as a transcription factor, it is likely that genes controlling hair follicle cycling are regulated by androgen (47). Without treatment, androgenetic alopecia is a progressive condition. Apart from various camouflage and surgical options, currently only two pharmaceutical agents are approved for the treatment of androgenetic alopecia in males: topical minoxidil [38304-91-5] and oral finasteride [98319-26-7].

minoxidil



finasteride

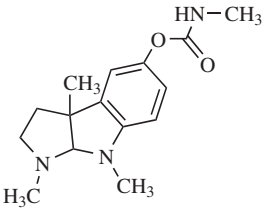


Minoxidil is a vasodilator originally used to treat high blood pressure but a topical formulation was developed when patients treated with the drug showed increased hair growth (52). Finasteride is a synthetic azo-steroid and is a highly selective and potent 5α-reductase type-2 inhibitor (53), thus lowering DHT levels in scalp and serum by >60% at a daily dose of only 1 mg (54). Future potential therapies may include the use of androgen-receptor blockers, but only in scalp follicles due to the potential risks of gynaecomastia, feminisation and impotence if administered systemically (47).

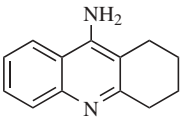
2.2. Central Nervous System. As the world population is aging, there are increasing incidences of dementia reported worldwide, with Alzheimer's disease (AD) being the most common form of dementia in the elderly. It is characterized by a gradual decline in three domains: cognition, behavior, and function (55). Ideally, an effective treatment would target all three types of impairment. However, available treatments for AD diminish only certain symptoms and cannot halt the dementing process. As scientists uncover the pathogenic mechanisms of AD, additional treatments will likely emerge. The AGE accumulation (discussed above) in the CNS, eg, may be related to the aging process and the degenerating process of AD neurons (56). New therapies currently being developed include therapeutic agents that target amyloid β peptide and downstream pathological changes (57). Inhibition of the formation of amyloid peptide from its precursor protein is an attractive target for blocking the cascade process leading to the development of neurodegenerative disease. Bafilomycin A [116764-51-3], eg, and its analogues are of interest, since they very effectively and selectively block the formation of amyloid peptide by an indirect inhibition of β-secretase activity (58). Other strategies in the search for new therapeutic approaches include: agents that compensate for the lowered activity of the cholinergic system, agents that protect nerve cells from the toxic metabolites formed in neurodegenerative processes, agents that affect the process of formation of neurofibrillary tangles, and antiinflammatory agents that prevent the negative response of nerve cells to the pathological process (59). Currently, there are only a few therapeutic agents on the market for the treatment of AD. The main pharmacological effect of most of the agents is to improve the cognitive functions decreased in AD due to hypofunction of the cholinergic transmitter system (59). The cholinesterase inhibitors are the first and most developed group of drugs for AD treatment whose main mechanism of action is thought to be increased activation of cholinergic receptors (which is decreased in AD pathology) due to an increase of both concentration and duration of action of the neurotransmitter acetylcholine. First generation cholinesterase inhibitors include physostigmine [57-47-6], tacrine [321-64-2], and amiridine [90043-86-0], which

show low selectivity for this enzyme, also inhibiting another enzyme in this group, butyrylcholinesterase (60). These compounds have significant side effects, such as sedation and hepatotoxicity, which limit their use (61).

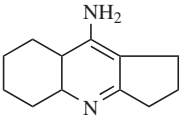
physostigmine



tacrine



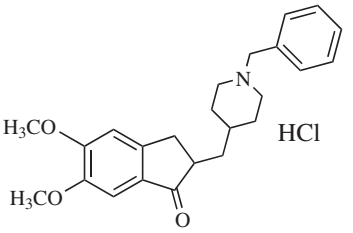
amiridine



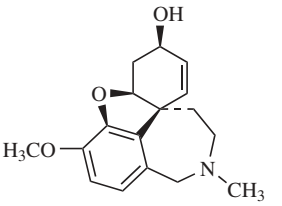
HCl

Newer inhibitors are more selective and include Aricept [120011-70-3] (62), Galanthamine [357-70-0] (63), and Eptastigmine [101246-68-8] (64). One drug, Rivastigmine [123441-03-2], successfully targets acetylcholinesterase in the brain as opposed to peripheral forms (65). These second generation inhibitors show considerably fewer side effects than their first generation predecessors.

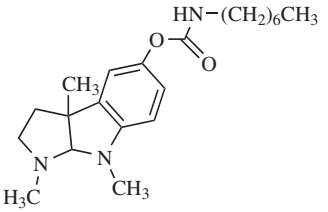
aricept



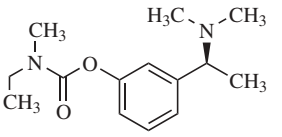
galanthamine



eptastigmine



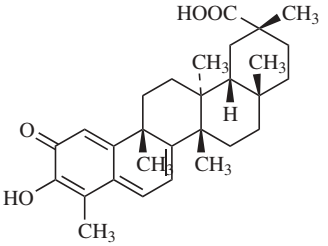
rivastigmine



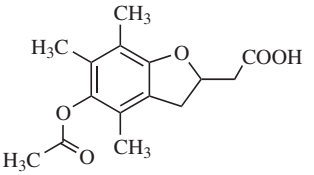
The close correlation between AD and the accumulation of reactive oxygen species (ROS) in CNS cells has been known for a long time. The role of ROS in AD has been thoroughly reviewed (66). The oxidative stress theory as it relates to AD involves homeostasis of intracellular calcium as well as the aggregation of protein, in this case, of amyloid peptide fibril (67). While nerve cells have an endogenous system that protects them from excessive ROS levels, an alternative approach to block the effects of ROS is the application of "external" antioxidants and/or the stimulation of the intracellular (endogenous) antioxidant systems.

Examples of such external antioxidants are the herbal triterpene Celastrol [34157-83-0] (68) and the vitamin E analog Raxofelast [128232-14-4] (69).

celastrol

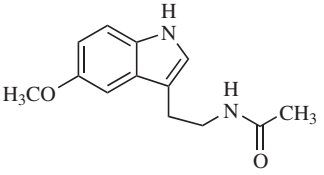


raxofelast

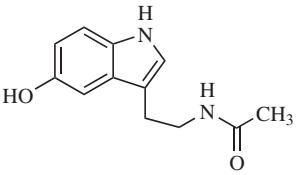


One of the promising approaches in the development of preventive therapies of AD is a design of agents based on derivatives and analogs of melatonin [73-31-4]. Melatonin is an endogenous hormone that has been shown to be effective against oxidative stress in the CNS (70). It has been shown in cell culture experiments and in animals that melatonin has a complex neuroprotective effect that includes both a specific antiamyloid component and a nonspecific gerantoprotective effect because of its strong radical-blocking properties. These protective functions together with the fact that melatonin levels decrease with age and in AD, suggest that this endogenous bioregulator has an important preventive function against the pathogenesis of AD. Melatonin is registered in the United States and most European countries as a food additive or supplement. A precursor of melatonin, *N*-acetylserotonin [1210-83-9], is a more effective agent than melatonin due to its superior properties of radical-scavenging ability (71), inhibition of lipid peroxidation (72), and antiamyloid activity (73). Dimebon [3613-73-8], a structurally rigid analogue of melatonin, is currently under development as a new therapeutic agent for the treatment of AD (74).

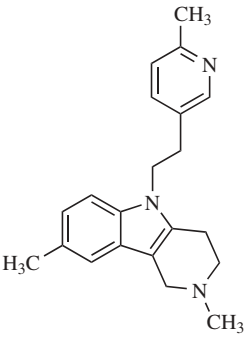
melatonin



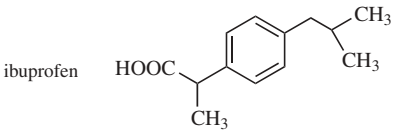
N-acetylserotonin

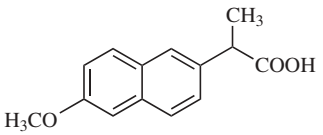


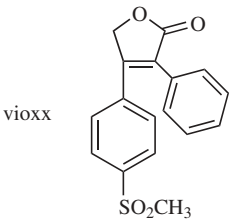
dimebon



Antiinflammatory agents are predicted to be of use in AD therapy because neurodegenerative changes in the AD brain are accompanied by inflammatory reactions of the CNS. Nonsteroidal antiinflammatory agents (NSAIDS) have been shown to decrease the risk of developing AD in epidemiological studies (75). Ibuprofen [15687-27-1] was the first in the series of NSAIDS to be suggested for AD therapy (76) and the activity of these types of compounds is thought to be due primarily to the nonspecific inhibition of the cyclooxygenases (COXs). Other promising NSAIDS being studied in AD are Naproxen [22204-53-1] and Rofecoxib (Vioxx) [162011-90-7] (77).



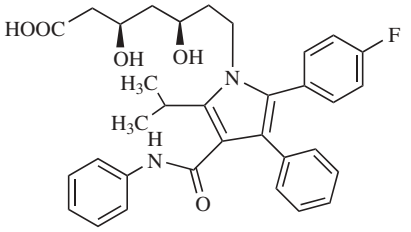




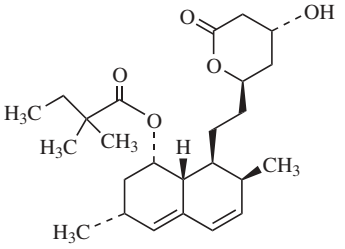
Finally, since amyloid-β peptide plays a key role in the pathogenesis of AD, the possibility of using immunotherapeutic approaches against this peptide have been investigated. Immunization with amyloid peptide or with antibodies to the peptide cleared or prevented amyloid peptide-containing plaque deposits in the brains of transgenic AD mouse models (78). Recently, a clinical trial of a vaccine consisting of a 42-amino acid form of amyloid peptide (called AN-1792 [401586-29-6]) in 360 patients with mild to moderate AD was halted after 15 patients developed meningoencephalitis (79). A neuropathological examination of the brain of a patient that died in the trial showed intriguing evidence of an effective immune response against amyloid peptide with virtually total clearance of amyloid peptide deposits from much of the cerebral cortex (80). These data suggest a powerful effect of the vaccination and provide the strongest evidence to date that an induced immune response can affect amyloid peptide pathology in human AD. However, these data do not prove the effectiveness of the vaccine against AD. It is still unknown whether symptoms improve after clearance of amyloid peptide and cognitive testing data acquired during the trial is still unavailable. There are many aspects of AD vaccination immunotherapy suggesting that this field is not yet prepared to move forward with this therapeutic approach in humans. It has been proposed that, although inflammation may be a component of AD vaccination therapy, it is short-lived phenomena and potentially integral to the eventual benefit of vaccination treatment. Although the experimental and Phase I clinical vaccination immunotherapy studies of AN-1792 were successful, there is still a need for a greater understanding of the inflammatory consequences of autoimmunization in both mice and eventually in humans (81).

2.3. Cardiovascular System. There is a large body of evidence that biological aging is related to a series of long-term catabolic processes resulting in decreased function and structural integrity of several physiological systems, among which is the cardiovascular system (82). Some of the cardiovascular deficits that accompany aging in health can be retarded by physical conditioning. Likewise, growth hormone (discussed above) has been found to exert potent effects on cardiovascular function in young animals and reverses many of the deficits in cardiovascular function in aged animals and humans (82). There is also the questionable role of AGEs in cardiovascular disease and aging as discussed above. The formation of AGEs on vascular wall and myocardial collagen causes cross linking of collagen molecules leading to loss of collagen elasticity, and subsequently to a reduction in arterial and myocardial compliance. Aminoguanidine, an inhibitor of AGE formation discussed above, is effective in slowing or preventing arterial stiffening and myocardial diastolic dysfunction in aging and diabetic animals. In aged and diabetic animals, agents that can chemically break pre-existing crosslinking of collagen molecules are capable of reverting indexes of vascular and myocardial compliance to levels seen in younger or nondiabetic animals (5). These studies suggest that collagen crosslinking is a major mechanism that governs aging and diabetes-associated loss of vascular and cardiac compliance. The development of AGEs cross-link breakers may have an important role for future therapy of isolated systolic hypertension and diastolic heart failure in these conditions. Aging of the population will undoubtedly result in a concomitant increase in the incidence of the most common chronic cardiovascular diseases, including coronary artery disease, heart failure, myocardial infarction, and stroke (83). These diseases are direct consequences of atherosclerosis (AS), a multifactorial process that is described as both an autoimmune and inflammatory condition (84). The immune system plays a major role in the development and progression of AS involving macrophages and activated lymphocytes. Once AS is regarded as an autoimmune and inflammatory condition, aims for its prevention and treatment should be focused not only on control of traditional risk factors for cardiovascular disease, but on immune modulation of the process as well (84). In this regard, new guidelines from the Adult Treatment Panel III (ATP III) of the National Cholesterol Education Program recommend blood lipid management beyond low density lipoprotein (LDL) lowering, including aggressive treatment of elevated triglycerides since recent studies show that elevated triglycerides significantly increase cardiovascular disease risk (85). High density cholesterol (HDL), on the other hand, appears to play a protective role against development of AS by several mechanisms, including "reverse cholesterol transport", inhibition of oxidation or aggregation of LDL, and modulation of inflammatory responses to favor vasoprotection (86,87). Thus, raising HDL while lowering LDL levels would be beneficial for prevention and treatment of AS. The beneficial effects of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase inhibitors (statins) in the treatment and prevention of cardiovascular disease have generally been attributed to their ability to lower cholesterol biosynthesis. The three most studied and widely used statins include atorvastatin [134523-03-8], simvastatin [79902-63-9], and pravastatin [81093-37-0].

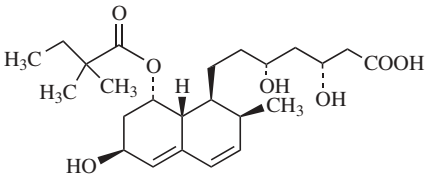
atorvastatin



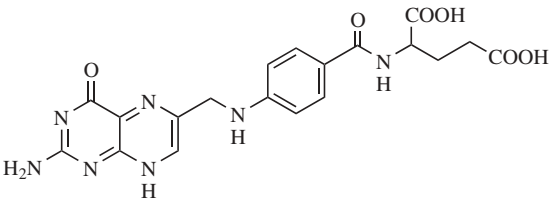
simvastatin



pravastatin



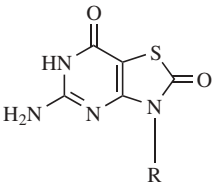
Besides their cholesterol-lowering properties, these statins show similar beneficial effects that include modification of thrombus formation and degradation, antiinflammatory response, plaque stabilization, and improved endothelial function (88). The statins have proven to be antiinflammatory by virtue of their effects on leukocyte adhesion and migration to sites of inflammation (89). Moreover, statins have been shown to lower C-reactive protein, a marker of systemic inflammation (90). This observation is significant because a growing body of evidence indicates that inflammation plays a substantial role in plaque progression and rupture and C-reactice protein appears to be a better biomarker than cholesterol levels for predicting cardiovascular risk in healthy persons as well as in persons with established cardiovascular disease (90). Another biomarker that has been shown in epidemiological studies to be an independent risk factor for AS, thrombosis, and hypertension is elevated homocysteine (91). Statins do not influence homocysteine plasma levels (92) but supplementation with B vitamins, and especially folic acid [75708-92-8], has been shown to be beneficial for treating hyperhomocysteinemia (93).



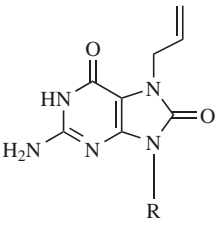
Finally, diet and exercise are well known to prevent or decrease the risk of cardiovascular diseases of all kinds. In addition to preventing obesity, which is a major risk factor for cardiovascular disease, physical activity slows down the age-associated loss of cardiopulmonary efficiency and has been shown to help prevent illness in old age (94). A diet that includes the "good" fats is also beneficial. Epidemiological studies over the past 40 years suggest that omega-3 fatty acids derived from fish and fish oil decrease the risk of coronary heart disease, hypertension and stroke, and their complications (95). Moreover, current evidence suggests that individuals with existing coronary artery disease may reduce their risk of sudden cardiac death by increasing their intake of long-chain omega-3 fatty acids by ~1 g/day (96).

2.4. Immune System. The functional capacity of the immune system gradually declines with age (97). The T lymphocytes are more severely affected than the B lymphocytes or antigen-presenting cells. The age-related alterations that occur in the immune system may be referred to as immunosenescence and involve both the innate and the adaptive immune responses. These alterations account for the increased susceptibility to certain microbial infections, autoimmune diseases, or malignancies in the elderly and contribute to increased morbidity and mortality with age. Hence, the restoration of immunological function is expected to have a beneficial effect in reducing pathology and maintaining a healthy condition in advanced age. A few intervention strategies are discussed here briefly. In animal studies, caloric restriction (discussed above) has been shown to be the most powerful modulator of the aging process (98) including its action on the immune system. Animal and human studies show that DHEA (discussed above) treatment results in stimulation of immune responses probably by induction of interleukin 2 (IL-2) production by T cells (99). Certain substituted guanosines, such as 7-thia-8-oxoguanosine [122970-40-5], 7-deazaguanosine [62160-23-0], and loxoribine [121288-39-9], activate the innate immune system through Toll-like receptor 7 in mouse and human cells (100).

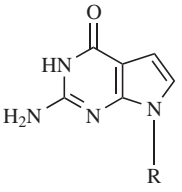
7-thia-8-oxoguanosine



loxoribine



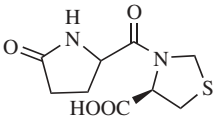
7-deazaguanosine



R = D-ribofuranose

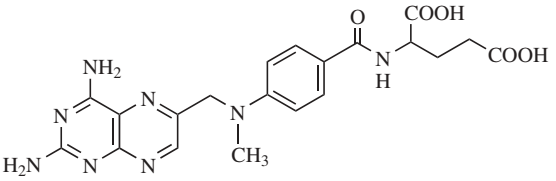
A synthetic thymic dipeptide, pidotimod [121808-62-6], stimulated the production of IL-2 in peripheral blood lymphocytes and splenocytes from old but not young rats (101). This has implications for restoration of immune function in the elderly.

pidotimod



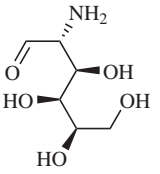
2.5. Musculoskeletal System. Several age-related conditions affecting the bones and joints are observed in both men and women. These include, among others, arthritis, both osteoarthritis (OA) and rheumatoid arthritis (RA), and bone loss including osteoporosis and fractures. A substantial part of the age-related decline in functional capabilities of the musculoskeletal system is not due to aging per se but to decreased and insufficient physical activity (102). Therefore, musculoskeletal disease prevention in the elderly must include physical exercise. Arthritis affects a large segment of the older population and is the leading cause of disability in the general population. Approximately 3% of the U.S. population suffers from RA (103) and RA is a systemic disease characterized by a chronic inflammatory reaction in the joint synovium, degeneration of cartilage, and erosion of adjacent joint bone. Many proinflammatory cytokines are expressed in diseased joints. One of the most effective agents for treatment of rheumatoid arthritis is etanercept (Enbrel [185243-69-0]) which is a biological disease-modifying antirheumatic drug (DMARD) that works by blocking the proinflammatory cytokine tumor necrosis factor-alpha (TNF-α) (104). Etanercept is a soluble TNF receptor fusion protein and is more effective than methotrexate [59-05-2], another DMARD and anticancer agent, at reducing the number of new erosions and joint-space narrowing in patients with active early rheumatoid arthritis (105).

methotrexate



Another biological that blocks TNF-α is infliximab (Remicade [170277-31-3]). Infliximab is a monoclonal antibody to TNF and is used effectively in Crohn's disease as well as RA (106). Combinations of methotrexate and these anti-TNF biologicals appear to be particularly effective in reducing the signs and symptoms of RA and, most importantly, in protecting joints against progressive structural damage (107). There is some evidence that omega-3 fatty acids in the diet can lessen the severity of rheumatoid arthritis as well as reduce bone loss in postmenopausal women (108). Whether or not a diet that includes omega-3 fatty acids is useful in prevention of RA or bone loss is not yet known. Glucosamine [3416-24-8], or its sulfate [29031-19-4], has been widely used to treat

osteoarthritis in humans and is thought to work by suppressing neutrophil function and activation (109).

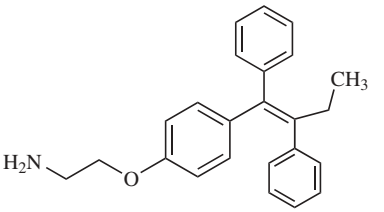


In addition, glucosamine also has been shown to inhibit inducible nitric oxide production via inhibition of inducible nitric oxide synthase expression (110) and thereby shows anti-inflammatory activity.

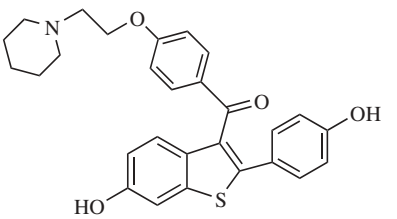
3. Cancer Prevention

Cancer is primarily a disease of aging even though there are many examples of cancer afflicting young individuals. For example, age is clearly the single most important risk factor for development of prostate cancer in men and breast cancer in women (111). Management options for women at high risk for breast cancer include close surveillance, chemoprevention, and prophylactic mastectomy. The optimal method remains to be determined. Chemoprevention refers to the use of specific natural or synthetic chemical agents to reverse, suppress, or prevent the progression to invasive cancer (112). The ideal chemopreventive agent is safe and nontoxic over the long term. Prevention of breast cancer is still under clinical investigation with only one drug, tamoxifen [10540-29-1], showing benefit in high risk patients (113). Tamoxifen is a nonsteroidal antiestrogen originally developed in 1996 as a contraceptive. Raloxifene [84449-90-1] is another nonsteroidal antiestrogen that is also being studied for potential chemoprevention of breast cancer.

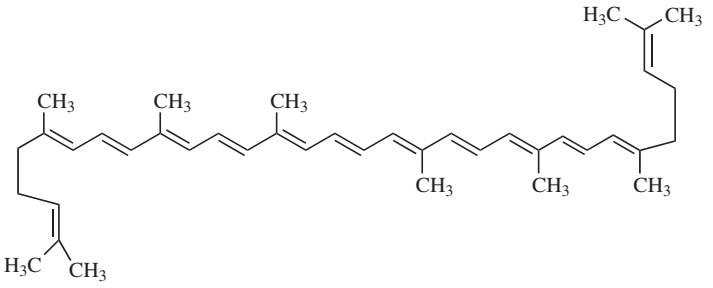
tamoxifen



raloxifene



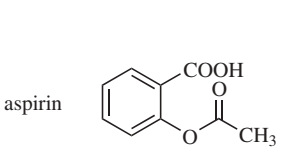
Both tamoxifen and raloxifene maintain bone density and raloxifene is now used to prevent osteoporosis and is also being tested as a preventive for coronary heart disease (114). Prostate cancer is the second leading cause of cancer death in the United States, largely because of the limitations of our current therapeutic options, especially once the cancer has metastasized (115). It is well established that the prostate is hormonally influenced. There is evidence suggesting that androgenic influences over a period of time encourages the process of prostate carcinogenesis (116). Moreover, early prostate tumors are often androgen dependent but androgen insensitive tumors inevitably develop that then have a very poor prognosis. This fact underscores the need for prevention strategies such as chemoprevention. Antiandrogens are among the promising chemopreventive agents for prostate cancer since prostate epithelium is androgen dependent. Studies of prostate biology support the concept that dihydrotestosterone (DHT) is the principal androgen responsible for normal and hyperplastic growth of the prostate gland, and therefore, inhibitors of DHT formation should be useful as chemopreventive agents for prostate cancer (117). As discussed above, 5α-reductase inhibitors, such as finasteride, are useful for inhibiting the formation of DHT. A large, randomized clinical chemoprevention trial supported by the National Cancer Institute is being conducted to test the efficacy of finasteride for prevention of prostate cancer incidence (118). Other trials that are ongoing include micronutients and phytochemicals such as lycopene [502-65-8], soy isoflavones, and vitamin E and selenium [7782-49-2], alone or in combination (118).

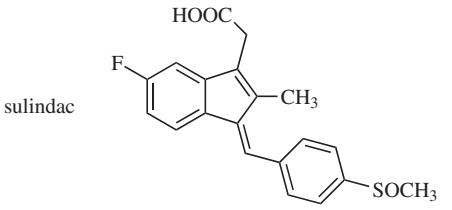


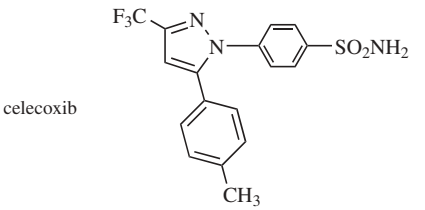
Lycopene is a carotenoid derived largely from tomato-based products. Recent epidemiological studies have suggested a potential benefit of this natural product against the risk of prostate cancer, especially the more advanced and aggressive form (119). In some studies, risk appeared to be lowered by 30–40% with high tomato or lycopene consumption. However, results are difficult to interpret until randomized controlled dietary intervention studies can be conducted (120).

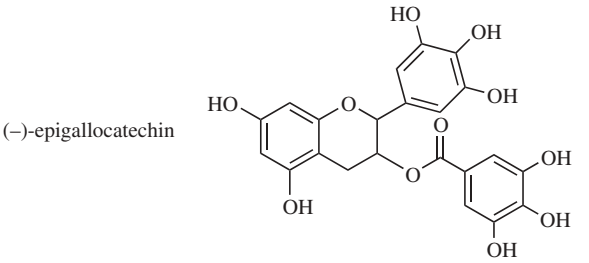
An impressive body of epidemiological data suggests an inverse relationship between colorectal cancer risk and regular use of nonsteroidal antiinflammatory drugs (NSAID), including aspirin [50-78-2] (121). Clinical trials with NSAIDs have demonstrated that NSAID treatment caused regression of preexisting colon adenomas in patients with familial adenomatous polyposis. In addition, several phytochemicals with antiinflammatory activity and NSAIDs act to retard, block or reverse colon carcinogenesis (121). Thus, these agents exert tumor-suppressive activity on premalignant lesions (polyps) in humans and on

established experimental tumors in mice. Trials using the NSAID sulindac [38194-50-2] reduced the number of polyps in patients with familial adenomatous polyposis (122). Some of the tumor-suppressive effects of NSAIDs depend on the inhibition of cyclooxygenase-2 (COX-2), a key enzyme in the synthesis of prostaglandins and thromboxane, which is highly expressed in inflammation and cancer (123). The selective COX-2 inhibitor celecoxib [169590-42-5] has been approved by the FDA for adjuvant treatment of familial adenomatous polyposis, and a large number of prevention and treatment trials of colorectal, prostate and breast cancer have been started (122). Green tea and (−)-epigallocatechin [989-51-5] are now acknowledged cancer preventives in Japan and there is new evidence that green tea and sulindac, in combination, have synergistic cancer preventive effects (124).









4. Applications

While there is a large number of products currently being sold by antiaging entrepreneurs who claim that it is now possible to slow, stop, or even reverse human aging, they have no scientifically demonstrated efficacy (1). Most biogerantologists believe that our rapidly expanding scientific knowledge holds the promise that methods may eventually be discovered to slow the rate of aging. If

successful, these interventions are likely to postpone age-related diseases and disorders and extend the period of healthy life. Because aging is the greatest risk factor for the leading causes of death and other age-related pathologies, successful efforts to slow the rate of aging would certainly have dramatic health benefits for the population, by far exceeding the anticipated changes in the health and length of life that would result from complete elimination of heart disease, cancer, stroke, and other age-associated diseases and disorders (1).

BIBLIOGRAPHY

"Antiaging Agents" in *ECT* 4th ed., Vol. 2, pp. 815–829, by W. B. Jolley, ICN Pharmaceuticals, Inc., and H. B. Cottam, University of California, San Diego; "Antiaging Agents" in *ECT* (online), posting date: December 4, 2000, by W. B. Jolley, ICN Pharmaceuticals, Inc., and H. B. Cottam, University of California, San Diego.

CITED PUBLICATIONS

1. S. J. Olshansky, L. Hayflick, and A. Carnes Bruce, *J. Gerontol. Ser. A, Biol. Sci. Med. Sci.* **57**, B292 (2002).
2. J. W. Baynes, *Exp. Gerontol.* **36**, 1527 (2001).
3. R. Singh, A. Barden, T. Mori and L. Beilin, *Diabetologia* **44**, 129 (2001).
4. S. Rahbar, and J. L. Figarola, *Curr. Med. Chem.: Immunol., Endocrine Metabolic Agents* **2**, 135 (2002).
5. D. Aronson, *J. Hypertension* **21**, 3 (2003).
6. P. C. Ulrich and D. R. Wagle, (1997) in PCT Int. Appl. (Alteon Inc., USA). Wo, pp. 40.
7. T. S. Kern and R. L. Engerman, *Diabetes* **50**, 1636 (2001).
8. Y. Du, M. A. Smith, C. M. Miller, and T. S. Kern, *J. Neurochem.* **80**, 771 (2002).
9. H.-P. Hammes, X. Du, D. Edelstein, T. Taguchi, T. Matsumura, Q. Ju, J. Lin, A. Bierhaus, P. Nawroth, D. Hannak, M. Neumaier, R. Bergfeld, I. Giardino, and M. Brownlee, *Nature Med. (New York, NY, United States)* **9**, 294 (2003).
10. K.-I. Mizutani, K. Ikeda, K. Tsuda, and Y. Yamori, *J. Hypertension* **20**, 1607 (2002).
11. R. Wada, Y. Nishizawa, N. Yagihashi, M. Takeuchi, Y. Ishikawa, K. Yasumura, M. Nakano, and S. Yagihashi, *Eur. J. Clin. Inv.* **31**, 513 (2001).
12. A. Stitt, T. A. Gardiner, N. L. Anderson, P. Canning, N. Frizzell, N. Duffy, C. Boyle, A. S. Januszewski, M. Chachich, J. W. Baynes, and S. R. Thorpe, *Diabetes* **51**, 2826 (2002).
13. J. M. Onorato, A. J. Jenkins, S. R. Thorpe, and J. W. Baynes, *J. Biol. Chem.* **275**, 21177 (2000).
14. P. A. Voziyan, T. O. Metz, J. W. Baynes, and B. G. Hudson, *J. Biol. Chem.* **277**, 3397 (2002).
15. M. A. Lane, D. K. Ingram, and G. S. Roth, *Sci. Am.* **287**, 36 (2002).
16. D. K. Ingram, R. G. Cutler, R. Weindruch, D. M. Renquist, J. J. Knapka, M. April, C. T. Belcher, M. A. Clark, C. D. Hatcherson, and B. M. Marriott, *J. Gerontol.* **45**, B148 (1990).
17. J. Wanagat and R. Weindruch, *Sci. Geriatrics (2nd Ed.)* **1**, 153 (2001).
18. J. J. Ramsey, R. J. Colman, N. C. Binkley, J. D. Christensen, T. A. Gresl, J. W. Kemnitz, and R. Weindruch, *Exper. Gerontol.* **35**, 1131 (2000).

19. R. Weindruch, K. P. Keenan, J. M. Carney, G. Fernandes, R. J. Feuers, R. A. Floyd, J. B. Halter, J. J. Ramsey, A. Richardson, G. S. Roth, and S. R. Spindler, *J. Gerontol., Ser. A: Biol. Sci. Med. Sci.* **56A**, 20 (2001).
20. A. Lane Mark, J. Mattison, K. Ingram Donald, and S. Roth George, *Microsc. Res. Tech.* **59**, 335 (2002).
21. M. P. Mattson, W. Duan, and Z. Guo, *J. Neurochem.* **84**, 417 (2003).
22. M. Brownlee, *Metabolism, Clin. Exper.* **49**, 9 (2000).
23. D. Harman, *Intern. Cong. Ser.* **782**, 3 (1988).
24. K. B. Beckman and B. N. Ames, *Physiol. Rev.* **78**, 547 (1998).
25. R. S. Sohal and R. Weindruch, *Science* **273**, 59 (1996).
26. C. B. Harley, A. B. Futcher, and C. W. Greider, *Nature (London)* **345**, 458 (1990).
27. R. C. Allsopp, H. Vaziri, C. Patterson, S. Goldstein, E. V. Younglai, A. B. Futcher, C. W. Greider, and C. B. Harley, *Proce. Natl. Acad. Sci. U. S. Am.* **89**, 10114 (1992).
28. G. B. Morin, *CELL* **59**, 521 (1989).
29. L. Hayflick, *Br. J. Cancer* **83**, 841 (2000).
30. C. P. Chiu and C. B. Harley, *Proc. Soc. Biol. Med.* **214**, 99 (1997).
31. A. G. Bodnar, M. Ouellette, M. Frolkis, S. E. Holt, C. P. Chiu, G. B. Morin, C. B. Harley, J. W. Shay, S. Lichtsteiner, and W. E. Wright, *Science* **279**, 349 (1998).
32. T. de Lange and T. Jacks, *Cell* **98**, 273 (1999).
33. J. Wang, G. J. Hannon and D. H. Beach, *Nature (London)* **405**, 755 (2000).
34. D. Rudman, A. G. Feller, H. S. Nagraj, G. A. Gergans, P. Y. Lalitha, A. F. Goldberg, R. A. Schlenker, L. Cohn, I. W. Rudman, and D. E. Mattson, *New England J. Med.* **323**, 1 (1990).
35. M. A. Papadakis, D. Grady, D. Black, M. J. Tierney, G. A. Gooding, M. Schambelan, and C. Grunfeld, *Ann. Inter. Med.* **124**, 708 (1996).
36. M. D. Johnson, R. A. Bebb and S. M. Sirrs, *Ageing Res. Rev.* **1**, 29 (2002).
37. B. Allolio and W. Arlt, *Trends Endocrinol. Metabolism* **13**, 288 (2002).
38. Y. Okano, *Nippon Koshohin Kagakkaishi* **23**, 340 (1999).
39. T. Fujimura, K. Tsukahara, and H. Kawada, *Fragrance J.* **30**, 38 (2002).
40. N. Sonehara and T. Nishiyama (2001), in *Jpn. Kokai Tokkyo Koho* (Shiseido Co., Ltd., Japan). Jp, pp. 11.
41. J. R. Schiltz (2001), in *PCT Int. Appl.* (Mary Kay, Inc., USA). Wo, pp. 34.
42. S. Sakaki and H. Masaki (1997), in *Jpn. Kokai Tokkyo Koho* (Noevir K. K., Japan). Jp, pp. 5.
43. S. Sakaki, T. Arashima, A. Iwamoto, Y. Okano, and H. Masaki, *J. SCCJ* **30**, 71 (1996).
44. N. Lowe, *Round Table Series—Royal Society of Medicine Press* **74**, 23 (2002).
45. J. C. Cather, J. C. Cather, and A. Menter, *Dermatologic Clinics* **20**, 749 (2002).
46. H. A. Khawaja and E. Hernandez-Perez, *Inter. J. Dermatol.* **40**, 311 (2001).
47. J. A. Ellis, R. Sinclair and S. B. Harrap (2002), *Expert Reviews in Molecular Medicine [online computer file]*, No pp. given.
48. J. A. Ellis, M. Stebbing, and S. B. Harrap, *J. Investigative Dermatol.* **116**, 452 (2001).
49. J. B. Hamilton, *Am. J. Anatomy* **71**, 451 (1942).
50. J. E. Griffin and J. D. Wilson (1989), in *The Metabolic Basis of Inherited Diseases.*, ed. C. R. Scriver, et al., eds (McGraw Hill, New York), pp. 1919–1944.
51. J. Imperato-McGinley, L. Guerrero, T. Gautier, and R. E. Peterson, *Science* **186**, 1213 (1974).
52. B. L. Devine, R. Fife, and P. M. Trust, *Br. Med. J.* **2**, 667 (1977).
53. E. Olsen, *Aust. J. Dermatol.* **38**, A316 (1997).
54. L. Drake, M. Hordinsky, V. Fiedler, J. Swinehart, W. P. Unger, P. C. Cotterill, D. M. Thiboutot, N. Lowe, C. Jacobson, D. Whiting, S. Stieglitz, S. J. Kraus, E. I. Griffin, D. Weiss, P. Carrington, C. Gencheff, G. W. Cole, D. M. Pariser, E. S. Epstein, W.

Tanaka, A. Dallob, K. Vandormael, L. Geissler, and J. Waldstreicher, *J. Am. Acad. Dermatol.* **41**, 550 (1999).
55. G. W. Small, *Am. J. Med.* **104**, 32S (1998); discussion 39S–42S.
56. A. Takeda, T. Yasuda, T. Miyata, K. Mizuno, M. Li, S. Yoneyama, K. Horie, K. Maeda, and G. Sobue, *Neurosci. Lett.* **221**, 17 (1996).
57. T. E. Golde, *J. Clin. Investig.* **111**, 11 (2003).
58. M. D. Kane, R. D. Schwarz, L. St. Pierre, M. D. Watson, M. R. Emmerling, P. A. Boxer, and G. K. Walker, *J. Neurochem.* **72**, 1939 (1999).
59. S. O. Bachurin, *Med. Res. Rev.* **23**, 48 (2003).
60. R. Cocabelos, A. Nordberg, J. Caamano, A. Franco-Maside, L. Fernandez-Novoa, M. J. Gomez, X. A. Alvarez, M. Takeda, J. Prous, Jr., T. Nishimura, and B. Winblad, *Drugs Today* **30**, 259 (1994).
61. P. B. Watkins, H. J. Zimmerman, M. J. Knapp, S. I. Gracon, and K. W. Lewis, *JAMA* **271**, 992 (1994).
62. H. M. Bryson and P. Benfield, *Drugs Aging* **10**, 234 (1997); discussion 240–241.
63. L. J. Scott and K. L. Goa, *Drugs* **60**, 1095 (2000).
64. B. P. Imbimbo, P. Martelli, W. M. Troetel, F. Lucchelli, U. Lucca, and L. J. Thal, *Neurology* **52**, 700 (1999).
65. A. Enz, R. Amstutz, H. Boddeke, G. Gmelin, and J. Malanowski, *Progress Brain Res.* **98**, 431 (1993).
66. C. Behl, *Prog. Neurobiol.* **57**, 301 (1999).
67. B. J. Tabner, S. Turnbull, O. El-Agnaf, and D. Allsop, *Curr. Top. Med. Chem.* **1**, 507 (2001).
68. A. C. Allison, R. Cacabelos, V. R. Lombardi, X. A. Alvarez, and C. Vigo, *Prog. Neuropsychopharmacology Biol. Psychiatry* **25**, 1341 (2001).
69. F. N. Bolkenius, J. Verne-Mismer, J. Wagner, and J. M. Grisar, *Eur. J. Pharmacol.* **298**, 37 (1996).
70. R. Reiter, L. Tang, J. J. Garcia, and A. Munoz-Hoyos, *Life Sci.* **60**, 2255 (1997).
71. A. Wolfler, P. M. Abuja, K. Schauenstein, and P. M. Liebmann, *FEBS Lett.* **449**, 206 (1999).
72. B. Longoni, W. A. Pryor, and P. Marchiafava, *Biochem. Biophys. Res. Communi.* **233**, 778 (1997).
73. S. Bachurin, G. Oxenkrug, N. Lermontova, A. Afanasiev, B. Beznosko, G. Vankin, E. Shevtzova, T. Mukhina, and T. Serkova, *Ann. N. Y. Acad. Sci.* **890**, 155 (1999).
74. S. Bachurin, E. Bukatina, N. Lermontova, S. Tkachenko, A. Afanasiev, V. Grigoriev, I. Grigorieva, Y. Ivanov, S. Sablin, and N. Zefirov, *Ann. N. Y. Acad. Sci.* **939**, 425 (2001).
75. M. Hull, K. Lieb, and B. L. Fiebich, *Expert Opinion Investigational Drugs* **9**, 671 (2000).
76. G. P. Lim, F. Yang, T. Chu, P. Chen, W. Beech, B. Teter, T. Tran, O. Ubeda, K. H. Ashe, S. A. Frautschy, and G. M. Cole, *J. Neurosci.* **20**, 5709 (2000).
77. H. Blain, J. Y. Jouzeau, A. Blain, B. Terlain, P. Trechot, J. Touchon, P. Netter, and C. Jeandel, *Presse Medicale* **29**, 267 (2000).
78. J. McLaurin, R. Cecal, M. E. Kierstead, X. Tian, A. L. Phinney, M. Manea, J. E. French, M. H. L. Lambermon, A. A. Darabie, M. E. Brown, C. Janus, M. A. Chishti, P. Horne, D. Westaway, P. E. Fraser, H. T. J. Mount, M. Przybylski, and P. St George-Hyslop, *Nat. Med. (New York, NY, United States)* **8**, 1263 (2002).
79. E. Check, *Nature (London)* **415**, 462 (2002).
80. J. A. R. Nicoll, D. Wilkinson, C. Holmes, P. Steart, H. Markham, and R. O. Weller, *Nat. Med. (New York, NY, United States)* **9**, 448 (2003).
81. G. M. Pasinetti, L. Ho, and P. Pompl, *Neurobiol. Aging* **23**, 683 (2002).

82. A. S. Khan, D. C. Sane, T. Wannenburg, and W. E. Sonntag, *Cardiovascular Res.* **54**, 25 (2002).
83. O. Bonow Robert, A. Smaha Lynn, C. Smith Sidney, Jr., A. Mensah George, and C. Lenfant, *Circulation* **106**, 1602 (2002).
84. Y. Sherer and Y. Shoenfeld, *Autoimm. Rev.* **1**, 21 (2002).
85. O. Bonow Robert, *Circulation* **106**, 3140 (2002).
86. O. Stein and Y. Stein, *Atherosclerosis (Shannon, Ireland)* **144**, 285 (1999).
87. J. X. Rong and E. A. Fisher, *Ann. Med. (Helsinki)* **32**, 642 (2000).
88. C. Joukhadar, N. Klein, M. Prinz, C. Schrolnberger, T. Vukovich, M. Wolzt, L. Schmetterer, and G. T. Dorner, *Thrombosis Haemostasis* **85**, 47 (2001).
89. G. Weitz-Schmidt, *Trends Pharmacol. Sci.* **23**, 482 (2002).
90. M. Ridker Paul, *Clin. Cardiology* **26**, III39 (2003).
91. L. E. Spieker, T. F. Luscher, and G. Noll, *HeartDrug* **1**, 160 (2001).
92. G. Miltiadous, J. Papakostas, G. Chasiotis, K. Seferiadis, and M. Elisaf, *Atherosclerosis (Shannon, Ireland)* **166**, 199 (2003).
93. W. G. Haynes, *Cardiovascular Drugs Therapy* **16**, 391 (2003).
94. E. Lang and B. M. Lang, *Z. Gerontol.* **26**, 429 (1993).
95. A. P. Simopoulos, *Environ. Health Preventive Med.* **6**, 203 (2002).
96. W. S. Harris, Y. Park, and W. L. Isley, *Curr. Opin. Lipid.* **14**, 9 (2003).
97. B. Grubeck-Loebenstein and G. Wick, *Adv. Immunol.* **80**, 243 (2002).
98. M. Pahlavani, *Frontiers Biosc. [Electronic Publication]* **5**, D580 (2000).
99. O. Khorram, *Dehydroepiandrosterone (DHEA)*, 57 (2000).
100. J. Lee, T.-H. Chuang, V. Redecke, L. She, P. M. Pitha, D. A. Carson, E. Raz, and H. B. Cottam, *Proc. Nat. Acad. Sci. United States of Am.* **100**, 6646 (2003).
101. A. Chiarenza, M. P. Iurato, N. Barbera, L. Lempereur, G. Cantarella, U. Scapagnini, and R. Bernardini, *Pharmacol. Toxicol. (Copenhagen)* **74**, 262 (1994).
102. I. Vuori, *Res. Quarterly Exercise Sport* **66**, 276 (1995).
103. R. M. Fleischmann, S. W. Baumgartner, E. A. Tindall, A. L. Weaver, L. W. Moreland, M. H. Schiff, R. W. Martin, and G. T. Spencer-Green, *J. Rheumatol.* **30**, 691 (2003).
104. A. Alldred, *Expert Opinion Pharmacotherapy* **2**, 1137 (2001).
105. J. M. Bathon, R. W. Martin, R. M. Fleischmann, J. R. Tesser, M. H. Schiff, E. C. Keystone, M. C. Genovese, M. C. Wasko, L. W. Moreland, A. L. Weaver, J. Markenson, and B. K. Finck, *New England J. Med.* **343**, 1586 (2000).
106. E. Valle, M. Gross, and S. J. Bickston, *Expert Opinion Pharmacotherapy* **2**, 1015 (2001).
107. P. C. Taylor, *Mol. Biotechnol.* **19**, 153 (2001).
108. G. Fernandes (2003) *Abstracts of Papers, 225th ACS National Meeting, New Orleans, LA, United States, March 23-27, 2003*, AGFD-019.
109. I. Nagaoka, J. Hua, S. Suguro, and K. Sakamoto, *Ensho, Saisei* **22**, 461 (2002).
110. C. J. Meininger, K. A. Kelly, H. Li, T. E. Haynes, and G. Wu, *Biochem. Biophys. Res. Commun.* **279**, 234 (2000).
111. H. Sakorafas George, *Cancer Treatment Rev.* **29**, 79 (2003).
112. R. S. Sandler, *Important Adv. Oncol.* 123 (1996).
113. K. A. Carolin and H. A. Pass, *Crit. Rev. Oncol./Hematol.* **33**, 221 (2000).
114. V. C. Jordan, *J. Steroid Biochem. Mol. Biol.* **74**, 269 (2000).
115. J. M. Kaminski, J. B. Summers, M. B. Ward, M. R. Huber, and B. Minev, *Cancer Treatment Rev.* **29**, 199 (2003).
116. O. W. Brawley, *Urologic Oncol.* **21**, 67 (2003).
117. G. J. Gormley, *J. Cellular Biochem.* 113 (1992).
118. O. Kucuk, *Develop. Oncol.* **81**, 331 (2002).
119. E. Giovannucci, *Exp. Biol. Med. (Maywood, NJ, United States)* **227**, 852 (2002).

120. C. Miller Elizabeth, E. Giovannucci, W. Erdman John, Jr., R. Bahnson, J. Schwartz Steven, and K. Clinton Steven, *Uro. Clinics North Am.* **29**, 83 (2002).
121. B. S. Reddy and C. V. Rao, *J. Environ. Pathol., Toxicol. Oncol.* **21**, 155 (2002).
122. K.-E. Giercksky, *Best Practice Res., Clin. Gastroenterol.* **15**, 821 (2001).
123. O. Dormond and C. Ruegg, *Drug Resistance Updates* **4**, 314 (2001).
124. H. Fujiki, M. Suganuma, K. Imai, and K. Nakachi, *Cancer Lett. (Shannon, Ireland)* **188**, 9 (2002).

HOWARD B. COTTAM
University of California