# Antioxidants and aging[1,2]

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**ABSTRACT** Aging in mammalian species appears to be the result of normal developmental and metabolic processes. In spite of the vast complexity of aging processes, relatively less complex processes such as longevity determinant genes (LDGs) may exist governing aging rate. Much experimental data exists indicating a causative role of oxyradicals in aging processes. In testing the hypothesis that antioxidants may represent LDGs, a positive correlation in the tissue concentration of specific antioxidants with life span of mammals was found. These antioxidants include superoxide dismutase, carotenoids, $\alpha$-tocopherol, and uric acid. We also found that the resistance of tissues to spontaneous autoxidation and the amount of oxidative damage to DNA correlates inversely with life span of mammals. These results suggest a role of oxyradicals in causing aging and that the antioxidant status of an individual could be important in determining frequency of age-dependent diseases and duration of general health maintenance. *Am J Clin Nutr* 1991;53:373S–9S.

**KEY WORDS** Aging, antioxidants, life span, carotenoids, retinoids, uric acid, free radicals

## Introduction

Humans have the longest life span of mammalian species and consume more energy over their life span on a per weight basis (1). These two unique biological characteristics reflect an unusually slow innate rate of aging of all physiological functions. Although much work in biogerontology involves studies on how humans and other animals age, little research has been oriented toward obtaining an understanding of what determines the longevity of humans and other animals. Consequently, a large gap exists in our knowledge about the unique characteristics of human biology that might help explain why human aging processes are so extraordinarily slow. Clearly, any insight into understanding why humans live as long as they do has the potential to contribute importantly toward developing effective means to ensure a healthy, productive life span for more individuals in our population and to provide new concepts for the development of treatments for the vast number of diseases initiated by aging processes affecting so many older citizens (2).

The vast complexity of the physiological aspects of aging impedes progress in understanding the biological mechanisms causing aging. This is particularly true since we still are far from understanding the normal nonaging characteristics of human biology. Moreover, increased knowledge limited only to understanding the mechanism of aging could lead to little increased insight as to how to develop more effective methods for treating age-related dysfunctions. That is, knowing what is wrong (the causes of aging) does not necessarily lead most efficiently to the best means of correcting the problem (decreasing aging rate).

In contrast to this inherent difficulty in studying aging mechanisms, a study of longevity rather than aging mechanisms may involve less complex biology and lead more directly to possible useful methods in the treatment of dysfunctions caused by aging processes (3). It is this latter approach to the problem of aging that has determined the direction of our research program.

## Longevity determinant genes

Comparative and evolutionary studies from our laboratory have suggested that the cause of aging is pleiotropic in nature, being the result of long-term toxic side effects of normal metabolic and developmental processes (4, 5). Thus, aging is not the result of a genetic program expressing specific genes to age an organism for some type of evolutionary-related benefit. From these results, it was reasoned that the aging rate of a species must be controlled by defense or protective processes acting against the unfavorable side reactions of normal and essential biological processes.

The genetic processes governing aging rate in different mammalian species may be remarkably less complex than the processes causing the aging process itself (3, 4, 6, 7). In fact, only small differences in the expression of key regulatory genes may be involved in controlling aging rate. These conclusions led to the proposal that a common set of longevity determinant genes may exist in all mammalian species independent of their life span. These longevity determinant genes would govern aging rate by changes in their timing and extent of expression. Thus, the genetic mechanisms of speciation are similar to those governing aging rate; that is, by gene regulatory processes and not by different kinds of genes (8).

The concept and data supporting the prediction that specific longevity determinant genes may exist are not generally well known. Instead it is usually thought that life span is governed largely by the biological status of the entire organism. This view is consistent with the concept that, because of the complexity of aging, there must be many or multiple causes of aging. Multiple causes of aging would also collectively govern aging rate. These concepts have generated the suggestion that significant decreases in human aging rate are not likely to be achieved by

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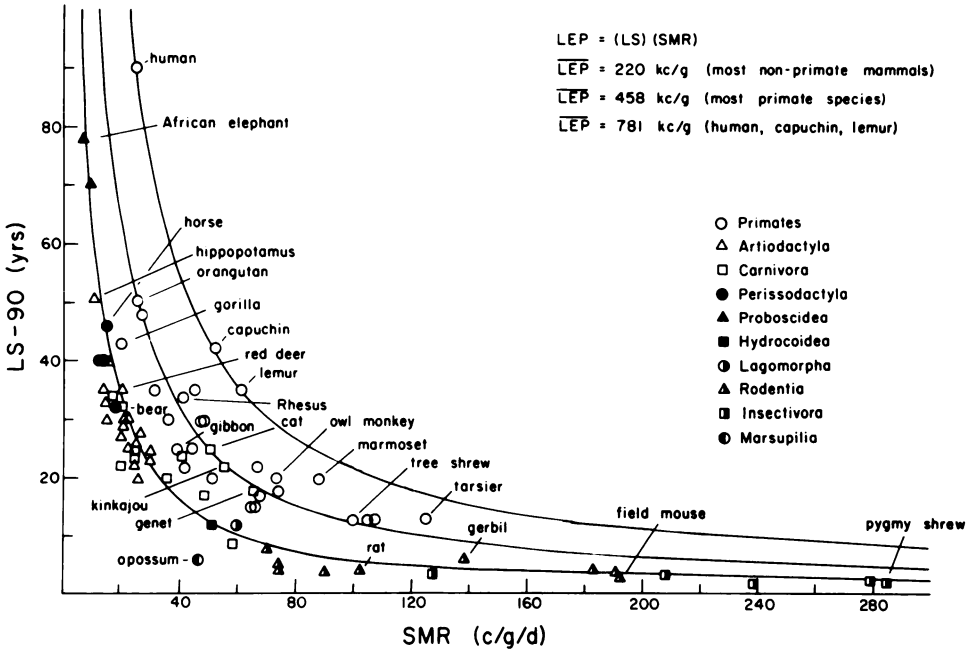


FIG 1. Life span energy potential (LEP) in mammalian species. SMR expressed in calories·g body $wt^{-1} \cdot d^{-1}$. LEP in kilocalories per gram body weight. Reprinted with permission from Cutler (10).

a few primary controlling factors. Thus, in view of these two contrasting perspectives on the mechanisms of aging and those processes governing aging rate and to their difficult predictions as to the feasibility of extending life span, it becomes particularly important to test the longevity determinant gene hypothesis.

## Oxygen radicals as a primary aging process

Comparative and evolutionary studies have indicated that aging may be the result of pleiotropic effects of two major biological processes (4, 5, 8, 9). These involve differentiation and devel-

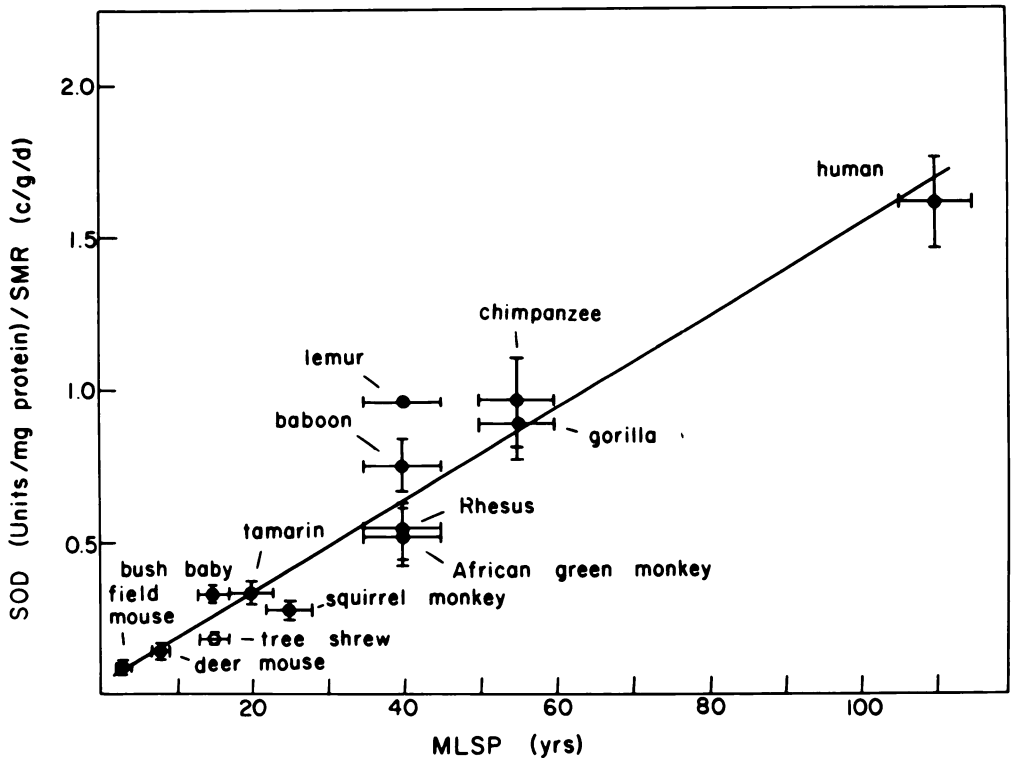


FIG 2. Superoxide dismutase concentration per SMR in liver of primate species as a function of MLSP. Reprinted with permission from Cutler (12).

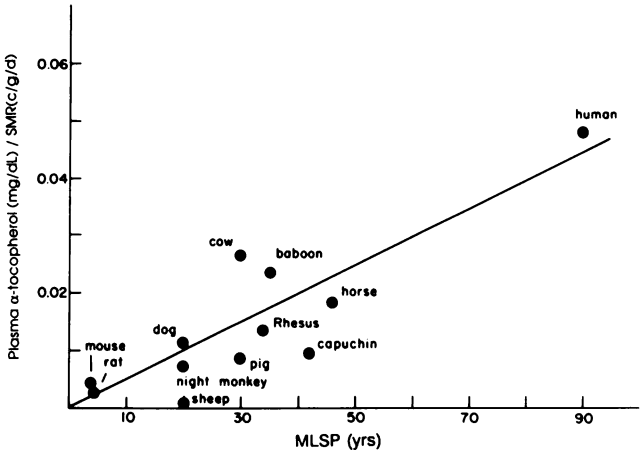


FIG 3. Plasma levels of vitamin E per SMR as a function of MLSP in mammalian species. Non-life span data from Altman and Dittmer (13) and Bernirschke et al (14). Reprinted with permission from Cutler (15).

opmental processes and energy metabolic pathways. Aging effects associated with developmental processes appear difficult to counter and may have been largely dealt with during the evolution of greater longevity in mammalian species by the decreasing rate of development. Aging rate is generally correlated with developmental rate. Thus, by slowing down rate of development, aging effects in the expression of development-related genes are simply postponed in time in longer-lived species.

However, much more is known about the possible aging effects of energy metabolism. It is well known that aging rate is generally proportional to metabolic rate. This correlation has led to the definition of a new longevity parameter called life span energy potential (LEP). The LEP value for a species is defined as the product of its average life-long specific metabolic rate (SMR) and its maximum life span potential (MLSP). Humans have the highest life span energy potential of ~800 kc/g, nonhuman primates ~400 kc/g; and for most other mammals it is ~200 kc/g (**Fig 1**). These data indicate that aging rate is related to metabolic rate or the rate oxygen is utilized per unit weight of tissue. Since rate of oxygen metabolism is positively correlated with rate of oxygen radical production, these data suggest that active oxygen species may be important as a causative factor in aging. If this is true, then various strategies acting to decrease the toxic effects of active oxygen species may represent a class of longevity determinant processes.

## Antioxidants and life span

This prediction was tested by comparing the tissue concentrations of various endogenous antioxidants in mammalian species as a function of their MLSP and LEP. Our first study found an excellent positive correlation in the concentration of superoxide dismutase (SOD) per SMR vs MLSP (11) (**Fig 2**). It is important to note that the correlation of SOD vs LEP is equivalent to the correlation of the ratio of SOD per SMR vs MSLP. Both types of analyses examine if the amount of antioxidant protection per amount of oxyradicals formed in a tissue is related to the aging rate of a species. The comparison of SOD per SMR vs MLSP over many different mammalian species is based on the concept that common strategies exist for the evolution of longevity within the domain of the mammalian species and that there is roughly a proportion between SMR and the actual amount of oxidative stress or oxyradicals being produced. Thus, the SOD experiments provide support for the hypothesis that primary aging processes (active oxygen species) may exist and that a simple increase in concentration of an antioxidant (a regulatory gene change) may act as a longevity determinant process.

Other antioxidants showing a similar positive correlation with life span are α-tocopherol (**Fig 3**), carotenoids (**Fig 4**), and urate (**Fig 5**). The results found for ascorbate were more complex to evaluate. Plasma concentrations of ascorbate showed no correlation, and some tissues like liver (**Fig 6**) showed an increase in ascorbate for short-lived species below MLSPs of 40 y. On the other hand, brain tissue did show a significant positive increase in ascorbate concentration with MLSP, including those of human (18) (**Fig 7**).

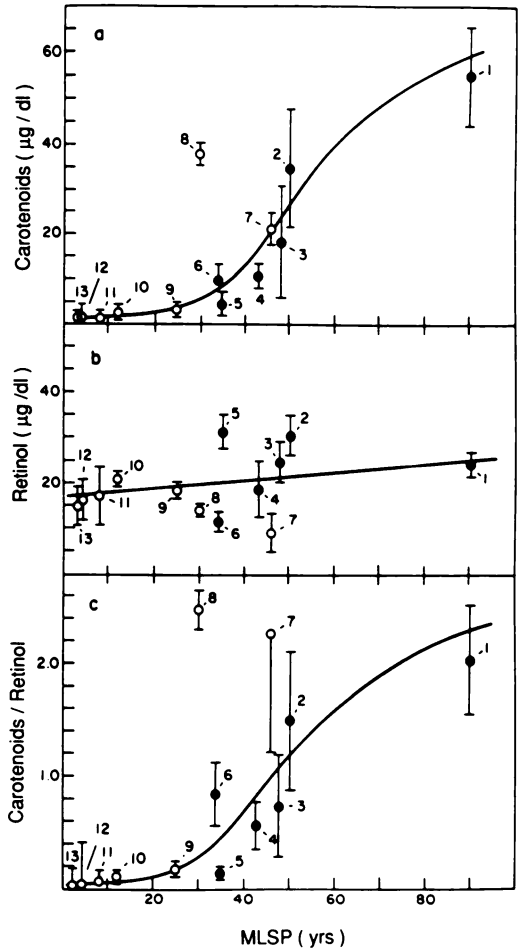


FIG 4. Serum carotenoids and retinol concentrations as a function of MLSP. ●, primates. ○, nonprimate species. Error bars represent SD. For primates only, linear correlation coefficients are: a. $r = 0.926$ ($P < 0.010$) b. $r = 0.163$ ($P > 0.10$), and c. $r = 0.905$ ($P < 0.020$). 1, human; 2, orangutan; 3, chimpanzee; 4, gorilla; 5, gibbon; 6, Rhesus; 7, horse; 8, cow; 9, goat; 10, rabbit; 11, deer mouse; 12, rat; and 13, field mouse. Reprinted with permission from Cutler (16).

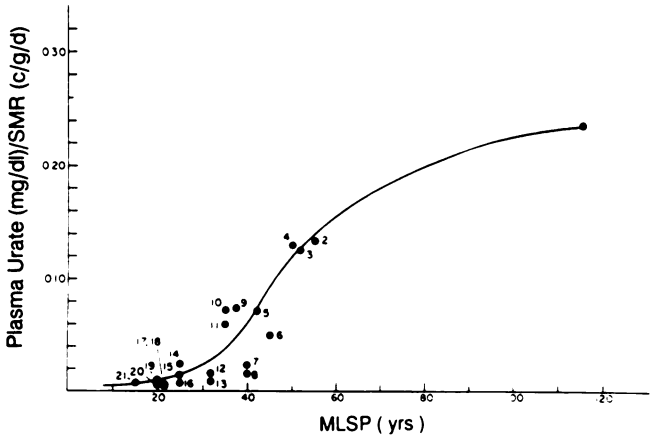


FIG 5. Plasma urate levels per SMR in primates as a function of MLSP. Values taken from the literature. Species: 1, human; 2, chimpanzee; 3, orangutan; 4, gorilla; 5, gibbon; 6, capuchin; 7, macaque; 8, baboon; 9, spider monkey; 10, Siamang gibbon; 11, woolly monkey; 12, langur; 13, grivet; 14, tamarin; 15, squirrel monkey; 16, night monkey; 17, potto; 18, patas; 19, galago; 20, howler monkey; 21, tree shrew. Reprinted with permission from Cutler (17).

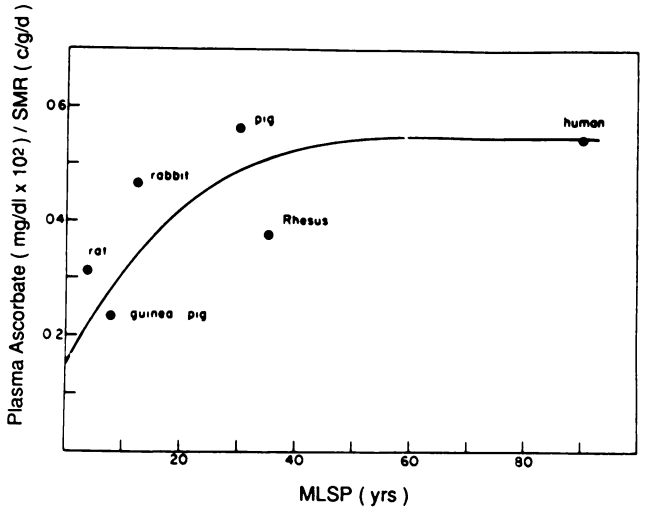


FIG 6. Ascorbate concentration per SMR in liver of mammalian species as a function of MLSP. Reprinted with permission from Cutler (12).

Some antioxidants such as catalase, glutathione, and glutathione peroxidase were found to have an inverse correlation with increased MLSP or LEP value (12). Also, total liver cytochrome P-450 content is inversely related to MLSP of mammalian species, which may prove interesting if P-450 is a potential source of oxyradicals (12).

These latter results were certainly unexpected and clearly need an explanation. In this regard, we investigated total tissue sensitivity to oxidative stress by measuring rate of spontaneous autoxidation of tissue homogenates (19). Brain tissues were taken from different species and their rates of spontaneous autoxidation showed an inverse correlation with MLSP (**Fig 8**); similar results were found for kidney tissues (data not included).

These data are summarized in **Table 1** and suggest that a simple increase in antioxidant concentration is not necessarily the only method that has evolved to cope with oxyradicals during the evolution of longevity. Other strategies appear to include a decrease in the rate of production of oxyradicals and an increase in the intrinsic resistance of tissue components (membranes) to peroxidative reactions.

## Aging is dysdifferentiation

If aging is caused in part by active oxygen species, then what is the mechanism? A common concept is that oxyradicals destroy cells and/or enzymes so that older animals simply run out of enough cells or enzymes to maintain optimum health status. There is, however, little data supporting this "wear-and-tear" hypothesis of aging. Indeed, tissues from older animals appear to have lost very few cells, and viability of the cells in older individuals appear quite normal in terms of housekeeping functions (3, 20, 21). Thus, if aging is not a result of an accumulation of damage causing improper cell function, then what could be the mechanism of aging at the cellular level? We have considered the possibility that active oxygen species could react with the genetic apparatus of cells, resulting in a slightly different and less efficient cell. This concept is represented by the dysdifferentiation hypothesis of aging, which states that improper gene regulation in those cells primarily involved in regulatory functions of the whole organism (eg, neuroendocrine system) could act as primary aging processes (21).

Such change in gene expression could conceivably account for aging as we observe it without gross cell impairment or cell loss. Of course, such changes in cell function or cell death could finally result, being an expression of the last stages of the dysdifferentiative effect. We have been testing this hypothesis by searching for evidence of improper gene expression. In these studies, an increased expression of hemoglobin, endogenous viral genes, and the c-*myc* oncogene in brain, liver, and other tissues was found with increasing age in mice (22–25). In addition, we have recently found that genomic 5-methyldeoxycytosine con-

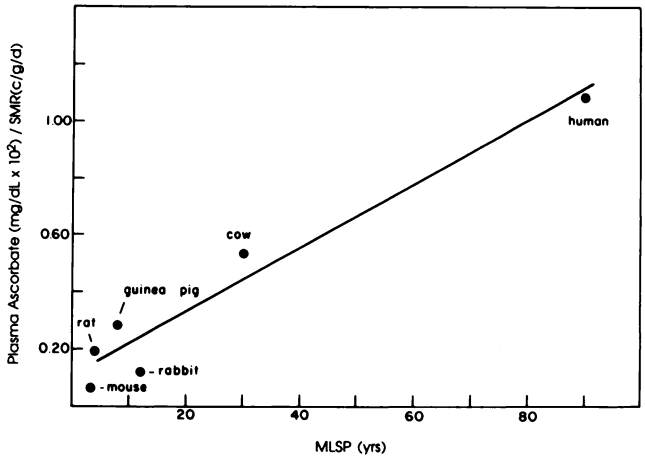


FIG 7. Ascorbate concentration per SMR in brain of mammalian as a function of MLSP. Reprinted with permission from Cutler (12).

TABLE 1
Summary of major experimental results searching for unique human biological characteristics as related to life span and oxyradicals

I. Antioxidants showing significant positive correlation in tissue concentrations per SMR vs life span, where human clearly has highest value
   1. Superoxide dismutase (CuZn and Mn)
   2. Carotenoids (including β-carotene)
   3. α-Tocopherol
   4. Uric acid
   5. Ascorbate (specific tissues only)
II. Antioxidants showing significant negative correlations in liver tissue concentration per SMR vs life span, where human clearly has least value
   1. Catalase
   2. Glutathione
   3. Glutathione peroxidase
III. Tissue concentration of liver cytochrome P-450 and glutathione transferase generally decrease with increasing life span of mammalian species, where human has lowest amount
IV. Rate of spontaneous autoxidation of tissues decreases with increasing life span of mammalian species, where human tissues are most resistant to autoxidation
V. Amount of steady-state oxidative damage in DNA (8-OHdG/dG) in liver tissue decreases with increasing life span of mammalian species

tent decreases with age in a number of different tissues and species and that the rate of this decrease was related to the aging rate of the animal (26). These data, in addition to morphological evidence from other laboratories indicating the actual presence of dysdifferentiated cells populating normal tissues, provide some credibility to the dysdifferentiation hypothesis of aging.

In the evaluation of these results it is important to note that oxygen radicals can alter the differentiated state of cells at extremely low concentrations and that antioxidants protect against these effects. The rate a cell undergoes dysdifferentiation may be related therefore to its steady state level of oxidative stress. Thus, defense and protective mechanisms acting against active oxygen species could represent a class of longevity determinant processes. This conclusion leads to the prediction that longevity would be governed by processes acting to stabilize proper gene expression and thus the stability of the differentiated state of the cell.

Unfortunately, very little is presently understood concerning the mechanisms acting to stabilize the proper differentiated state of cells. Perhaps antioxidants and other strategies used in a cell to lower oxidative stress represents a major mechanism stabilizing proper differentiation. In this role they would consequently represent a class of longevity determinant processes. To understand stabilizing mechanisms of differentiation is a problem now relevant to biogerontology, and a major aim of our research program is to focus on this new emerging area of research.

## Steady-state level of oxidative damage in DNA

If oxidative stress is actually less in longer-lived mammalian species and if the genetic apparatus plays a key role in determining the proper functional state of a cell, then we would expect that longer-lived species would have a lower level in steady-state oxidative damage. This possibility is presently being investigated by measuring the amount of 8-hydroxydeoxyguanosine per deoxyguanosine in liver DNA and urine samples taken from different mammalian species (27). Preliminary results are shown in **Figure 9,** confirming the prediction of a general inverse correlation of oxidative damage with increasing life span, with human having the least amount of oxidative damage.

## Summary and conclusion

Unique characteristics of human biology may exist that could determine their extraordinary capacity for general health maintenance and longevity as compared with all other mam-

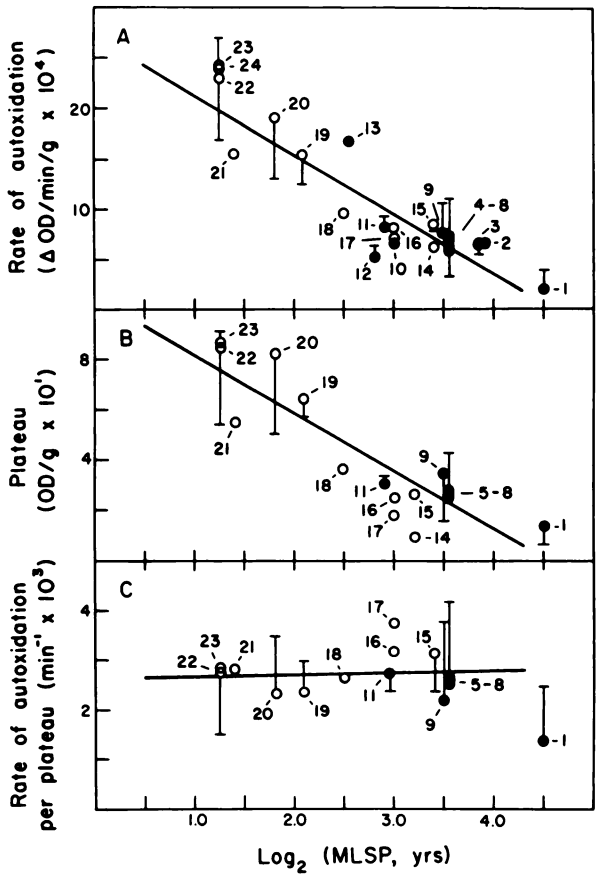


FIG 8. Characteristics of autoxidation of brain homogenate as a function of MLSP. ●, primates. ○, nonprimate species. 1, human; 2, orangutan; 3, chimpanzee; 4, gibbon; 5, baboon; 6, Rhesus; 7, pig-tailed macaque; 8, African green monkey; 9, mangabey; 10, marmoset; 11, squirrel monkey; 12, galago; 13, tree shrew; 14, cow; 15, pig; 16, dog; 17, sheep; 18, rabbit; 19, white-footed mouse; 20, deer mouse; 21, rat; 22, field mouse; 23, C57B:/6J mouse; 24, summation of 23 mouse strains. Reprinted with permission from Cutler (19). Error bars represent SD. Linear correlation coefficients of lines drawn and probability that there is no correlation are as follows: (A) $r = -0.891$, $P < 0.001$; (B) $r = -0.884$, $P < 0.001$; (C) $r = 0.0779$, $P > 0.1$. Reprinted with permission from Cutler (19).

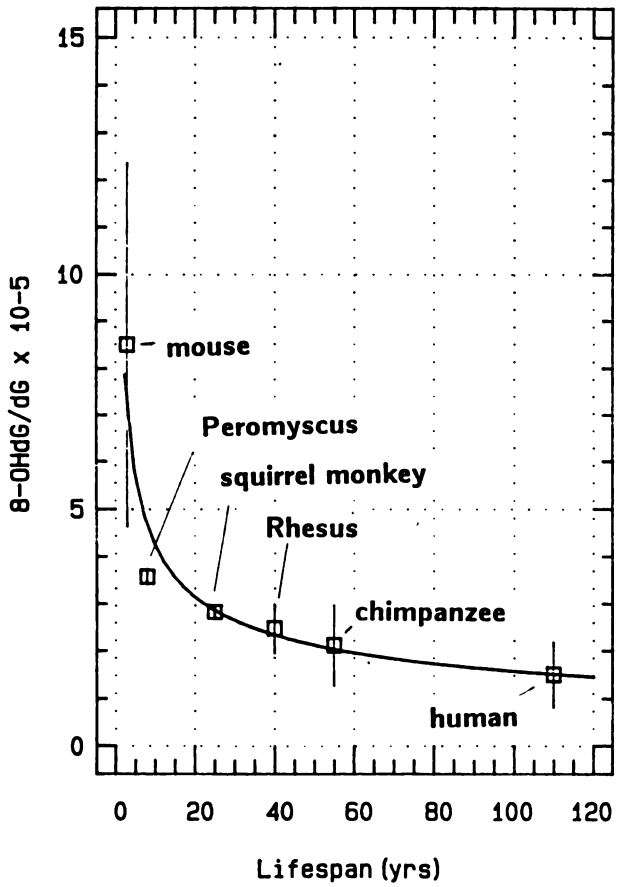


FIG 9. 8-Hydroxydeoxyguanosine content in liver DNA as a function of life span in mammals. 8-OHdG was determined in purified DNA sample hydrolyzed to nucleosides by enzymatic digestion. Nucleoside preparations were analyzed by HPLC using an electrochemical detector at 600 mv.

malian species. This prediction is based on comparative and evolutionary studies suggesting that a common set of specific longevity determinant processes exists in all mammals. Our work in testing this hypothesis has centered on two basic but interrelated questions: *1*) is the cause of aging largely the result of dysdifferentiative processes and *2*) is the rate of aging governed by mechanisms acting to stabilize the proper differentiated state of cells?

Stability of gene regulation has been investigated by searching for improper gene expression (endogenous retroviruses, oncogenes). Results suggest that improper expression of some genes does occur throughout life span.

Active oxygen species may be a cause of improper gene regulation, and therefore mechanisms to decrease their presence would lower the rate dysdifferentiation would occur. This possibility has been investigated in different mammalian species by measuring *1*) the steady-state concentration levels of various antioxidants, *2*) the rate of autoxidation of tissues, and *3*) the concentration of specific oxidative damage products in DNA (8-hydroxydeoxyguanosine). Results of these experiments indicate that tissues of longer-lived species have higher innate resistance to oxidation and consequently less oxidative damage.

Taken together, these results support the longevity determinant gene hypothesis where oxidative stress plays a causal role in aging and life span is governed by a number of different mechanisms acting to lower oxidative stress in cells. However, it should be emphasized that our results so far are largely of a correlative nature and do not demonstrate cause and effect relationships. Experimental work is now critically needed to more definitively test the longevity determinant gene hypothesis through direct intervention of potential longevity determinant genes now identified.

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