Age, Vol. 6, 86-94, July, 1983

FREE RADICAL THEORY OF AGING: CONSEQUENCES OF MITOCHONDRIAL AGING

Denham Harman
University of Nebraska College of Medicine
Departments of Medicine and Biochemistry
Omaha, Nebraska 68105

Abstract

Mitochondria may serve as biologic clocks. This paper provides plausible explanations, based on mitochondrial aging, of some aging phenomenon: a) the inverse relationship between basal metabolic rate and life span; b) the antioxidants studies thus far which increase the average life span of mice, depress body weight and fail to lengthen maximum life span; c) the association of degenerative diseases with the terminal part of the life span; d) the exponential nature of the mortality curve, and e) the beneficial effect of caloric restriction on degenerative diseases and life span. A short discussion is also presented of the effect of exercise on life span and aging of muscle mitochondria.

Introduction

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 (1). These changes are attributed to the aging process. This process may be the deleterious, irreversible changes produced by the ubiquitous free radical reacitons (1-5). Defenses have evolved to limit such damage. They include antioxidants, such as the tocopherols (6) and carotenes (7) — like tocopherols, good quenchers of singlet oxygen, heme-containing proxidases (8) — catalase is an important member of this group, selenium-containing glutathione peroxidase (8), superoxide dismutases (9, 10), elevated serum uric acid levels (11), and DNA repair mechanisms (12, 13). Studies demonstrate that these defenses can be augmented. For example, dietary manipulations which would be expected to lower the rate of production of free radical reaction damage have been shown: 1) to increase the life span of mice (14-19), rats (20), fruit flies (18, 19), nematodes (21), and rotifers (22), as well as the "life span" of neurospora (23); 2) to inhibit development of some forms of cancer (24-26); 3) to enhance humoral and cell-mediated responses (27); and 4) to slow development of amyloidoses (28, 29) and the autoimmune disorders (30) of NZB and NZB/NZW mice. In addition, studies strongly suggest that such dietary changes will slow the deterioration of the cardiovascular (31-14) and central nervous systems with age (35-37).

Although antioxidants have been shown to increase both the mean and maximum life spans of fruit flies and nematodes, antioxidants have failed to increase the maximum life span of mice. Consideration of these failures led to the suggestion (5) that mitochondria may serve as biological clocks, the rate of mitochondrial aging being largely independent of the environment, i.e., influenced little by normal environmental fluctuations, except for food restriction, with 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. Others (38-42) have also stressed the role of mitochondria in determining life span. The major purpose of this paper is to provide plausible explanations, based on mitochondrial aging, of some mammalian aging phenomena: a) the inverse relationship between the basal metabolic rate and life span, b) the observation that antioxidants, which increase the life span of mice, depress body weight and fail to increase maximum life span, c) the association of degenerative diseases with the terminal part of the life span, d) the exponential nature of the mortality curve, and e) the beneficial effect of caloric restriction on life span and degenerative diseases; (a), (c) and (e) were discussed briefly in an earlier paper (5). A short discussion is also presented on the effect of exercise on life span and mitochondrial aging in muscle.

Discussion

Mitochondrial Aging

Mitochondria would be expected to be particularly subject to free radical induced change as over 90 percent of the oxygen utilized by mammals takes place in them. The rate of mitochondrial aging should be roughly proportional to the rate of oxygen consumption, which in turn is presumably largely under both nuclear and mitochondrial genetic control. The rate of production of mitochondrial damage may be essentially independent of the environment, except for the caloric intake, because of the highly selective permeability of the inner mitochondrial membrane (43, 44).

Changes with age in the steady state of the inner membrane components, presumably resulting from the presence of oxy-radicals (5, 45-48) — such as the superoxide radical, $O_2^{\bar{}}\bullet$, and HO• — generated as a result of reactions involving oxygen, include: a) increasing levels of peroxidized lipids (45, 49, 50), b) decreases in membrane fluidity (47, 49, 50), c) increased saturation of lipids (45, 50), d) changes in enzyme activities (47, 51-56), and

e) decreases in the rate of carrier-coupled flow of ATP and ADP (57) through the membrane. Free radical reactions may also contribute to changes in the inner membrane with age due to effects on both mitochondrial and nuclear DNA. Mitochondrial DNA codes for some inner membrane components (58); the remaining components, the majority, are coded for by nuclear DNA (58). Mitochondrial DNA is synthesized on the matrix side (59) of the inner membrane, an area that should be readily subject to free radical damage, while mitochondrial DNA that has been altered by free radical reactions could be lost due to the poor excision repair capacity (60) of mitochondria. Hence, changes in the inner membrane with age may be in part due to decreased coding capacity of mitochondrial DNA owing to progressive loss of mitochondrial DNA (61-64) and/or to altered function. Although the amount of nuclear DNA apparently does not decrease with age (65), coding capacity — including that for mitochondrial inner membrane components — may be progressively impaired with advancing age owing, at least in part, to free radical reactions (66), such as those responsible for the formation of disulfides (67, 68), in the chromatin. The changes observed in the inner mitochondrial membrane with age are most likely due to one or more of the following possibilities: 1) synthesis to replace "damaged" components is too slow; synthesis of mitochondria inner membrane proteins declines with age (69), 2) alterations in the membrane inhibit or prevent replacement, or 3) the "new" components are defective.

Collectively the above free radical induced changes in the steady state of mitochondria with age reasonably account for the decreases in mitochondrial number (70-72) with age and increased fragility (73) as well as the accumulation of lipofuscin-like material (74-77) in the mitochondria of postmitotic cells — these cells have been estimated to constitute more than 90 percent of all mammalian cells (78); apparently cell division "rejuvenates" mitochondria as only minor changes occur with age in the mitochondria of dividing cells (79). The increased flux of $O_2^{-}\bullet$ from mitochondria with age (45) is probably also a reflection of free radical damage and/or decreased scavenging by superoxide dismutase (45).

Basal Metabolic Rate and Life Span

The potential maximum life span, under "optimal living conditions" and in the absence of overt disease, of a given organism is the time required for intrinsic deleterious processes to cause the death and/or dysfunction of cells critical for function of the organism as a whole, e.g., cells in the respiratory center. The potential maximum average life span at birth is the average of the individual potential maximum life spans while the potential maximum life span of a species is that of the individual with the largest potential maximum life span. The actually determined maximum average life expectancy at birth and maximum life span should be somewhat less than the potential values because it seems very unlikely that living things ever live under "optimal living conditions" throughout life. In the case of man, the average maximum life expectancy at birth is about 85 years while the longest authenticated life span is about 114 years (80); the maximum life span is usually taken as 100 years because only a very small percentage of a given cohort live beyond that age. It is very likely that the rate of the gradual impairment of State 3 respiration (47, 51, 52, 54) with advancing age, most probably the result of deleterious free radical reactions, determines the maximum life span of an organism. With time the progressive impairment of State 3 respiration should eventually result in stress-induced episodic periods of inadequate production of ATP for cell maintenance and function. These episodic periods of ATP deficiency would be expected to increase progressively in both frequency and duration with advancing age and cause death due to the loss and/or dysfunction of cells critical for function of the organism as a whole, e.g., as indicated above, cells in the respiratory center. Since the rate of impairment of State 3 respiration should be higher, the higher the basal metabolic rate — more than 90 percent of the oxygen utilized by an organism is consumed in the mitochondria — the foregoing discussion provides a reasonable explanation for the inverse relationship between the maximum life spans of mammalian species and their basal metabolic rates (81).

Effect of Antioxidants on Body Weight and Average and Maximum Life Spans

Attempts to reduce the rate of free radical reaction induced "aging" of mitochondria with inhibitors are hampered as these inhibitors also depress (82-85) respiration and oxidative phosphorylation. These effects, which may largely account for the approximately 10 percent decrease in body weight (14, 15) of mice given effective anti-aging antioxidants, such as 2-mercaptoethylamine (2-MEA) or ethoxyquin, could arise in part by the reaction of the antioxidants with $O_2^{-}\bullet$ and/or HO• followed by combination of the antioxidant free radical with ubisemiquinone (86-88) or other free radicals involved in the respiratory chain.

Apparently as the dietary level of an antioxidant is increased average life span rises, owing to inhibition of free radical reactions involved in disease processes and production of non-specific damage, until the trend is halted and then reversed by the accompanying progressive decreases in oxidative phosphorylation. The dietary concentrations of antioxidants which result in the highest average life spans for groups of mice do not seem to be high enough to significantly depress mitochondrial aging as none that have

been evaluated increase the maximum life expectancy, with the possible exception of two pyridine derivatives: 1) 2-ethyl-6-methyl-3-hydroxypyridine (16) and 2) 2,6-dimethyl-3,5-diethoxycarbonyl-1,4-dihydropyridine (89).

In contrast to mice, both the average and maximum life spans of *Drosophila* (18, 19) and of nematodes (21) are increased by antioxidants. These effects, at least in *Drosophila,* are associated with decreased respiration (85) and, presumably, also of oxidative phosphorylation. As indicated above, antioxidants which have a beneficial effect on the maximum life spans of *Drosophila* and nematodes may do so largely by significantly decreasing the rate of production of mitochondrial damage by free radical reactions at a dietary level which does not seriously depress oxidative phosphorylation; this may also account for the approximately 25 percent increases in maximum life span of mice attributed to the two pyridine derivatives named above.

Degenerative Diseases and 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 both oxygen consumption and mitochondrial degeneration (5). As the rate of utilization of O_2 increases in different mammalian species, the necessary higher rate of O_2 transport is facilitated by an increasing ease of dissociation of O_2 from oxyhemaglobin (81) and an increased Bohr effect (81), i.e., more O_2 is dissociated from oxyhemoglobin by a given decrease in pH. The increased exposure of cellular and extracellular areas to O_2 resulting from higher O_2 transport should lead to an increase in the rate of oxidative change in these areas. The increasing flux of $O_2^{-\bullet}$ (45) from mitochondria with age should further contribute to the "base level of deleterious free radical reactions"; these increases should be offset to some extent by decreases in mitochondrial number (70-72). Further additions to the free radical reaction level with advancing age would be expected as ATP production, and with it reductive synthesis — for example, of free radical reaction inhibitors such as glutathione and NADPH, apparently declines with age owing to decreases in both mitochondrial number (70-72) and function (47, 51-57). Hence, to the extent that free radical reactions are involved in the pathogenesis of degenerative diseases (1, 3, 4) — such reactions are definitely associated with the development of some forms of cancer and are strongly implicated in the disorders of 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.

The Exponential Nature of the Mortality Curve

Decreases in reductive synthesis secondary to mitochondrial aging may be largely responsible for the exponential nature of the mortality curve (90, 91) in a manner analogous to the autoxidation of a lipid containing an antioxidant (92, 93). The rate of oxidative change in the lipid is slow at first, becoming progressively faster as the antioxidant is consumed through inhibition of free radical reactions. In living organisms the "antioxidant" level seemingly decreases with age because the production of ATP needed for reductive synthesis declines as mitochondria age, leading to progressive increases in free radical reaction levels, free radical damage and mortality.

Effect of Caloric Restriction on Life Span and Degenerative Diseases

The maximum life span can be increased by food restriction (94); this may be due in part to a decrease in the rate of mitochondrial aging. Increases in life span associated with life-long food restriction started after weaning, are accompanied by adaptive changes (94-99) — decreases in body weight and temperature, and changes in body composition and cellular metabolism — which presumably reflect attempts of the organism to maintain function with fewer calories. The reduction in body weight is probably largely a result of decreases in cell size, particularly of postmitotic cells, and only to a lesser extent of cell numbers; for example, liver and kidney cells of female C57BL/6J (99) mice, reduced in body weight by feeding *ad libitum* a 4 percent protein diet from weaning to 7 months or from 17 to 24 months of age, were smaller than corresponding cells of mice fed a 24 percent protein diet. Thus on the average it would be expected that restricted animals have more cells per unit of weight than those on an unrestricted diet. The rate of oxygen consumption per gram body weight by restricted animals is about the same, or somewhat greater, than for *ad libitum* fed ones 94, 95); restricted animals use more oxygen throughout life per gram body weight than nonrestricted animals as they live longer. Hence, since restricted animals contain more cells per gram of body weight than nonrestricted ones, the rate of oxygen utilization per cell is less. Further, assuming that both groups of animals have approximately the same number of cells, the total amount of oxygen consumed per cell in restricted animals throughout life is approximately the same as for cells in nonrestricted animals, for lifetime oxygen consumption by the longer-lived, lighter, restricted animals is about equal to that of *ad libitum* fed animals (95). In view of the foregoing and the comments above on oxygen consumption and mitochondrial aging, the beneficial effect of food restriction on life span may be mainly due to decreases in the rates of mitochondrial degradation and of degenerative

disease pathogenesis secondary to decreased rates of O_2 consumption.

There is a limit to the beneficial effect of food restriction on life span. Progressive decreases in the percentage of the *ad libitum* diet fed to groups of animals throughout life are associated with progressive increases in life span to some maximum value, while further dietary reduction results in decreases back to the *ad libitum* life span or shorter. The foregoing would be expected, even if the organism were provided with adequate intake of vitamins, minerals and essential fatty acids, because as calories are increasingly restricted, the rate of production of ATP is eventually reduced to levels below that needed to sustain reductive synthesis at a rate compatible with body maintenance and function and/or "adequate" defenses against free radical reactions so that with further food restriction the organism ages progressively faster.

Exercise and Life Span

Exercise of low work intensity, i.e., running, for prolonged periods, has been reported to increase the average, and possibly also, the maximum life span of rats (100). In man there is no certain evidence, although much suggestive evidence, that physical activity may extend average life expectancy (101-103) and little, if any, that it will increase the maximum life span. Apparently the expected adverse effects of increased O_2 utilization (104, 105) required to attain and maintain the "trained state" are offset, and probably more than offset, by beneficial effects of the widespread changes (106) — for example, decreases in cardiovascular risk factors (102, 107, 108) — induced in the body in response to the need to sustain prolonged periods of increased work.

The exercise-induced beneficial changes may include slower aging of mitochondria in the involved muscles. Endurance training increases the oxidative capacity of muscle through increases in the number of mitochondria — they can be doubled in rats (109). Whole body O_2 consumption is about the same in trained and untrained individuals under conditions of submaximal activity (110). Hence, the more numerous mitochondria in trained muscle should individually utilize less O_2 than those in untrained muscle. Deleterious changes arising from increased O_2 utilization (104, 105) during the relatively short fraction of the day needed to attain, and then maintain, the "trained state" are likely to be more than compensated for by the lower than normal O_2 consumption per mitochondria during submaximal activity. Thus, mitochondria in trained muscle may age at a slower than normal rate and so help to preserve normal muscle function until later in life.

Further Discussion of Mitochondrial Aging

Mitochondrial aging is superimposed on a dynamic process (111). All mitochondrial components are normally in a steady state with respect to synthesis and turnover. Increase in mass of mitochondria is through incorporation of increased amounts of newly synthesized constituents while the number of mitochondria, under physiological control, is altered by fusion or simple division. The rate of repair of mitochondrial damage, including that of mitochondrial DNA, in somatic cells is apparently less than that of damage production so that changes — aging — accumulate with time. These changes seemingly lead to progressive losses in mitochondrial function and eventual conversion of mitochondria to age pigment (74-77).

Mitochondrial aging is seen most prominently in postmitotic cells, particularly in fixed postmitotic cells, and to a minimal degree in normal dividing cells (79). These latter cells appear to have either more effective repair capabilities than postmitotic cells and/or, more likely, cell division partially rejuvenates cells, including their mitochondria. Dividing cells show only slight changes in fine structure with increasing age of the organism (79) and in transplantation studies individual replicating cell populations outline their host (112). Slower aging in dividing cells may be due in part to recombinational repair (113, 114) of both nuclear and mitochondrial DNA during mitosis; the 50-100 copies of mitochondrial DNA per mitochondria (115) may serve to facilitate recombinational repair. The foregoing also applies to cancer cells, but here mitochondrial repair must be complete in at least some cells for the cells to be propagated indefinitely. Likewise, meiosis (113, 114) in some manner restores mitochondria as well as nuclear DNA to the "base state" for species are essentially immortal.

Thus aging can be viewed as largely the result of inadequate repair of cellular damage to somatic cells, primarily in postmitotic cells, and in particular, damage to their mitochondria.

Suggestions for Future Studies

Future increases in the average life span of man, and hopefully of maximum life span, should be achieved by caloric-restricted diets, the components being selected to minimize adverse free radical reactions in the tissues and cells while at the same time providing essential nutrients — essential fatty acids, trace elements, "high quality" protein, etc., to which have been added one or more free radical reaction inhibitors. The diets and amounts and kinds of inhibitors should be selected to optimize the combination of, a) maximum reduction of adverse free radical reactions in the mitochondria and elsewhere and b) minimal reduction of ATP production, so as to allow the greatest increase in functional life span; these efforts should be offset to some extent by decreases in the concentration of oxy-radical protective enzymes as they are induced by peroxides (115, 116). The foregoing is not to say that additional means of combating the aging process may

not be found in the future but only that the approaches outlined briefly above, coupled with increased efforts to prevent and/or compensate for environmental contributions to functional decline — for example, of hearing and vision, provide opportunities today to delay the onset of diseases and to increase the span of healthy productive life.

References

1. Harman, D.: The aging process. Proc. Natl. Acad. Sci. USA, 78: 7124-7128, 1981.

2. Harman, D.: Aging: a theory based on free radical and radiation chemistry. J. Gerontol., 11: 298-300, 1956.

3. Harman, D.: Role of free radicals in mutation, cancer, aging, and the maintenance of life. Radiation Res., 16: 753-763, 1962.

4. Harman, D.: Prolongation of life: role of free radical reactions in aging. J. Amer. Geriatrics Soc., 17: 721-735, 1969.

5. Harman, D.: The biologic clock: the mitochondria? J. Amer. Geriatrics Soc., 20: 145-147, 1972.

6. Tocopherol, Oxygen, and Biomembranes, edited by de Duve, C., and Hayaishi, O., New York, Elsevier North-Holland, 1978.

7. Klebanoff, S. J.: Oxygen metabolism and the toxic properties of phagocytes. Ann. Intern. Med., 93: 480-489, 1980.

8. Flohé, L., Günzies, W. A., and Ladenstein, R.: Glutathione peroxidase, in Glutathione: Metabolism and Function, edited by Arias, I. M., and Jakoby, W. B., New York, Raven Press, 1976, pp. 115-138.

9. Fridovich, I.: Oxygen radicals, hydrogen peroxide, and oxygen toxicity, in: Free Radicals in Biology, Vol. 2, edited by Pryor, W. A., New York, Academic Press, 1977, pp. 239-277.

10. Superoxide and Superoxide Dismutases, edited by Michelson, A. M., McCord, J. M., and Fridovich, I., New York, Academic Press, 1977.

11. Ames, B. N., Cathcart, R., Schiviers, E., and Hochstein, P.: Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. Proc. Natl. Acad. Sci. USA, 78: 6858-6852, 1981.

12. Hart, R. W., D'Ambrosio, S. M., Ng, K. J., and Modak, S. P.: Longevity, stability and DNA repair. Mech. Ageing Dev., 9: 203-223, 1979.

13. DNA Repair Processes, edited by Nichols, W. W., and Murphy, D. G., Miami, Miami Symposium Specialists, 1977.

14. Harman, D.: Free radical theory of aging: effect of free radical inhibitors on the mortality rate of male LAF_1 mice. J. Gerontol., 23: 476-482, 1968.

15. Comfort, A.: Effect of ethoxyquin on the longevity of C3H mice. Nature, 229: 254-255, 1971.

16. Emanuel, N. M.: Free radicals and the action of inhibitors of radical processes under pathological states and aging in living organisms and in man. Quart. Rev. Biophysics, 9: 283-308, 1976.

17. Clapp, N. K., Satterfield, L. C., and Bowles, N. D.: Effects of the antioxidant butylated hydroxytoluene (BHT) on mortality in BALC/C mice. J. Gerontol., 34: 497-501, 1979.

18. Miquel, J., Economos, A. C.: Favorable effects of the antioxidants sodium and magnesium thiazolidine carboxylate on the vitality and life span of *Drosophila* and mice. Exper. Gerontol., 14: 279-285, 1979.

19. Miquel, J., Johnson, Jr., J. E.: Effects of various antioxidants and radiation protectants on the life span and lipofuscin of *Drosophila* and C57BL/6J mice. The Gerontologist, 15, II: 25, 1975 (abstr.).

20. Bun-Hoi, N. P., Ratsunamanga, A. R.: Age retardation in the rat by nordihydroguaiaretic acid. C. R. Seances Soc. Biol., Paris, 153: 1180-1182, 1959.

21. Epstein, J., Gershon, D.: Studies on aging in nematodes, IV. The effect of antioxidants on cellular damage and life span. Mech. Ageing Dev. 1: 257-264, 1972.

22. Enesco, H. E., Verdone-Smith, C.: α-Tocopherol increases lifespan in the rotifer *Philodina*. Exper. Gerontol., 15: 335-338, 1980.

23. Munkres, K. D., Minssen, M.: Aging of *Neurospora Crassa*. 1. Evidence for the free radical theory of aging from studies of a natural death mutant. Mech. Ageing Dev., 5: 79-98, 1976.

24. Harman, D.: Prolongation of the normal lifespan and inhibition of spontaneous cancer by antioxidants. J. Gerontol., 16: 247-254, 1961.

25. Harman, D.: Free radical theory of aging: effect of vitamin E on tumor incidence. The Gerontologist, 12, No. 3, Part 3: 33, 1972 (abstr.).

26. Wattenberg, L. L.: Inhibition of chemical carcinogenesis by antioxidants, in Carcinogenesis, Vol. 5, Modifiers of Chemical Carcinogenesis, edited by Slaga, T. J., New York, Raven Press, 1980, pp. 85-98.

27. Harman, D., Heidrick, M. L., and Eddy, D. E.: Free radical theory of aging: effect of free-radical-reaction inhibitors on the immune response. J. Amer. Geriatrics Soc., 25: 400-407, 1977.

28. Harman, D., Eddy, D. E., and Noffsinger, J.: Free radical theory of aging: inhibition of amyloidosis in mice by antioxidants; possible mechanism. J. Amer. Geriatrics Soc., 24: 203-210, 1976.

29. Harman, D.: Secondary amyloidosis and antioxidants. Lancet, 2: 593, 1980.

30. Harman, D.: Free radical theory of aging: beneficial effect of antioxidants on the life span of male NZB mice: role of free radical reactions in the deterioration of the immune system with age and in the pathogenesis of systemic lupus erythematosus. Age, 3: 64-73, 1980.

31. Harman, D.: Atherosclerosis: hypothesis concerning the initiating steps in pathogenesis. J. Gerontol., 12: 199-202, 1957.

32. Harman, D.: Atherogenesis in minipigs: effect of dietary fat unsaturation and of copper, in Atherosclerosis. Proceedings of the Second International Symposium, edited by Jones, R. J., New York, Springer-Verlag, 1970, pp. 472-475.

33. Moncada, S.: Prostacyclin and arterial wall biology. Atherosclerosis, 2: 193-207, 1982.

34. Ludwig, P. W., Hunninghake, D. B., and Hoidal, J. R.: Increased leukocyte oxidative metabolism in hyperlipoproteinaemia. The Lancet, 2: 348-350, 1982.

35. Harman, D., Eddy, D. E., and Seibold, J.: Free radical theory of aging: effect of dietary fat on central nervous system function. J. Amer. Geriatrics Soc., 24: 301-307, 1976.

36. Eddy, D. E., and Harman, D.: Free radical theory of aging: effect of age, sex and dietary precursors on rat brain docosahexanoic acid. J. Amer. Geriatrics Soc., 25: 220-229, 1977.

37. Brizzee, K. R., and Ordy, J. M.: Age pigments, cell loss and hippocampal function. Mech. Ageing Dev., 9: 143-162, 1979.

38. Fleming, J. E., Miquel, J., Cottrell, S. F., Yengoyan, L. S., and Economos, A. C.: Is cell aging caused by respiratory-dependent injury to the mitochondrial genome? Gerontology, 28: 44-53, 1982.

39. Miquel, J., and Fleming, J. E.: Experimental support for the mitochondrial mutation theory of cell aging. Age, 5: 142-143, 1982 (abstr.).

40. Miquel, J., Economos, A. C., Fleming, J., and Johnson, Jr., J. E.: Mitochondrial role in cell aging. Exper. Gerontol., 15: 575-591, 1980.

41. Munkres, K. D.: Ageing of *Neurospora Crassa.* IX. Microviscosity properties of mitochondrial membranes during normal and abnormal growth and development of an inositol auxtroph. Mech. Ageing Dev., 10: 173-197, 1979.

42. Huemer, R. P., Lee, K. D., Reeves, A. E., and Bickert, C.: Mitochondrial studies in senescent mice. II. Specific activity, buoyant density, and turnover of mitochondrial DNA. Exper. Gerontol., 6: 327-334, 1971.

43. Ernster, L., and Kuylenstierna, B.: Structure, composition and function of mitochondrial membranes, in Federation of European Biochemical Societies Symposium, Vol. 17, Mitochondria: Structure and Function, edited by Ernster, L., and Drahota, Z., New York, Academic Press, 1969, pp. 5-31.

44. Tzagoloff, A.: Mitochondria. New York, Plenum Press, 1982, pp. 199-233.

45. Nohl, H., and Hegner, D.: Do mitochondria produce oxygen radicals *in vivo*? Eur. J. Biochem., 82: 863-867, 1978.

46. Chance, B., Sies, H., and Boveris, A.: Hydroperoxide metabolism in mammalian organs. Physiol. Rev., 59: 527-605, 1979.

47. Nohl, H., Breuninger, V., and Hegner, D.: Influences of mitochondrial radical formation on energy-linked respiration. Eur. J. Biochem., 90: 385-390, 1978.

48. Nohl, H., Jordan, W., and Hegner, D.: Mitochondrial formation of HO• radicals by an ubisemiquinone-dependent reaction: an alternative pathway to the iron-catalyzed Haber-Weiss cycle. Hoppe-Seyler's Z. Physiol. Chem., 363: 599-607, 1982.

49. Vladimirov, Y. A., Olenev, V. I., Suslova, T. B., and Cheremisina, Z. P.: Lipid peroxidation in mitochondrial membrane. Adv. Lipid Res., 17: 173-249, 1980.

50. Hegner, D.: Age-dependence of molecular and functional changes in biological membrane properties. Mech. Ageing Dev., 14: 101-118, 1980.

51. Chiu, Y. J. D., and Richardson, A.: Effect of age on the function of mitochondria isolated from brain and heart tissue. Exper. Gerontol. 15: 511-517, 1980.

52. Chen, J. C., Warshaw, J. P., and Sanadi, D. R.: Regulation of mitochondrial respiration in senescence. J. Cell. Physiol., 80: 141-148, 1980.

53. Berdanier, C. D., and McNamara, S.: Aging and mitochondrial activity in BHE and Wistar strains of rats. Exper. Gerontol., 15: 519-525, 1980.

54. Murfitt, R. R., and Sanadi, D. R.: Evidence for increased degeneration of mitochondria in old rats. A brief note. Mech. Ageing Dev., 8: 197-201, 1978.

55. Abu-Erreish, G. M., and Sanadi, D. R.: Age related changes in cytochrome concentration of myocardial mitochondria. Mech. Ageing Dev., 7: 425-432, 1978.

56. Vann, A. C., and Webster, G. C.: Age-related changes in mitochondrial function in *Drosophila Melanogaster.* Exper. Gerontol., 12: 1-5, 1977.

57. Nohl, H., and Kramer, R.: Molecular basis of age-dependent changes in the activity of adenine nucleotide translocase. Mech. Ageing Dev., 14: 137-144, 1980.

58. Tzagoloff, A.: Mitochondrial biogenesis, in Mitochondria, New York, Plenum Press, 1982, pp. 235-266.

59. Shearman, C. W., and Kalf, G. F.: DNA replication by a membrane-DNA complex from rat liver mitochondria. Arch. Biochem. Biophys., 182: 573-586, 1977.

60. Fukunaga, M., and Yielding, K. L.: Fate during cell growth of yeast mitochondrial and nuclear DNA after photolytic attachment of the monoazide analog of ethidium. Biochem. Biophys. Res. Comm., 90: 582-586, 1979.

61. Massie, H. R., Colacicco, J. R., and Williams, T. R.: Loss of mitochondrial DNA with aging in the Swedish C strain of *Drosophila Melanogaster.* Age, 4: 42-46, 1981.

62. Polson, C. D., and Webster, J. C.: Loss of mitochondrial DNA in mouse tissues with age. Age, 5: 133, 1982 (abstr.).

63. Murray, M. A., and Balcavage, W. Y.: Changes in mitochondrial DNA during aging. Mech. Ageing Dev., 20: 233-241, 1982.

64. Litoshenko, A. Y., and Levitsky, E. L.: Replication of total and mitochondrial DNA in rat liver with aging. Exper. Gerontol., 16: 213-218, 1981.

65. Barrows, Jr., C. H., and Kokkonen, G. C.: Effect of age on the DNA, RNA and protein content of tissues, in CRC Handbook of Biochemistry in Aging, edited by Florini, J. R., Boca Raton, Florida, CRC Press, 1981, pp. 115-134.

66. Harman, D.: Chromatin template capacity: effect of oxygen. The Gerontologist, 7, No. 3, Part 2: 29, 1967 (abstr.).

67. Tas, S., Tam, C. F., and Walford, R. L.: Disulfide bonds and the structure of the chromatin complex in relation to aging. Mech. Ageing Dev., 12: 65-80, 1980.

68. Tas, S., and Walford, R. L.: Increased disulfide-mediated condensation of the nuclear DNA-protein complex in lymphocytes during postnatal development and aging. Mech. Ageing Dev., 19: 73-84, 1982.

69. Marcus, D. L., Ibrahim, N. G., and Freedman, M. L.: Age-related decline in the biosynthesis of mitochondrial inner membrane proteins. Exper. Gerontol., 17: 333-341, 1982.

70. Herbener, G. H.: A morphometric study of age-depedent changes in mitochondrial populations of mouse liver and heart. J. Gerontol., 31: 8-12, 1976.

71. Stocco, D. M., and Hutson, J. C.: Quantitation of mitochondrial DNA and protein in the liver of Fischer 344 rats during aging. J. Gerontol., 33: 802-809, 1978.

72. Tachi, H., and Sato, T.: Age changes in size and number of mitochondria of human hepatic cells. J. Gerontol., 23: 454-461, 1968.

73. Weinbach, E. C., and Garbus, J.: Oxidative phosphorylation in mitochondria from aged rats. J. Biol. Chem., 234: 412-417, 1959.

74. Miquel, J., Oro, J., Bensch, K. G., and Johnson, Jr., J. E.: Lipofuscin: fine-structural and biochemical studies, in Free Radicals in Biology, Vol. 3, edited by Pryor, W. A., New York, Academic Press, 1977, pp. 133-182.

75. Miquel, J., Lundgren, D. R., and Johnson, Jr., J. E.: Spectrophotofluorometric and electron microscopic study of lipofuscin accumulation in the testes of aging mice. J. Gerontol., 33: 5-19, 1978.

76. Koobs, D. H., Schultz, R. L., and Jutzy, R. V.: The origin of lipofuscin and possible consequences to the myocardium. Arch. Pathol. Lab. Med., 102: 66-68, 1978.

77. Lambert, J. R., Shing, C. L., and Pritzker, K.P.H.: Brown bowel syndrome in Chron's disease. Arch. Pathol. Lab. Med., 104: 201-205, 1980.

78. Kohn, R. R.: Intrinsic aging of postmitotic cells, in Aging Gametes, edited by Blandau, R. J., New York, S. Karger, 1975, pp. 1-18.

79. Miquel, J., Economos, A. C., Bensch, K. G., Atlan, H., and Johnson, Jr., J. E.: Review of cell aging in *Drosophila* and mouse. Age, 2: 78-88, 1979.

80. Comfort A.: The Biology of Senescence, 3rd edition. New York, Elsevier, 1979, pp. 81-86.

81. Schmidt-Nielsen, K.: Energy metabolism, body size, and problems of scaling. Fed. Proc., 29: 1524-1532, 1970.

82. Lelièvre, P., and Liébecq, C.: Metabolic effects of sulfur-containing radioprotective agents, in International Encyclopedia of Pharmacology and Therapeutics. Section 79. Sulfur-Containing Radioprotective Agents, edited by Bacq, Z. M., New York, Pergamon Press, 1975, pp. 239-259.

83. Pardini, R. S., Heidker, J. C., and Fletcher, D. C.: Inhibition of mitochondrial electron transport by nor-dihydroguaiaretic acid (NDGA). Biochem. Pharmacol., 19: 2695-2699, 1970.

84. Pardini, R. S., Cotlin, J. C., Heidker, J. C., and Folkers, K.: Specificity of inhibition of Coenzyme Q — enzyme systems by lipoidal benzoquinone derivatives. J. Med. Chem., 15: 195-197, 1972.

85. Miquel, J., Fleming, J., and Economos, A. C.: Antioxidants, metabolic rate and aging in *Drosophila*. Arch. Gerontol. Geriatrics, 1: 159-165, 1982.

86. Cadenas, E., Boveris, A., Ragan, C. I., and Stoppani, A. O.M.: Production of superoxide radicals and hydrogen peroxide by NADH-ubiquinone reductase and ubiquinol-cytochrome c reductase from beef-heart mitochondria. Arch. Biochem. Biophys., 180: 248-257, 1977.

87. Boveris, A., Cadenas, E., and Stoppani, A. O.: Role of ubiquinone in the mitochondrial generation of hydrogen peroxide. Biochem. J., 156: 435-344, 1976.

88. Ingledew, W. J., Salerno, J. C., and Ohnishi, T.: Studies on electron paramagnetic resonance spectra manifested by a respiratory chain hydrogen carrier. Arch. Biochem. Biophys. 177: 176-184, 1976.

89. Emanuel, N. M., Duburs, G., Obukhov, L. K., and Uldrikis, J.: Drug for prophylaxis of aging and prolongation of lifetime. Chem. Abst., 94: 9632a, 1981.

90. Dublin, L. L., Lotka, A. J., And Spiegelman, M.: Length of Life. New York, The Ronald Press, 1949, p. 16.

91. Upton, A. C.: Pathobiology, in Handbook of the Biology of Aging, edited by Finch, C. E., and Hayflick, L., New York, Van Nostrand Reinhold, 1977, pp. 513-535.

92. Lundberg, W. O.: Mechanisms, in Lipids and Their Oxidation, edited by Schultz, H. W., Day, E. A., and Sinnhuber, R. O., Westport, The Avi Publ. Co., 1962, pp. 31-50.

93. Augustin, M. A., and Berry, S. K.: Effectiveness of antioxidants in refined, bleached and deodorized palm olein. J. Amer. Oil Chem. Soc., 60: 105-108, 1983.

94. Barrows, Jr., C. H., and Kokkonen, G. C.: Diet and life extension in animal model systems. Age, 1: 130-142, 1978.

95. Masoro, E. J., Yu, B. P., and Bertrand, H. A.: Action of food restriction in delaying the aging process. Proc. Natl. Acad. Sci., USA, 79: 4239-4241, 1982.

96. Yu, B., Masoro, E. J., Murata, I., Bertrand, H. A., and Lynd, F. T.: Life span study of SPF Fischer 344 male rats fed *ad libitum* or restricted diets: longevity, growth, lean body mass and disease. J. Gerontol., 37: 130-141, 1982.

97. Berg, B. N., and Simms, H. S.: Nutrition and longevity in the rat. III. Food restriction beyond 800 days. J. Nutr., 74: 23-32, 1961.

98. Ross, M. H.: Aging, nutrition and hepatic enzyme activity patterns in the rat. J. Nutr., 97: Suppl. 1, Part II, 563-602, 1969.

99. Barrows, Jr., C. H., and Kokkonen, G.: The effect of various dietary restricted regimes on biochemical variables in the mouse, Growth, 42: 71-85, 1978.

100. Goodrick, C. L.: Effects of long-term voluntary wheel exercise on male and female Wistar rats. Gerontology, 26: 22-33, 1980.

101. Shephard, R. J.: Physical Activity and Aging. Chicago, Year Book Publ. Co., 1978, p. 273.

102. Leon, A. S., and Blackburn, H.: The relationship of physical activity to coronary heart disease and life expectancy. Ann. N. Y. Acad. Sci., 301: 561-578, 1977.

103. Brown, K. S., and Milvy, P.: A critique of several epidemiological studies of physical activity and its relationship to aging, health, and mortality. Ann. N. Y. Acad. Sci., 301: 703-719, 1977.

104. Davies, K. J. A., Quintanilha, A. T., Brooks, G. A., and Packer, L.: Free radicals and tissue damage produced by exercise. Biochem. Biophys. Res. Comm., 107: 1198-1205, 1982.

105. Dillard, C. J., Litov, R. E., Savin, W. M., Dumelin, E. E., and Tappel, A. L.: Effects of exercise, vitamin E, and ozone on pulmonary function and lipid peroxidation. J. Appl. Physiol., 45: 927-932, 1978.

106. Cantu, R. C., Means, R. H., Micheli, L. J., Siegel, A. J., and Vinger, P.: Sports Medicine in Primary Care. Toronto, Collamore Press, 1982, p. 14.

107. Bassler, T. J.: Marathon running and immunity to atherosclerosis. Ann. N. Y. Acad. Sci., 301: 579-592, 1977.

108. Hartung, G. H., Foreyt, J. P., Mitchell, R. E., Vlasek, I., and Gotto, Jr., A. M.: Relation of diet to high-density-lipoprotein cholesterol in middle-aged marathon runners, joggers, and inactive men. New Engl. J. Med., 302: 357-361, 1980.

109. Davies, K. J. A., Packer, L., and Brooks, G. A.: Biochemical adaptation of mitochondria, muscle, and whole-animal respiration to endurance training. Arch. Biochem. Biophysics, 209: 539-554, 1981.

110. Patch, L. D., and Brooks, G. A.: Effects of training on $\dot{V}O_2$ max and $\dot{V}O_2$ during two running intensities in rats. Pflugers Arch., 386: 215-219, 1980.

111. Tzagoloff, T.: Mitochondria. New York, Plenum Press, 1982, p. 237.

112. Schneider, E. L., Sternberg, H., Tice, R. R., Senula, G. C., Kram, D., Smith, J. R., and Bynum, G.: Cellular replication and aging. Mech. Ageing Dev., 9: 313-324, 1979.

113. Medvedev, Z. A.: On the immortality of the germ line: genetic and biochemical mechanisms. A Review. Mech. Ageing Dev., 17: 331-359, 1981.

114. Bernstein, H.: Recombinational repair may be an important function of sexual reproduction. Biosci., 33: 326-331, 1983.

115. Tzagoloff, T.: Mitochondria. New York, Plenum Press, 1982, p. 268.