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Free Radicals in Molecular Biology, Aging, and Disease

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Preface

All organisms go through stages of development, maturity, aging, and death. Historically, scientific observations on the declines associated with aging focused first on such clearly demonstrable end points as increasing mortality with age, expressed in the classic Gompertz Plot, and the progressive decline in vigor, health, sensory, cognitive, and motor functions; these age-related changes are observable at the organismic level. During the last decade, considerable scientific information has accumulated on age-related changes observable at organ, tissue, and cell levels. Recent advances in molecular biology have made it possible to examine whether the overt physical manifestations of aging may be causally related to basic mechanisms of aging at the molecular level. The dramatic increase in knowledge provided by molecular biology may make it possible to focus on basic mechanisms of aging or, at least, begin to study cause and effect sequences in aging from molecular to organismic levels. This volume presents the rapidly emerging knowledge concerning the role of free radicals in molecular biology with particular emphasis on free radical activity in age-pigment accumulation and cell loss during normal aging, and in specific age-related diseases.

Despite significant differences in life spans among species, correlations among life span, body weight, brain weight, and particularly metabolic rate have provided the initial clues that different species may age by similar mechanisms. Correlations among metabolic rate, rate of lipofuscin accumulation, cell loss, and life span have suggested that lipofuscin pigment of postmitotic cells may represent a basic cellular marker for normal aging, as well as for a variety of age-related diseases. Considerable evidence has also emerged recently to suggest a causal or, at least, a close link between age pigments and the free radicals that are generated as a result of univalent reduction of oxygen during aerobic respiration. As yet, evidence for oxygen free-radical damage of cells *in vivo* is considerable but indirect. However, recent clues suggest that abnormal free radical activity with increasing age and in certain diseases may represent a significant cause of cellular breakdown. Age-related changes in molecular oxygen, trace metals, and antioxidant activity have been implicated in the generation of free radicals, lipopigment accumulation, and cell loss. Free radicals are believed to cause peroxidation of polyunsaturated lipids of membranes resulting in lipopigment accumulation, cell loss, and tissue breakdown. Thus, age- and disease-related tissue and cell breakdown may represent a consequence of increasingly abnormal free radical activity. In turn, this cellular breakdown may trigger greater free radical activity and tissue damage.

As there is age-related breakdown of cell structure and function, the topic of free radicals has become increasingly important not only in molecular biology but also in aging, nutrition, pharmacology, pathology, and even in clinical practice. The upsurge of interest in free radical damage of cell structure and function has occurred

in researchers of widely different disciplines. As a result, current information on the role of free radicals in aging and disease is widely scattered in the literature.

This volume brings together both the more basic and the most recent information on free radicals in aging and disease. The chapters deal with free radical theories of aging, basic chemistry of free radical reactions, effects on lipopigments, free radicals in age-related diseases, and antioxidant defense mechanisms. The chapters in this volume should make it possible to plan and conduct further research on the causes or mechanisms of aging, ranging from the molecular to the organismic levels. Due to the significant increase in the proportion of the elderly in our population, the search for causes of aging in molecular biology will attract increasing numbers of investigations from many disciplines.

This volume will be of great interest to students, scientists, physicians, nutritionists, pharmacologists, and other individuals who are interested in the rapid progress that will be made in future studies of basic mechanisms of aging and prospects of nutritional and chemical intervention.

J. Mark Ordy
Chairman, AGE Symposia

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Free Radicals and the Origination, Evolution, and Present Status of the Free Radical Theory of Aging

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The term "radical" comes from the early days of chemistry. Lavoisier (33) in his theory of acids designated the element or group of elements which combined with oxygen in the acid a "radical". This theory was soon discarded but the word "radical" continued to be used to signify a group of elements which retained their identity through a series of reactions, e.g., a methyl radical (CH_3). Today a "radical" is defined as an atom or group of atoms with an unpaired electron, e.g., $Cl\bullet$, $HC_3\bullet$, or $HO\bullet$.

Numerous "radicals" were discovered in the early 1800's including the cacodyl radical, C_2H_6As. In 1849 it was found that heating zinc with ethyl iodide in a sealed tube produced a gas which was thought to be "free ethyl". When measurement of molecular weight by the method of vapour densities was established, it was soon realized that groups such as methyl or ethyl did not persist in the free state but combined to form dimers.

The first stable free radical found, triphenylmethyl, was prepared in benzene in 1900. In 1911 tetraphenylhydrazine was shown to disociate into two identical stable free radicals.

By the 1920's free radicals were being proposed as intermediates in gas-phase reactions. In the 1930's the body of knowledge of free radical reactions began to increase rapidly as a result of studies of halogenation, oxidation, formation of polymers from vinyl chloride, addition of hydrogen bromide and of bisulfite to olefins, etc. My introduction to free radical chemistry was a course in photochemistry in 1939. Subsequently I spent most of the period 1943-1949 studying free radical reactions involving compounds of sulfur and phosphorus, as well as the reaction of O_2 with organic compounds. By the 1950's free radical chemistry was a well-established field of chemistry (36, 43).

Prodded in part by interest in the biological properties of some of the compounds I had synthesized, I entered medical school in the fall of 1949. Five years later, in July 1954, I was

fortunate to have time to address the question of why all living things age and die. I had first become interested in this problem in December of 1945 after reading the article, "Tomorrow You Will be Younger", by William L. Laurence, science editor of the New York Times (34). This article was concerned with the work of Dr. Alexander A. Bogomolets of Kiev. An English translation of Dr. Bogomolet's book, The Prolongation of Life, was published in 1947 (2). The free radical theory of aging was formulated (9,10) in the first part of November, 1954, after four frustrating months in the library. This theory 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 disease. By chance this theory was put forth at the time when the rate of increase in average life expectancy at birth in the United states began to decrease; it is now 73.7 years and progressing slowly toward a plateau value of 74-76 years.

EARLY STUDIES

Direct validation of the free radical theory of aging did not seem feasible. Hence attempts were started to obtain support for the theory indirectly by determining if dietary changes designed to lower adverse endogenous free radical reactions would increase the life span.

The first study, published in 1957 (12) was encouraging. Addition of one of several free radical reaction inhibitors to the diet at a level of 0.5 - 1.0% by weight throughout life increased the average life span of C3H female mice and of AKR male mice. This study was fortunate as the effect would probably not have been observed at lower or higher dietary concentrations.

Studies prompted by the free radical hypothesis were summarized twice in the 1960's, in 1962 (14) and 1969 (18). Knowledge of free radical reactions in biological systems was still so meager in 1962 that information such as the following was included to support the probable presence of $HO\cdot$ and $HO_2\cdot$: "Investigation of the action of xanthine oxidase, with either sulfite oxidation or luminal used to detect free radicals, indicate that $HO\cdot$ and/or $HO_2\cdot$ are formed. The organic free radicals, detected by ESR arising in several dehydrogenase systems, in the action of peroxidase and H_2O_2 on a number of substrates, and in illuminated choroplast preparations, would be expected to react, to a greater or lesser degree, depending on the availability of oxygen and the resonance stability of the free radical, with oxygen with the formation of radicals such as $RO_2\cdot$ and $HO_2\cdot$." All of the available information was summarized with the statement, "The foregoing, taken as a whole, strongly indicates that chemically active free radicals of the nature of $HO\cdot$ and $HO_2\cdot$ are produced in living things in the course of normal metabolism".

By the early 1970's it had become evident that dietary measures

designed to decrease endogenous free radical reaction levels in mice tended to increase the average life expectancy by as much as 20-30 percent, but had little, if any, effect on maximum life span. Consideration of such data resulted in the suggestion (22) in 1972, expanded in 1983 (27), that the mitochondria might serve as the "biologic clock"; slowing mitochondrial degradation may increase the maximum life span.

PRESENT SUPPORT

Support for the free radical theory of aging, summarized in part again in 1981 (26), now includes (27,28): 1) studies on the origin of life and evolution, 2) life span experiments in which adverse free radical reactions were expected to be lowered by dietary manipulations, 3) the plausible explanations it provides for aging phenomena, and 4) the growing number of studies which implicate free radical reactions in the pathogenesis of specific diseases.

ORIGIN OF LIFE AND EVOLUTION

Life apparently arose spontaneously (26) 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 atmosphere by free radical reactions, which were initiated mainly by ionizing radiation from the sun. It seems likely that evolution was made possible initially by the constant presence of ionizing radiation, which served on the one hand to provide compounds in the environment necessary for the survival and growth of the first protocells and on the other to produce more-or-less random changes throughout the cells.

From the beginning, the evolution of more complex cells apparently occurred through the gradual selection and development of: a) defenses against deleterious chemical reactions (e.g., free radical reactions), and b) means to repair or replace cellular components (e.g., DNA, RNA, proteins) that were rendered defective by such adverse reactions.

Defenses that now help limit free radical damage include antioxidants, such as tocopherols and carotens, heme-containing peroxidases, the selenium-containing glutathione peroxidase, superoxide dismutases, and elevated serum uric acid levels.

The first process evolved to help restore altered DNA to its original form was probably excision repair, followed later by recombinational repair (28). The evolution of germ cells (cells capable of encoding for themselves as well as somatic cells, cells with similar basic functions on which are superimposed differences allowing for the growth and development of multicellular organisms suitable for the continuation and evolution of the germ cells) has been accompanied by the development of more complex systems, e.g., meiosis, for restoring the "purity" of the DNA in the zygote to the degree necessary for the formation of a "normal" member of the species while still allowing changes

in DNA that might lead to further evolution of the germ cells and of the somatic cells encoded in them. The foregoing implies that evolution, driven almost entirely by energy from the sun, is the selection and improvement of measures to increase the probability of survival of germ cell DNA.

The original basic pattern of evolution does not appear to have changed except that the sun-initiated free radical reactions, which were essential for the origination and early evolution of the protocells, have largely been replaced by those derived from enzymatic and non-enzymatic reactions. Enzymatic reactions serving as sources of free radicals include those involved in the respiratory chain, in phagocytoses, in prostaglandin synthesis, and in the cytochrome P-450 system. Free radicals also arise in the non-enzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing radiation. Changes attributed to free radical reactions include: 1) accumulative oxidative alterations in the long-lived molecules collagen, elastin, and chromosomal material; 2) breakdown of mucopolysaccarides through oxidative degradation; 3) accumulation of metabolically inert material such as ceroid and age pigment through oxidative polymerization reactions involving lipids, particularly polyunsatured lipids, and proteins; 4) changes in membrane characteristics of such elements as mitochondria and lysosomes because of lipid peroxidation; and 5) arteriolocapillary fibrosis secondary to vessel injury by products resulting from peroxidation of serum and vessel-wall components.

The somatic and germ cells resulting from the "readout" of the zygote DNA all age and die except for those germ cells which are rejuvenated by entering into zygote formation. Presumably the cells age and die in much the same manner as the early protocells except that now the adverse free radical reactions, such as those listed above, arise largely from within the cells rather than as a result of ionizing radiation from the sun. Collectively the defenses that have evolved against changes that could have deleterious effects on germ cells, and hence on evolution, have permitted the life spans of multicellular organisms to increase by enabling somatic cells, particularly those critical to the existence of the organism as a whole (e.g., cells of the respiratory center or of the myocardium) to function longer.

DIETARY MANIPULATIONS

Dietary (23) manipulations expected to lower the rate of production of free radical reaction damage are also in accord with the possibility that more-or-less random damage produced by free radical reactions constitute the basic aging process; these include: 1) minimizing dietary components - such as copper and polyunsaturates, which tend to increase free radical reaction levels, 2) adding to the diet compounds able to inhibit free radical reaction-induced damage, e.g., 2-mercaptoethylamine (2-MEA), α-tocopherol, butylated hydroxytoluene (BHT), and 1,2-dihydro-6-ethoxy-2,2,4-trimethlquinoline (ethoxyquin).

For example, dietary antioxidants increase the life span (26) of mice, rats, fruit flies, nematodes, and rotifers, as well as the "life span" of neurospora. In the case of mice, addition of 1.0% (wt/wt) 2-mercaptoethylamine to the diet of male LAF_1 mice (17), starting shortly after weaning, increased the average life span of 30%; this increase is equivalent to raising the human life span from 73 to 95 years. Corresponding increases produced by 0.5% ethoxyquin in the diet of male and female C3H mice (4), were 18.1% and 20.0%, respectively. Although it has been relatively easy to increase the average life span of mice, the increases were not accompanied by any certain extension of maximum life spans.

AGING PHENOMENA

The free radical theory of aging is also supported by the plausible explanations it provides for aging phenomena, including the:

(a) Inverse relationship between the average life spans of mammalian species and their basal metabolic rates (27).
(b) Observation that antioxidants which increase the average life span of mice, depress body weight and fail to increase maximum life span (27).
(c) Clustering of degenerative diseases in the terminal part of the life span (27).
(d) Exponential nature of the mortality curve (27).
(e) Beneficial effect of caloric restriction on life span and degenerative diseases (27).
(f) Increase in autoimmune manifestations with age (25).
(g) Greater longevity of females (24).

Thus, for example, the greater longevity of females may be due, at least in part, to the greater protection of female embryos from free radical damage during a period (about 48 hours in the mouse) 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 state 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. Glutathione, the major cellular sulphydryl compound, serves to minimize free radical damage to the organism by acting as a free radical reaction inhibitor and as a hydrogen donor for glutathione peroxidase in the reduction of hydrogen peroxide and hydroperoxides.

The increase in autoimmune manifestation with age is probably largely due to a disproportionate decrease in the radiosensitive T-suppressor cell function owing to the increasing levels of free radical reactions associated with advancing age.

Compounds that increase average life span, such as 2-MEA and ethoxyquin, tend to depress body weight and fail to increase maximum life. These effects are most likely mainly caused by decreased ATP production resulting from the interaction of the antioxidants with free radicals in the respiratory chain.