Aging: Overview

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ABSTRACT: Aging is a universal process that began with the origination of life about 3.5 billion years ago. Accumulation of the diverse deleterious changes produced by aging throughout the cells and tissues progressively impairs function and can eventually cause death. Aging changes can be attributed to development, genetic defects, the environment, disease, and an inborn process—the aging process. The chance of death at a given age serves as a measure of the average number of aging changes accumulated by persons of that age, that is, of physiologic age, and the rate of change of this measure as the rate of aging. Chances for death are decreased by improvements in general living conditions. As a result, during the past two millennia average life expectancy at birth (ALE-B), determined by the chances for death, of humans has risen from 30 years, in ancient Rome, to almost 80 years today in the developed countries. Chances for death in the developed countries are now near limiting values and ALE-Bs are approaching plateau values that are 6–9 years less than the potential maximum of about 85 years. Chances for death are now largely determined by the inherent aging process after age 28. Only 1.1% of female cohorts in Sweden die before this age; the remainder die off at an exponentially increasing rate with advancing age. The inherent aging process limits ALE-B to around 85 years, and the maximum life span (MLS) to about 122 years. Past efforts to increase ALE-B did not require an understanding of aging. Such knowledge will be necessary in the future to significantly increase ALE-B and MLS, and to satisfactorily ameliorate the medical, economic, and social problems associated with advancing age. The many theories advanced to account for aging should be used, to the extent it is feasible, to help with these important practical problems, including applications of the free radical theory of aging. Past measures evolved by societies to ensure adequate care for older individuals are rapidly becoming inadequate because of changes in life style, the growing percentage of older people, declining fertility rates, and the diminishing size of the work forces to provide for the elderly. Measures are being advanced to help with this problem. Prospects are bright for further increases in the span of functional life and improvements in the lives of the elderly.

KEYWORDS: Aging; Evolution; Mitochondria; Free radical reactions; Retirement and care of the elderly

INTRODUCTION

Aging and evolution began with the apparent spontaneous origination of life about 3.5 billion years ago.[1] Aging is the accumulation of more or less random di-

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verse changes with time. Some changes[2,3] are inheritable, whereas the majority increase the chance of disease and death with advancing age. Together these aging changes ensure evolution. This paper is largely limited to a discussion of aging in mammals, and in particular, to humans.

The rate of accumulation of aging changes under optimal living conditions limits average life expectancy at birth (ALE-B) to about 85 years[4-6] and the maximum life span (MLS) to around 122 years.[7] These limits have been slowly approached over the past 2000 years, owing to gradual improvements in living conditions, for example, nutrition, housing, and medical care. ALE-B, a rough measure of the span of healthy, productive life, has increased from about 30 years in ancient Rome[8] to almost 80 years today in the developed countries.[9] The MLS has apparently remained unchanged.

The accumulation of aging changes throughout the cells and tissues concomitantly progressively lower the ability to cope with work and the stresses and strains of life.[10,11] During recorded history societies continued to develop measures to help minimize the decrements of age. These measures are now under severe strain in many countries,[12] owing in part to the increasing numbers of older persons associated with increasing ALE-Bs.

Future efforts to significantly increase the span of healthy productive life, that is, the functional life span, and to ameliorate problems associated with disproportionate increases in the percentages of older persons in the populations, will require a greater understanding of aging. The purpose of this paper is to contribute to the above goal.

DISCUSSION

Aging

Definition

Aging is the accumulation of diverse deleterious changes in the cells and tissues with advancing age that increase the risk of disease and death.[2,3,9] Aging changes can be attributed to developmental and genetic defects, the environment, disease processes, and an inherent process, referred to as the aging process. The chance of death of an individual of a given age in a population—readily available from vital statistics data—serves as a measure of (1) the average number of adverse changes, that is, aging changes, accumulated by persons of that age, and (2) physiologic age, that is, "true age," in contrast to chronological age. The chances for death in a population determine the ALE-B. ALE-B is a rough measure of the span of healthy, productive life, that is, the functional life span.

Effect of Improved Living Conditions on Aging

Conventional means (CM) of increasing the ALE-B of a population by decreasing the chances for death through improvements in general living conditions—for example, improved nutrition, housing, and medical care—are becoming increasingly futile.[7,9] This is illustrated in FIGURE 1 by the curves of the logarithm of the chance of death versus age for Swedish females for various periods from 1751 to 1992;[13,14] a

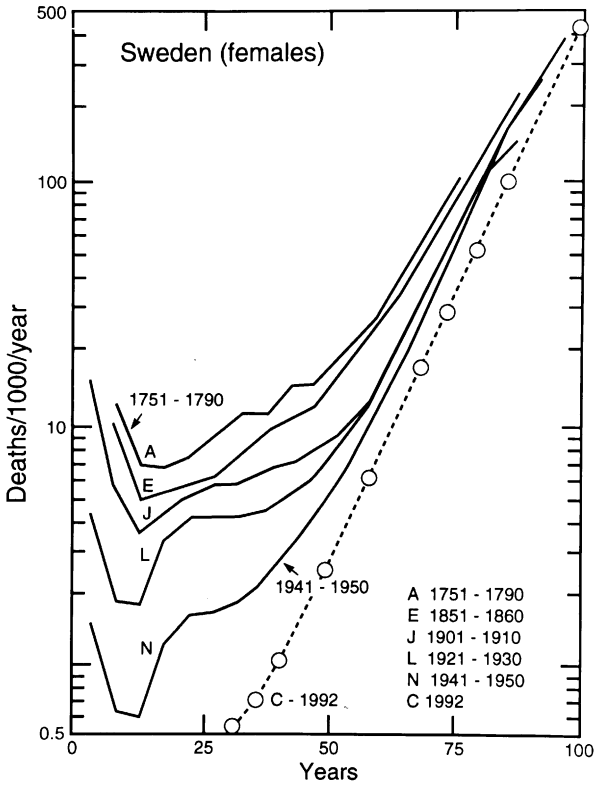

FIGURE 1. Age-specific death rates of Swedish females in various periods from 1751 to 1950 (adapted from H.R. Jones[13]) and for 1992.[14]

straight line represents exponential increases with age. The chances for death in the developed countries are now near limiting values, and ALE-Bs approach plateau values of around 76 years for males and 82 years for females.[7,9]

Thus, as living conditions in a population approach the optimum, and premature deaths the minimum, the logarithmic curve of the chance of death versus age shifts towards a limit determined by the sum of (1) the irreducible contributions to the chance of death by aging changes that can be prevented to varying degrees by CM, for example, those due to the environment and disease, and (2) contributions that can be influenced little, if at all, by CM, that is, those due to the innate aging process. The now-near limiting chances for death rise almost exponentially after about age 28. Only 1–2% of a cohort die before this age.[14]

The Aging Process

The inherent aging process is the major risk factor for disease and death in the developed countries after age 28.[7] It limits ALE-B to about 85 years[4-6] and the MLS to around 122 years.[7] Aging rates are low early in life but rapidly increase with age,

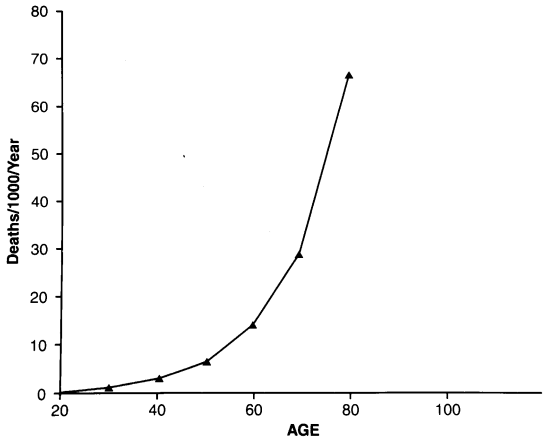

FIGURE 2. The chance of dying in 1985 as a function of age for the total population of the United States.[7]

illustrated in FIGURE 2 by a plot of the chances for death in 1985 for the United States population as a function of age.[7] The innate aging process is caused by chemical reactions that arise in the course of normal metabolism that, collectively, produce aging changes that exponentially increase the chance of death with advancing age even under optimal living conditions.

Theories of Aging: Free Radical Theory

Many theories[7,9] have been advanced to account for the inherent aging process. No theory is generally accepted. The free radical theory of aging (FRTA)[9,15,16] shows promise; the subsequent discussion is based on the assumption that it is correct. The FRTA, and the simultaneous discovery of the important, ubiquitous involvement of free radicals in endogenous metabolic reactions, was proposed in 1954.[15,16] The theory arose from a consideration of aging phenomena from the premise that a single common process, modifiable by genetic and environmental factors, was responsible for the aging and death of all living things.

The FRTA postulates that the common aging process is the initiation of free radical reactions (FRRs). These reactions, however initiated, could be responsible for the progressive deterioration of biological systems over time due to their innate ability to produce random change. The theory was extended in 1972[17,18] with the suggestions that most FRRs were initiated by the mitochondria at an increasing rate with age, and that the life span is determined by the rate of free radical damage to the mitochondria. Collectively, the FRRs initiated by the mitochondria constitute the inherent aging process.

Free Radical Reactions

Free radical reactions can be divided into three stages:[19] initiation, propagation, and termination (FIG. 3). The amount of a compound involved in a free radical reac-

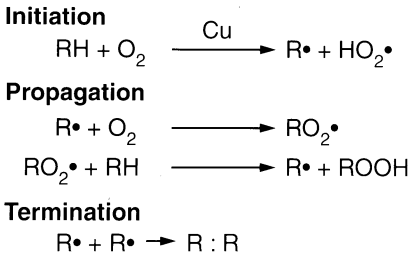

FIGURE 3. Free radical reactions: reaction of O_2 with organic compounds.[19]

tion that is converted to products per unit of time depends on the rate of initiation and the number of times the propagation phase is repeated before termination, that is, the chain length.

Major sources of FRRs today in mammals include the respiratory chain, phagocytosis, prostaglandin synthesis, the cytochrome P-450 system, nonenzymatic reactions of O_2, and ionizing radiation. Defenses that have evolved to minimize free radical–induced damage include antioxidants (e.g., tocopherols and carotenes), heme-containing peroxidases (e.g., catalase), glutathione peroxidase, superoxide dismutases, and DNA repair mechanisms.

Importance of FRRs in Biological Systems

FRRs are ubiquitous in living systems. A reasonable explanation for the presence of this class of chemical reactions is provided by studies on the origin and evolution of life;[1,20] these are summarized in TABLE 1. Life apparently originated spontaneously about 3.5 billion years ago from amino acids, nucleotides, and other basic chemicals of living things produced from the simple, reduced components of the

TABLE 1. Overview of the origin and evolution of life

Years Ago	Main Events
3.5 Billion	Basic chemicals of life formed by free radical reactions, largely initiated by ionizing radiation from the sun. Life begins, excision and recombinational repair processes evolve.
	Ferredoxin appears: RH or $H_2S + CO_2 \rightarrow (h\alpha) \rightarrow CH$
2.6 Billion	Blue-green algae appear.
	$2H_20 \rightarrow (h\alpha) \rightarrow 4H + O_2$
1.3 Billion	Atmospheric O_2 reaches 1% of present value. Anaerobic prokaryotes disappear. Eukaryotes become dominate cells. Eukaryotes plus blue-green algae → the green leaf plants. Eukaryotes plus a prokaryote able to reduce O_2 to H_2O → animal kingdom. Emergence of multicellular organisms and plants. Meiosis evolves.
500 Million	Atmospheric O_2 reaches 10% of present value. Ozone screen allows emergence of life from the sea.
65 Million	Primates appear.
5 Million	Humans appear.

primitive oxygen-free atmosphere by free radical reactions, initiated mainly by ionizing radiation from the sun.

A picture emerges from the growing knowledge of the role of FRRs in biological systems that naturally follows from their chemical nature. It would appear that life originated as a result of FRRs, selected FRRs to play major metabolic roles, and used them to provide for aging, mutation, and death, thereby assuring evolution. Further, life span evolved in parallel with the ability of organisms to cope with damaging free radical reactions. In short, the origin and evolution of life may be due to free radical reactions and, in particular, to their ability to induce random change. If so, it is remarkable that life with its beautiful order owes its origin to, and is sustained by, a class of chemical reactions whose outstanding characteristic is their unruly nature.

The aging process may be simply the sum of the deleterious FRRs going on continuously throughout the cells and tissues. The process may never have changed; in the beginning the reactions were apparently largely initiated by UV radiation from the sun, and to a lesser extent by volcanic activity; and now they arise from enzymatic and nonenzymatic free radical reactions.

Application of the Free Radical Theory of Aging

The FRTA suggests that measures to decrease the chain lengths of FRRs and/or their rates of initiation can decrease aging changes, even under optimal living conditions, and in turn the rate of aging and of disease pathogenesis. Many studies now support this possibility.[9,16,21–24]

The above studies also indicate that CM increase ALE-B by decreasing the rate of accumulation of aging changes associated with suboptimal living conditions. For example, (1) better nutrition provides more compounds to decrease free radical damage; (2) housing improvements may decrease the amount of food metabolized—and thus free radical production, needed to maintain body temperature; (3) in the case of disease, free radicals are widely involved,[16,21,22] so that measures to improve prevention and treatment would be expected to have beneficial effects on life span; and (4) tissue injury causes free radical formation; thus better accident prevention measures should reduce the accumulation of aging changes.

Increasing Inhibition of Free Radical Reactions

Measures that decrease chain lengths of FRRs include the following: (1) antioxidant enzymes (i.e., superoxide dismutase (SOD), catalase, glutathione peroxidase, and SOD mimics); (2) spin traps; and (3) chain-breaking compounds (i.e., such synthetic compounds as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), 2-mercaptoethylamine (2-MEA), ethoxyquin, 21-aminosteroids, and 2-methylaminochromans (lazaroids); and natural compounds as α-tocopherol, ascorbic acid, β-carotene, melatonin, and α-lipoic acid).

Antioxidant enzymes. Studies with short- and long-lived strains of *Neurospora crassa*,[25] *Drosophila melanogaster*,[26] and *Caenorhabditis elegans*,[27] have shown that activities of antioxidant enzymes are higher in the longer-lived strains. Overexpression in *Drosophila* of both SOD and catalase, which acting in tandem provide the primary enzymatic antioxidant defenses in *Drosophila*, extended the life span by as much as one third.[28]

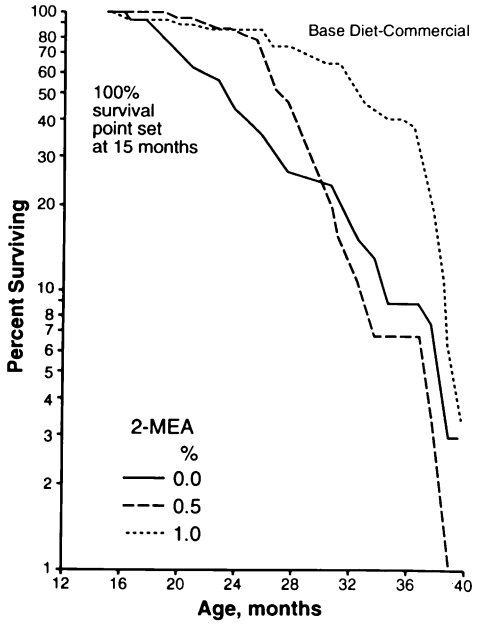

FIGURE 4. Effect of adding 0.5 and 1.0 wt % of 2-MEA to the diet, starting shortly after weaning, on the life span of male LAF_1 mice.[29]

Chain breaking compounds.

- *Dietary antioxidants, from weaning throughout life*. Antioxidants reduce free radical reaction damage by decreasing chain lengths.[19] Most animal studies directed to increasing the life span have used dietary antioxidant supplements to augment the natural defenses against FRR damage. For example, addition of 1% by weight of 2-mercaptoethylamine hydrochloride (2-MEA) to the diet of male LAF mice (FIG. 4), started shortly after weaning, increased ALE-B by 30%;[29] the MLS was increased little, if at all. When 0.5% of ethoxyquin was added to the diet of male and female C3H mice, the increase in ALE-B was 20% for both groups with no change in the MLS.[30] Thus, antioxidant supplements in mice—living under conditions of good animal care, that is, production of free radical reaction–induced aging changes secondary to suboptimal living conditions is small—increased the percentage of older animals in the studies and decreased the senescence periods,[29,30] as there was little, if any, effect on the MLS. The latter is true because the exponentially increasing production of aging changes with age by the inherent aging process progressively nullify the beneficial effects of the antioxidants, keeping increases in maximum life span minimal.
- *Exposure to antioxidants during early life*. FRRs may also produce significant life-shortening changes during the short period of high mitotic and metabolic rate of early life. These reactions, aside from those associated with the envi-

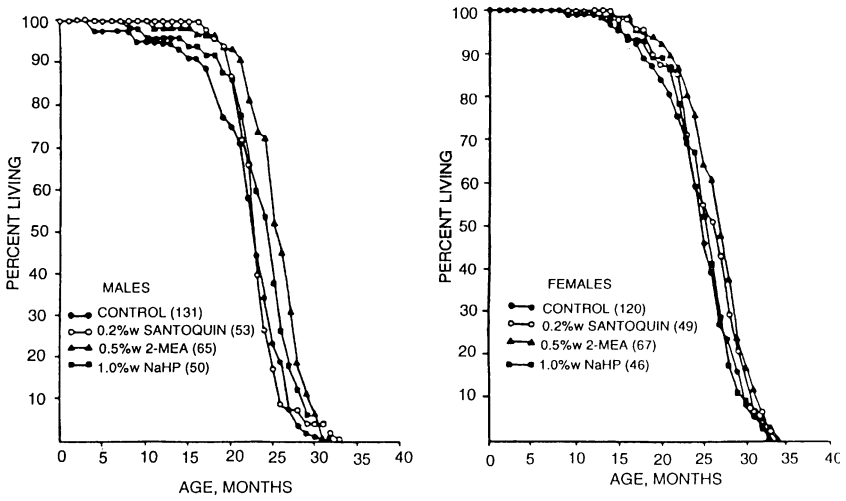

FIGURE 5. Effect of adding antioxidants to the maternal diet on the life spans of their offspring.[35]

ronment, apparently first arise with the onset of mitochondrial function and the associated formation of superoxide radicals in the course of normal metabolism. This occurs on gestational day 10 in the rat.[31] There are at least two other sources of FRRs in early life: (1) steroid estrogens, elevated in pregnancy,[32] can be converted to catechol estrogens that serve as a source of superoxide radicals via the quinone/hydroquinone redox system,[33] and (2) sporadic episodes of transient increases in superoxide radicals, secondary to ischemia-reperfusion injury, due to uteroplacental hypoperfusion.[31,34] The above possibility was assessed twice. In the second experiment[35] groups of female Swiss mice were maintained on a semisynthetic diet, with and without an added antioxidant supplement (0.2 wt % ethoxyquin, 0.5 wt % 2-MEA, or 1.0 wt % sodium hypophosphite), from one month before mating until the offspring were weaned. The male and female offspring were separated and placed on a pelleted commercial diet (no added antioxidants). Survival curves were obtained. Addition of 2-MEA, the most effective of the compounds evaluated, to the maternal diet increased the ALE-B of male offspring by 15% and of females by 8% (FIG. 5). The results of both experiments were similar. The second study confirmed the unexpected results of the first one—the percentage increase in life span was greater for male offspring than for female offspring. The antioxidants had no apparent adverse effects on the pregnancies. The above increases in offspring life spans are attributed to decreases by the 2-MEA in free radical–induced nonspecific aging changes and life-shortening mutations. The lesser effect observed in females may have been largely due to a normally higher level of protection of female embryos from free radical damage during a short period, about 48 hours in the mouse, just prior to the random inactivation of one of the two functioning X chromosomes in the late

blastocyst stage of development. The X chromosome codes for glucose-6-phosphate dehydrogenase, a key enzyme in the production of NADPH; NADPH acts to maintain glutathione in the reduced state. Also contributing to the gender difference in longevity are the lower body stores of iron in females prior to the menopause secondary to iron loss during menstruation.[36]

- *Association of disease with age*. The adverse effects of FRRs after conception provide a plausible explanation for the association of disease with age.[23] The ubiquitous FRRs would be expected to produce deleterious changes that accumulate progressively with age. The nature and locations of the changes are influenced by genetic and environmental factors. Those that are more or less common to all persons determine the "normal" sequential alterations with time. Superimposed on this common pattern of change are patterns that differ from individual to individual owing to genetic and environmental differences that modulate FRR damage, for example, defective Cu/Zn SOD in Lou Gehrig disease[37] and probable higher rates of formation of superoxide radicals in the vessel walls in essential hypertension.[38] The superimposed patterns of change may become progressively more discernable with time, and some may eventually be recognized as diseases, at ages influenced by genetic and environmental risk factors. In agreement with the preceding: (1) FRRs have been implicated in the pathogenesis of a growing number of disorders—the free radical diseases[23,24] (i.e., atherosclerosis, cancer, Alzheimer disease, Parkinson disease, essential hypertension, cataracts, Fanconi anemia, Bloom syndrome, amyloidosis, diabetes mellitus, Laennec cirrhosis, and amyotrophic lateral sclerosis), and (2) accumulating recent data demonstrate that many diseases of adulthood that shorten life[39,40]—for example, breast and prostate cancer, coronary heart disease, hypertension, and diabetes mellitus—have their origins in events of early life.[39,40] This is also probably true for Alzheimer disease.[39]
- *Decreasing the incidence of free radical diseases*. Although the exact etiologies of the free radical diseases are not known, the probability of developing any one of them should be decreased by lowering levels of the more or less random FRRs by any of the following means: (1) during early development, enhance antioxidant content of maternal diets, reduce catechol estrogens, and increase efforts to lower episodes of uteroplacental ischemia; (2) food restriction; (3) minimization of exposure to ionizing radiation; (4) increasing antioxidant intake (dietary and/or supplements); and (5) in the case of a specific disease, decreasing environmental risk factors, for example, cholesterol in atherosclerosis and carcinogens in cancer. In some instances it may be necessary to "target" the inhibitor(s) in order to achieve effective inhibitor concentrations. Thus, the blood pressure of spontaneously hypertensive rats is not lowered by intravenously injected Cu/Zn SOD, but it is lowered when the enzyme is coupled with a basic peptide to form HB-SOD.[38] Unlike SOD, HB-SOD undergoes transendothelial transport and localizes within arterial walls.
- *Failure to increase the maximum life span*. The general failure of antioxidants to increase maximum life span is attributed to[9,39] (1) depression of mitochondrial function by the compounds at concentrations below those needed to slow free radical damage to the mitochondria (that is, as the dietary concentration of an antioxidant is increased it significantly impairs mitochondrial function

before it slows mitochondrial aging)—2-mercaptoethanol[41] and two pyridine compounds[42,43] may be exceptions, and/or (2) progressive increases in the initiation of free radical reactions with age by the mitochondria eventually nullify the beneficial effects of the added antioxidant.

Decreasing FRR Initiation Rates

Measures to reduce FRR initiation rates, and thus increase life span, can be divided into two categories: those that produce increases in ALE-B but have little, if any, effect on MLS, and those that produce increases in both ALE-B and MLS. The following measures fit into the first category.

Measures That Increase ALE-B.

- *Minimize intake of dietary componments prone to increase inintiation rates*. For example, copper, iron, and manganese, polyunsaturated lipids, and easily peroxidized amino acids. Thus, increasing the the dietary content of easily peroxidized amino acids might increase FRR damage and thereby decrease life expectancy. In agreement, when 1 wt % of either histidine or lysine was added to a semisynthetic diet containing 20 wt % of casein as the sole source of protein, average life expectancy was decreased by 5 and 6%, respectively.[44] Conversely, replacing casein by a soybean protein containing a lesser amount of easily oxidized amino acids increased life expectancy by 13 percent.
- *Cruciferous vegetables.* Cruciferous vegetables[9] contain compounds that induce enzymes that catalyze the two-electron reduction of dietary quinones to hydroquinones, thereby decreasing the likelihood that the compounds would undergo one electron reduction to the semiquinone radical. These can react spontaneously with O_2 to form the superoxide radical and regenerate the quinones, thus resulting in oxidative stress by redox cycling.
- *Phlebotomy and chelation.* The life span may be increased further by measures such as phlebotomy[36,45] and chelation.[46–48] These minimize accumulation in the body of metals capable of initiating adverse free radical reactions, for example, iron, copper, and manganese, as well as of such heavy metals as lead, mercury, and cadmium that can impair activities of sulfur and selenium-containing enzymes.
- *Peroxisomal activity.* Peroxisomes[49,50] are organelles that have large complements of flavin oxidases and catalase. Normally a small amount of the H_2O_2 formed by the oxidases escapes catalase action and diffuses from the organelle. This peroxisomal-derived H_2O_2 can be increased, for example, by peroxisomal proliferators such as clofibrate and high fat diets. Thus, minimizing peroxisomal proliferator activity should have a beneficial effect on life span.

Measures That Increase both ALE-B and MLS. The rate of mitochondrial superoxide radical formation is the major determinant of the life span. The major source of endogenous free radical reactions are superoxide radicals arising from the mitochondrial respiratory chain in the course of normal metabolism. The rate of mitochondrial superoxide radical formation increases with age.[17,22,51–58] This makes it progressively

more difficult, and eventually impossible, to prevent free radical damage to the body by dietary means, including the use of dietary supplements. Decreases in caloric intake are associated with (a) decreases in O_2 utilization and in superoxide radical formation, and (b) increases in life span. Comparing birds and mammals of similar metabolic rates, the much longer life spans of the birds is related to a lower rate of formation of superoxide radicals by the mitochondria.[59,60] This also occurs in two closely related rodent species.[61] Life spans of different mammalian species[53] are related to the rates of mitochondrial superoxide radical formation. The frequency of the mitochondrial genotype, Mt5178A, in Japan is higher in centenarians than in healthy blood donors.[62] The high frequency of this genotype in the Japanese population (45%) may be related to the fact that the Japanese are the longest lived of the the world's populations. As expected, because mitochondria are of maternal origin, siblings of centenarians live longer than the normal life span.[63] In accord with the foregoing, oxidative stress has been reported to be less in healthy centenarians than in younger individuals.[64]

The following measures can/may decrease mitochondrial superoxide formation without lowering ATP production below a level compatible with normal life. These are followed by a further comment on decreasing damage during the early period of life.

• *Caloric restriction.* Decreases in caloric intake are associated with proportionate decreases in O_2 utilization. Over 90% of the O_2 consumed by mammals is used by mitochondria; of this, a small fraction is diverted to form superoxide radicals, causing a corresponding decrease in ATP production. Thus, food restriction decreases both superoxide radical formation and ATP production. The former decreases the aging rate,[9,21,22] thereby increasing life span, whereas the latter decreases energy input, resulting in adapted metabolic changes in an effort to sustain body maintenance and function. The foregoing provides the most parsimonious explanation of the numerous changes[65] found in caloric restriction studies.

In accordance with the above possibility, decreasing the daily caloric intake of rats by 40%, while maintaining essential nutrients,[66] decreased body weight by 40% and increased average life span by 40% and maximum life span by 49 percent. The metabolic rate, that is, the caloric consumption per day per unit of body weight, was the same for the two dietary groups because the percentage decrease in the restricted group, in caloric intake per day and in body weight, was equal. This study demonstrated that caloric restriction slows production of aging changes by the inherent aging process: the slope of the curve of the log of the chance of death versus age for restricted animals is less than that of the controls.[9,21] A similar study has been initiated with primates.[67]

Caloric restriction can almost certainly increase the MLS of humans. However, the increase associated with a tolerable level of restriction would undoubtedly be small. The goal for humans is to significantly increase our healthy life span while living a normal life. Efforts to achieve this goal should also include some acceptable degree of caloric restriction.

Compounds that compete with O_2 for access to "electron-rich areas" of the mitochondria.

• *Spin traps*. Spin traps commonly used in biological systems are nitrones ($\rightarrow$N $\rightarrow$ O) or nitroso (>N = O) compounds.[68] They react with free radicals to form

relatively stable nitroxides (>N − O•) that are readily reduced to hydroxylamines (>N − OH). Although spin traps are antioxidants, in a biological system their antioxidant activity seems small in comparison to the apparent ability of the nitroxide derivatives, at least that of *N-tert*-butyl-″-phenylnitrone (PBN), to inhibit initiation of FRRs. PBN gradually enhanced performance of old gerbils[69,70] in a radial-arm maze test (a measure of memory) to near that of young gerbils, over a two-week period of twice a day intraperitoneal injections of 32 mg/kg PBN (the threshold dose for protection against ischemia/reperfusion brain injury).[69] This was accompanied by increases in the brain of the ratio of unoxidized to oxidized protein and of the activities of glutamine synthetase and neutral protease. After injections were stopped these measures returned slowly over two weeks to the original values. The above results are those to be expected if PBN had disproportionately lowered the FRR level in old gerbils to near that of the young. This would have increased activities of both neutral protease and glutamine synthetase. The protease would have increased the turnover rate of oxidized proteins,[71,72] changing the ratio of normal protein to oxidized protein towards that of the young and improving maze performance. Assuming the foregoing is correct, it was in part a consequence of the free radical scavenging effect of PBN. However, most likely the major action of PBN was to decrease the initiation rate of adverse FRRs. This could probably be accomplished by the joint action of the following: (a) Addition of a free radical, for example, superoxide radical, to PBN followed by association of the resulting nitroxide with a mitochondrial respiratory chain,[73] where, in competition with O_2 for electrons, a hydroxylamine is formed instead of a superoxide radical. The hydroxylamine could then diffuse away from the mitochondria and be readily converted back to the nitroxide by reacting with a free radical, for example, a hydroxyl radical, thus resulting in cyclic oxidation of the respiratory chain. In essence, the spin trap in a cyclic fashion causes the removal of a free radical while preventing formation of a new one. (b) Decrease formation of the hydroxyl radical by helping to maintain cellular iron in the ferric state;[74,75] (c) the nitroxide serving as a superoxide dismutase mimic; and (d) depressing hydroxyl radical formation inside Cu/Zn SOD.[74] In accordance with the above discussion:

(i) C57BL male mice at 24.5 months of age were divided into two groups of 50 each.[76] The experimental group received 0.25 mg/mL of PBN in their drinking water. The mean life spans for the control and treated groups were 29.0 and 30.1 months, respectively, whereas the corresponding maximum life spans (last survivors) were 31.7 and 33.3 months.

(ii) Eleven 24-month-old male Sprague-Dawley rats were started on daily ip injections of 32 mg/kg of PBN for the 9.5-month study period; the 12 controls of the same age were similarly injected with 0.9% saline solution.[77] The average life span of the controls was 28 months, whereas that of the experimental group was 30. At the end of the study period, 36% (4 rats) of the PBN group was alive, but only 8% (1 rat) of the controls was alive. PBN improved cognitive performance in several tasks associated with decreased oxidative brain damage.

(iii) Daily intraperitoneal injection of senescence-accelerated mice (SAM-P8)—mice that seem to age at a higher than normal rate—with 30 mg/kg of PBN increased both average and maximum life spans.[78] This study suggests that the increased FRR levels in SAM-P8 mice[79] are caused by higher than normal rates of mitochondrial formation of superoxide radical.

- *Nitroxides and hydroxylamines*. In view of the above discussion, these two classes of compounds should act like nitrones and nitroso compounds.

Blocking agents. Compounds that can associate with the electron-rich areas of the mitochondrial respiratory chain, but not react significantly with it or elsewhere, may block access of O_2 to these areas to some extent and thus decrease superoxide radical formation. A search for such substances may be productive.

- The "free radical sponge,"[80] buckyball—for example, fullerene (C_{60}), or some of its derivatives—may have the foregoing properties. These empty ball-like compounds show promise as neuroprotective agents.[81]

- The antiviral agent, amantadine,[82] may also be useful. This soluble, stable 10-carbon amine prevents cellular entry of a virus,[83] possibly by coating the virus. This compound is excreted unchanged in the urine.

Compounds that may decrease loss of mtDNA function with age.

- *Coenzyme Q_{10}.* This compound is an essential component of the electron transport chain and serves also as an important antioxidant in both mitochondria and lipid membranes. Levels of coenzyme Q_{10} in both animals and humans decrease with age. Dietary supplementation of rats with coenzyme Q_{10} significantly increased mitochondrial content of the compound.[84] The decline in the level of this substance with age hinders the transfer of electrons from complexes I and II to complex III.[85] This increases the electron density of complexes I and II, resulting in a higher rate of formation of superoxide radical. Thus coenzyme Q supplementation should both decrease the block and the oxidative damage to the mitochondria. Apparently very few long-term studies have been made with coenzyme Q_{10}. A lifelong study of mice and rats supplemented with coenzyme Q_{10} found no increase in life span, and no shortening;[86] further studies are indicated.
- *R-″-Lipoic acid*. This form of the disulfide lipoic acid is the naturally occurring enantiomer in mammalian cells. It is a coenzyme for the mitochondrial dehydrogenases for pyruvate and α-ketoglutarate.[87] Supplementation of the diet of rats with (R)-α-lipoic acid "significantly attenuates the age-related increase in hepatocellular oxidant production as well as lipid peroxidation."[88] Supplementation also increased the cellular levels of glutathione and ascorbic in old rats to be like those of the young. Further, "feeding acetyl-L-carnitine (ALCAR) in combination with lipoic acid effectively increases mitochondrial metabolism without an increase in oxidative stress," which was observed with using ALCAR alone.[89] Thus, (R)-α-lipoic acid may serve, at least in part, to decrease mitochondrial superoxide radical formation.

- *Glutathione*. Increases in oxidative damage to mtDNA with age are associated with decreases in mitochondrial glutathione (GSH) content.[90,91] The changes are reversed with oral antioxidants—for example, thiazolidine carboxylate (TC)[90] and a *Ginkgo biloba* extract (EGb 761).[91] Other measures directed to increasing mitochondrial GSH[92] include providing GSH esters, and precursors of substrates for GSH synthetase and γ-glutamylcysteine synthetase. The MLS[93] of *Drosophila melanogaster* was increased about 18% when maintained on a diet supplemented with either sodium or magnesium thiazolidine carboxylate.
- *Aminoethylcysteine ketimine decarboxylated dimer.* This compound[94] may slow mitochondrial superoxide radical formation by inhibiting oxidation of mitochondrial components at concentrations that do not inhibit function of complex I.
- 2-Mercaptoethanol[41] and two pyridine compounds[42,43] have been reported to increase the MLS (see above). If these studies can be reproduced they should prompt evaluation of other antioxidants.

Genetic change. Birds have high metabolic rates compared to comparable sized mammals and yet have relatively long life spans. This is attributed to a genetically determined diversion of a smaller fraction of the oxygen they use to superoxide radicals than do mammals.[59,60] Likewise,[61] and apparently for the same reason, the white-footed mouse (*Peromyscus leucopus*) lives longer than the common mouse (*Mus musculus*).

Efforts to determine the cause(s), such as more detailed knowledge of the structure of the complexes[95] of these differences in O_2 diversion to superoxide radicals, may result in measures to decrease the diversion in humans. These efforts may be helped by studies of neuroleptics; these compounds may alter mitochondrial gene expression.[96,97]

The short life span of SAM-P8 mice, discussed above, is probably also caused by a mutation(s) that increased mitochondrial superoxide formation. Recently it has been demonstrated that a single targeted mutation of the mouse $p66^{shc}$ gene[98] induces resistance to oxidative stress and increases life span by about 30% without apparent negative side effects.[98] A reasonable possibility is that mutation of the $p66^{shc}$ gene decreases the fraction of O_2 diverted to superoxide radicals by the mitochondria (see above).

Rate of Mitochondrial Superoxide Radical Formation

Decreasing Ill Effects of Early Life

Accumulating data implicates abnormal changes in early life that predispose to diseases of adulthood that shorten life.[39,40] Measures to ameliorate these changes have been briefly discussed above. In addition, efforts to minimize the rise[99,100] in estrogen levels in pregnancy, for example, by a low fat diet,[101] may also increase the life span of offspring.

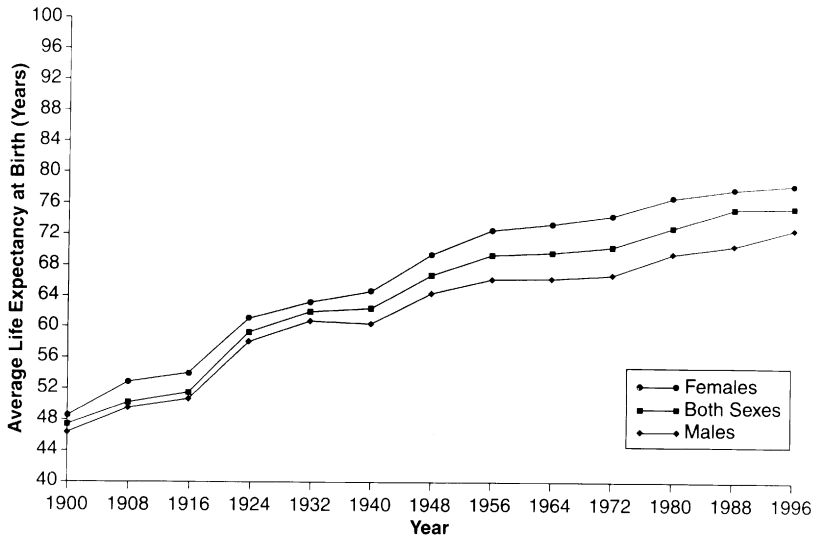

FIGURE 6. Average life expectancy at birth: United States, 1900–1996.[102]

Satisfactorily Accommodating Disproportionate Increases in the Numbers of Older Persons in the Populations

Increases in longevity by continued improvements in CM[102] (FIG. 6) are accompanied by progressive increases in the percentages of older people in the population[10] (FIG. 7), illustrated by data for the United States for 1900–1996. Thus, in the United States the ALE-B rose from 69.7 years in 1960 to 75.4 years in 1990.[102] The 38.7% increase in the total population during this 30-year period was accompanied by an 86.7% increase in the 65+ group (the elderly), and the 85+ group (the oldest old) grew by 225.2%.[10,103] Such disproportionate increases in the numbers of older members, particularly of the oldest old, of many populations are now beginning to (1) severely stress the measures that societies have evolved to help make the lives of older individuals worth living,[12,104–106] and (2) stimulate efforts to cope.

It is in the self-interest of all individuals in a society that it have acceptable, sustainable measures that satisfactorily ameliorate the medical, economic, and social problems of the elderly. Because everyone eventually benefits, everyone should contribute. These measures must recognize that each individual has different abilities, opportunities, and interests, while each individual must accept the responsibility for his/her life and those of their children. These responsibilities include efforts to ensure "successful aging"[104] and to put their children on a course to do likewise.

A sustainable, and probable acceptable, measure in many societies would mandate each individual to save and invest for retirement throughout their working life. This is akin to the present successful plans[107] in England, Australia, and Chile. Life

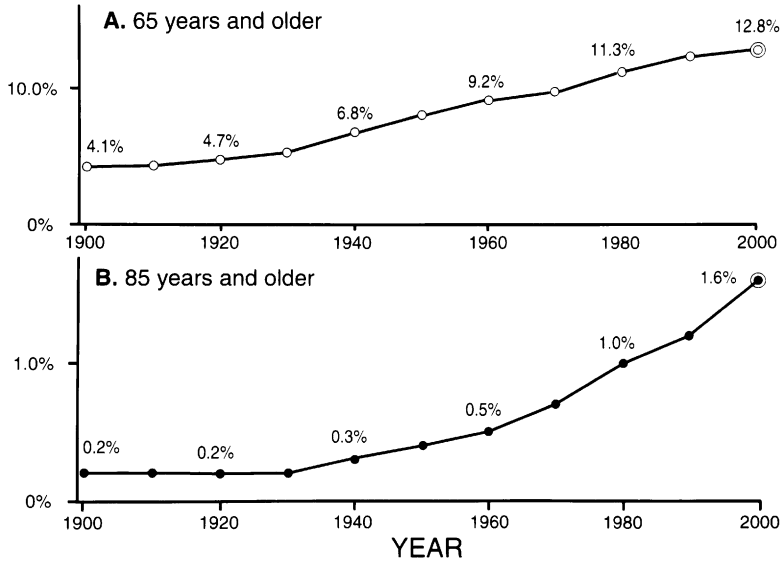

FIGURE 7. Percentage of the population of the United States from 1900 to 2000[7] that are (A) aged 65 and older, and (B) aged 85 and older.

is not perfect. Society must continue to make provisions for those who are unable or unwilling, for whatever reason, to take care of themselves. When necessary, society should provide a means-tested safety net. Humankind has the ability to both extend human life and to make the extensions worthwhile.

COMMENT

Future efforts to increase ALE-B will be largely confined to slowing the inherent aging process because attempts to do so by CM are now almost futile. Some measures that may significantly increase the MLS and/or the ALE-B without lowering ATP below acceptable levels are suggested above. More will be available in the future from the growing biomedical gerontology data base, for example, the recent study mentioned above with the $p66^{shc}$ gene,[98] and the synthesis of a nonpeptidyl mimic of superoxide dismutase with therapeutic activity in rats.[108] Although the measures evolved by societies to make the latter part of life worthwhile are now under increasing stress, efforts are already under way to replace them by ones that are acceptable and sustainable.

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