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The aging process

(free radicals/evolution/antioxidants/degenerative diseases/longevity)

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ABSTRACT **Aging is the progressive accumulation of changes with time that are associated with or responsible for the ever-increasing susceptibility to disease and death which accompanies advancing age. These time-related changes are attributed to the aging process. The nature of the aging process has been the subject of considerable speculation. Accumulating evidence now indicates that the sum of the deleterious free radical reactions going on continuously throughout the cells and tissues constitutes the aging process or is a major contributor to it. In mammalian systems the free radical reactions are largely those involving oxygen.**

Dietary manipulations expected to lower the rate of production of free radical reaction damage have been shown (*i*) to increase the life span of mice, rats, fruit flies, nematodes, and rotifers, as well as the "life span" of neurospora; (*ii*) to inhibit development of some forms of cancer; (*iii*) to enhance humoral and cell-mediated immune responses; and (*iv*) to slow development of amyloidosis and the autoimmune disorders of NZB and NZB/NZW mice. In addition, studies strongly suggest that free radical reactions play a significant role in the deterioration of the cardiovascular and central nervous systems with age.

The free radical theory of aging provides reasonable explanations for age-associated phenomena, including (*i*) the relationship of the average life spans of mammalian species to their basal metabolic rates, (*ii*) the clustering of degenerative diseases in the terminal part of the life span, (*iii*) the beneficial effect of food restriction on life span, (*iv*) the greater longevity of females, and (*v*) the increase in autoimmune manifestations with age.

It is not unreasonable to expect on the basis of present data that the healthy life span can be increased by 5–10 or more years by keeping body weight down, at a level compatible with a sense of well-being, while ingesting diets adequate in essential nutrients but designed to minimize random free radical reactions in the body.

Aging is the progressive accumulation of changes with time associated with or responsible for the ever-increasing susceptibility to disease and death which accompanies advancing age. These time-related changes are attributed to the aging process. This process may be common to all living things, for the phenomenon of aging and death is universal. If so, both aging and the rate of the aging process are under genetic control to some extent for the manifestations of aging, and life span differs between species and individual members of a species. Further, like all chemicals and chemical reactions, the manifestations of aging—which reflect chemical composition—and the rate of the aging process should be subject to environmental influences.

Aging and death of single cells then can be viewed as being due to the aging process, the changes with time and their rates of production being under genetic control but subject to modification by the environment, with death ensuing when one or more activities vital to the cell are depressed below some critical level. Similarly, aging at the multicellular level may be considered the result of the aging processes proceeding in all the cells, with environmental influences now including the effects of the aging cells on each other and the changes with time of the connective tissues. Death of multicellular life occurs because of death or dysfunction, or both, of cells involved in functions vital to the cells as a whole (e.g., functions in mammals such as those of the respiratory center or of the myocardium).

The nature of the aging process has been the subject of considerable speculation (1). Suggested possibilities include (*i*) encodement of aging in DNA (made manifest in a manner similar to development), (*ii*) progressive breakdown in accuracy in protein synthesis, (*iii*) crosslinkage of macromolecules, (*iv*) in higher organisms, "attack" of the immune system on self-antigens, and (*v*) free radical reaction damage. This paper is mainly limited to a discussion of the last-named possibility not only because accumulating evidence indicates that aging is largely due to free radical reaction damage but also because it shows promise of serving as a useful guide in the search for practical means of further increasing the healthy human life span.

The free radical theory (2–6) of aging assumes that there is a single basic cause of aging, modified by genetic and environmental factors, and postulates that free radical reactions are involved in aging and age-related disorders. These reactions arise upon exposure to ionizing radiation, from nonenzymatic reactions, and from enzymatic reactions, particularly those of the two major energy-gaining processes employed by living things—photosynthesis (7) and the reduction of O_2 to water (8–10). They probably also arise as well in the reduction of some terminal electron acceptors employed by anaerobes: probably with NO_3^- (11, 12), possibly with CO_2 (11), and maybe with SO_4^{2-} (11). Although this theory is applicable to all life, the following comments are directed largely to mammalian aging, in which O_2 is the main source of damaging free radical reactions, because of the importance attached to slowing the aging process in man.

The ubiquitous free radical reactions are initiated continuously throughout cells and tissues from both enzymatic and nonenzymatic reactions; examples include enzymatic reactions involved in the respiratory chain (8, 9, 13, 14), in phagocytosis (10), and in the cytochrome *P*-450 system (15); nonenzymatic reactions of oxygen (16, 17) with organic compounds; and nonenzymatic reactions initiated by ionizing radiation (18). Because of the high chemical reactivity of the intermediates, free radicals, it would be expected that all components of the body would be constantly subject to some degree of chemical change in a more-or-less random manner, somewhat like the effects produced by the free radicals formed by ionizing radiation (18). These expected changes include: (*i*) accumulative oxidative alterations in the long-lived molecules collagen (19), elastin (20), and chromosomal material (21, 22); (*ii*) breakdown of mucopolysaccharides through oxidative degradation (23); (*iii*) accumulation of metabolically inert material such as ceroid and age pig-

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ment through oxidative polymerization reactions involving lipids, particularly polyunsaturated lipids, and proteins (24, 25); (*iv*) changes in membrane characteristics of such elements as mitochondria and lysosomes because of lipid peroxidation (26, 27); and (*v*) arteriolocapillary fibrosis secondary to vessel injury by products resulting from peroxidation of serum and vessel-wall components (28). Defenses have evolved that help to limit the production of free radical damage to "tolerable" levels. They include antioxidants, such as the tocopherols (29) and carotenes (10) (like tocopherols, good quenchers of singlet oxygen); heme-containing peroxidases (30) (catalase is an important member of this group); selenium-containing glutathione peroxidase (30); superoxide dismutases (31); and DNA repair mechanisms (32, 33).

Dietary manipulations expected to further lower the rate of production of free radical reaction damage, briefly summarized recently (6), give results in accord with the free radical theory of aging.

For example, dietary antioxidants increase the life span of mice (34–38), rats (39), fruit flies (37, 38), nematodes (40), and rotifers (41), as well as the "life span" of neurospora (42). In the case of mice, addition of 1.0% (wt/wt) 2-mercaptoethylamine to the diet of male LAF_1 mice, starting shortly after weaning, increased the average life span by 30% (34); this increase is equivalent to raising the human life span from 73 to 95 years. Corresponding increases produced by 0.5% 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (Santoquin; Monsanto, St. Louis, MO) in the diet of male and female C3H mice were 18.1% and 20.0%, respectively (35). Although it has been relatively easy to increase the average life span of mice, the increases were not accompanied by any certain extensions of maximum life spans.

A number of studies have shown that antioxidants inhibit cancer development in some model systems (43–46). Similarly, the declining death rate from gastric carcinoma in the United States may be related to the introduction of breakfast cereals, particularly wheat cereals, rich in tocopherols (47). Likewise, in areas where the selenium intake is relatively high, the incidence of some forms of cancer tends to be low (48–50); presumably the effect is mediated through a reduction in free radical reactions. Selenium is a component of glutathione peroxidase (30), an enzyme that lowers free radical reactions by reducing H_2O_2 to water and organic hydroperoxides to alcohols.

Humoral and cell-mediated immune responses are enhanced by adding antioxidants to the diet of mice (51). Santoquin and 2-mercaptoethylamine were particularly effective; addition of 0.25% Santoquin or 0.5% 2-mercaptoethylamine increased the humoral response of female C3HB/FeJ mice spleen cells by 94% and 79%, respectively. Selenium (52) also increases immune system activity.

Development of amyloidosis (53–55), and the autoimmune disorders of NZB and NZB/NZW mice (56), is slowed by antioxidants. Thus, addition of 0.25% Santoquin to the diet markedly inhibited amyloid formation in LAF_1 male mice, whereas the same compound increased the average life span of male NZB mice by 32%. Further, the water-soluble antioxidant *N*-(carboxyphenyl)-4-choloroanthranilic acid (disodium salt), had a beneficial effect on life span and kidney disease in NZB/NZW mice (57). These results were probably in part due to a slower rate of loss of T-cell suppressor function in the presence of antioxidants (56).

Further, dietary changes designed to lower the free radical reaction damage rate should also have a beneficial effect on the cardiovascular and central nervous systems.

There long has been data implicating free radical reactions in the pathogenesis of atherosclerosis and hypertension (5, 6, 58, 59). More recently, lipid peroxides have been found to inhibit prostacyclin synthetase (60, 61); prostacyclin is a potent inhibitor of platelet aggregation (61)—believed to be one of the events contributing to the development of atherosclerosis (62)—and a strong vasodilator (61, 63). Further, studies of selenium deficiency in man (64) and pigs (65, 66)—the deficiency is associated with myocardial and vascular damage—and a study of the effect of selenium and vitamin E on myocardial necrosis induced by isoprenaline treatment or coronary artery ligation (67) indicate that free radical reactions are continuously present in the cardiovascular system and that the rate of accumulation of damage produced by them is limited to tolerable values largely by glutathione peroxidase. Likewise, the incidence of the common forms of cardiovascular disease is low in areas where the dietary intake of selenium is high and vice versa (49, 68, 69). The ability of glutathione peroxidase to "buffer" free radical reaction damage may largely account for the inconclusive results (70) obtained in studies designed to alter atherogenesis rates in minipigs by diets formulated to change the level of free radical reactions in the serum; the diets contained varying amounts of polyunsaturated fats and of copper.

Lipofuscin (age pigment) accumulates with age (71) in the various areas of the central nervous system (CNS) in parallel with the activities of oxidative enzymes (72, 73). Age pigment is formed by oxidative polymerization of lipids, probably largely mitochondrial, and proteins (74). The accumulation of lipofuscin can be slowed by antioxidants (75). Relatively large amounts of lipofuscin may be associated with adverse effects in the central nervous systems, including loss of neurons (72, 76); large deposits of melanin (produced by free radical reactions) also appear to be associated with detrimental changes (77). Vitamin E-deficient diets increase CNS lipofuscin and depress function (78). Further, free radical reactions may be significantly involved in formation of the neuritic plaques (senile plaques) associated with senile dementia of the Alzheimer type (79, 80). The plaques present in the cortex and basal ganglia of normal old people are increased in senile persons. The first changes seen in the development of the plaques are alterations in the mitochondria of the axon terminals (81). These mitochondrial changes may be due to peroxidation, for the mitochondria have both a high degree of lipid unsaturation and a high rate of O_2 utilization. The foregoing could be the basis for the observation that rats receiving a semisynthetic diet containing distilled triglycerides of menhaden oil performed less well in a Hebb-Williams maze than had been anticipiated from mortality data (80).

The free radical theory of aging provides plausible explanations for aging phenomena, including the relation of the average life spans of mammalian species to their basal metabolic rates, the clustering of degenerative diseases in the terminal part of the life span, the beneficial effect of food restriction on life span, the greater longevity of females, and the increase in autoimmune manifestations with age.

Over 90% of the oxygen consumed by a mammal is utilized in the mitochondria. Presumably the rate of O_2 consumption is under genetic control, both nuclear and mitochondrial. It is reasonable to assume that some of the free radicals generated during the reactions involving oxygen (8, 9, 13, 14), such as the superoxide radical ($O_2^{-}\cdot$), would produce changes in the mitochondria at a rate related to the rate of oxygen consumption. Such alterations (for example, in the mitochondrial DNA) could have an accumulative deleterious effect on mitochondrial functions, leading to the observed mitochondrial changes in number (82, 83), morphological characteristics (74), and enzymatic activity (13, 84–86). Thus, the relationship between life span and basal metabolic rate—to a first approximation, constant throughout life—may reflect the overall rate of accumulation of mitochondrial damage secondary to free radical reactions (8).

This rate may be largely shielded from external influences (except for food intake) by the highly selected permeability of the mitochondrial inner membrane (87). In agreement with this possibility, measures expected to decrease free radical reaction levels have resulted in significant increases in the average life expectancy of mice but no certain increases in maximum life expectancy. In other words, it would appear that damage from extramitochondrial free radical reactions can be more readily decreased than can that from reactions arising within the mitochondria. Thus, mitochondria may serve as a "biologic clock," death ensuing when mitochondrial function as a whole or in key areas such as parts of the central nervous system drops below a critical level. Supporting this possibility is a recent study (88) of two strains of *Drosophila melanogaster* which demonstrated that mitochondrial DNA (in contrast to nuclear DNA) declined dramatically with age, beginning at 20 days of age in the shorter-lived strain and at 30–35 days of age in the longer-lived strain. Another recent study (89) directed to lowering the free radical flux in isolated mitochondria may aid efforts to increase the maximum human life span.

The association of the degenerative disorders with the terminal portion of the life span in each mammalian species may be related to the rate of oxygen consumption (8). The tissue and cellular concentrations of O_2 and, thus, the level of free radical reactions increase as O_2 utilization rises. Hence, to the extent that free radical reactions are involved in the pathogenesis of degenerative diseases (free radical reactions are definitely associated with the development of some forms of cancer and are strongly implicated in the disorders of the cardiovascular and central nervous systems), the shorter life spans associated with higher basal metabolic rates should also be accompanied by more-or-less proportionally higher rates of development of degenerative diseases.

Restriction of food consumption tends to increase life span (90). It also decreases oxygen consumption. Thus, in view of the above comments on mitochondria and oxygen consumption, the beneficial effects of food restriction may be simply due to a decreased rate of both mitochondrial degradation and degenerative disease pathogenesis related to decreased levels of free radical reactions.

A recent study of the effect of maternal antioxidants on the life span of their offspring (91) has led to a possible explanation for the greater longevity of females. Addition of 2-mercaptoethylamine·HCl, the most effective of the compounds evaluated, at a level of 0.5% in the maternal diet increased the average life span of their male offspring by 15% and that of the females by 8%; the offspring were fed a pellet control diet throughout life. The greater longevity of the females over the males was decreased from 11.5% in the control offspring to 6% in the 2-mercaptoethylamine-fed offspring. Consideration of the results of this study in the light of events in the early stages of life led to the suggestion that the greater longevity of females is due, at least in part, to the greater protection of female embryos from free radical reaction damage during a period of about 48 hours of both high mitotic and metabolic activity just prior to the random inactivation of one of the two functioning female 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 form. Gluthathione serves as a free radical reaction inhibitor and as a substrate for glutathione peroxidase, keeping the cellular concentrations of H_2O_2 and hydroperoxides low.

There is growing evidence that tolerance to self-antigens is actively maintained mainly by suppressor T-cells (92). T-cell suppressor function is relatively easily depressed by free radical reactions (93). It is likely (56) that the increase in autoimmune manifestations with age is due, at least in part, to a relative depression of T-cell suppressor function resulting from the increasing levels with age (6) of endogenous free radical reactions.

Free radical reactions are ubiquitous in living systems. A reasonable explanation for the presence of this class of chemical reactions is provided by studies (94–97) on the origin and evolution of life; the pertinent data are briefly presented below.

Life apparently arose 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 primitive oxygen-free atmosphere by free radical reactions, initiated mainly by ionizing radiation from the sun. The protocells gradually developed biochemical machinery to free them of environmental sources of "building blocks" and to help provide protection from damaging radiation. Energy for these purposes was first derived from reactions involving organic compounds. Later the first ferredoxin appeared, permitting photosynthetic reduction of CO_2 with organic compounds or H_2S as a source of hydrogen, followed by the blue-green algae about 2.6 billion years ago, which utilized water as a hydrogen source. Still later, as the O_2 liberated by the blue-green algae began to build up in the atmosphere, some cells developed the ability to obtain energy from the reduction of O_2 to water.

Approximately 1.3 billion years ago the atmospheric concentration of O_2 reached about 1% of the present value, the toxic level for the anaerobes. The anaerobic procaryotes disappeared, except for a few in O_2-deficient niches, and the more complex eucaryotes, better equipped to minimize damaging O_2 reactions, appeared and became the dominant cells. The progenitor of the green-leaf plants apparently acquired a blue-green algae to assist with its energy needs, whereas that of the animal kingdom took in a procaryote able to reduce O_2 to water. Subsequently, colonies of cells appeared that evolved into multicellular organisms and plants.

All plant and animal life was confined to the sea to escape the destructive UV radiation from the sun until about 500 million years ago. About this time absorption of UV radiation by the increasing amounts of atmospheric oxygen reduced the radiation reaching the surface to a level compatable with existence on land. Evolution then accelerated. Primates appeared about 65 million years ago, and man appeared some 4–5 million years ago.

Inheritable change is necessary for evolution. Free radical reactions undoubtedly have been involved in production of inheritable change since the beginning of life; the reactions were initiated at first by external radiation and later by radicals arising largely from photosynthesis and the reduction of O_2 to water. Because survival and evolution of the first protocells depended on protection from damaging free radical reactions initiated by ionizing radiation, the selection and development of defenses against these reactions probably also began when life originated. Later, these defenses and others that evolved, served to protect the cells from free radical reactions arising within the cell from enzymatic and nonenzymatic reactions.

From the growing knowledge of the role of free radical reactions in aging and degenerative diseases, mutation, and the origin of life, a picture emerges that naturally follows from the chemical nature of these reactions. It would appear that life originated as a result of free radical reactions, selected free radical reactions to play major metabolic roles, and utilized them to provide for 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 free radical reactions going on continuously throughout the cells and tissues. The process may never have changed; in the beginning the free radical reactions were initiated by UV radiation from the sun and now free radicals arise within from enzymatic and nonenzymatic free radical reactions.

The free radical theory of aging was first proposed in November of 1954 (2). In the ensuing years, considerable data have been accumulated which demonstrate that free radical reactions do contribute to the degradation of biological systems, and a beginning has been made with the aid of the rapidly increasing general knowledge of free radical reactions in biology (98, 99) to determine the mechanisms by which the changes are made. However, the question remains, now as in 1954: are free radical reactions the sole cause of aging, the major cause, or a minor contributor to the process? Although an answer to this question that is satisfactory to all may never be reached, it now seems very likely that the assumption that there is a basic cause of aging is correct and that the sum of the deleterious free radical reactions going on continuously throughout the cells and tissues is the aging process or a major contributor to it. Whatever the final answer, it is apparent that the free radical theory of aging can serve as a useful guide for efforts to increase the healthy life span. This is not to say that other approaches to increasing the useful span of human life may not be found in the future, but only that it now seems feasible to augment it to some degree by interfering with what appears to be the natural process which leads to death.

Underscoring the need for new approaches to the problem of increasing the productive life span is the fact that life expectancy at birth in the developed countries is plateauing; because the average period of senescence is not known, the average life span at birth serves as a rough measure of the healthy, productive life span. Thus, in the United States life expectancy increased rapidly from a value of 47.2 years in 1900 to 67.2 years in 1954–1955 and then increased progressively more slowly to the present value of 73.3 years (100) on an advance towards a limiting figure of about 74–76 years. At best, complete elimination of overt causes of death would increase life expectancy to about 85 years (101, 102), the maximum average life expectancy of man; "conquest" of cancer could add about 2 years to the present life expectancy, whereas the corresponding figure for cardiovascular disease is around seven.

The free radical theory of aging predicts that the healthy life span can be increased by minimizing deleterious free radical reactions while not significantly interferring with those essential to the economy of the cells and tissues. The data now available indicates that this can be done by keeping body weight down, at a level compatible with a sense of well-being, while ingesting diets adequate in essential nutrients but designed to minimize random free radical reactions in the body. Such diets would contain minimal amounts of components prone to enhance free radical reactions, for example copper and polyunsaturated lipids, and increased amounts of substances capable of decreasing free radical reaction damage, such as α-tocopherol, ascorbic acid, selenium, or one or more synthetic antioxidants. It is reasonable to expect that this approach will decrease the morbidity and mortality due to degenerative diseases and nonspecific age changes and possibly also increase slightly the maximum life span, so as to result in an extension of 5 or more years in the span of healthy productive life.

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