Peroxide-producing potential of tissues: Inverse correlation with longevity of mammalian species

(aging/antioxidants/autoxidation/lipid peroxidation/oxygen radicals)

RICHARD G. CUTLER

Gerontology Research Center, National Institute on Aging, Francis Scott Key Medical Center, Baltimore, MD 21224

ABSTRACT   Peroxidation reactions may cause many of the dysfunctions associated with aging. Accordingly, the 30-fold differences in aging rate among the mammalian species could be determined in part by peroxidation defense processes. This possibility was tested by measuring the spontaneous autoxidation of aerobically incubated brain and kidney tissue homogenates of 24 different mammalian species as a function of their maximum lifespan potential. Results show a statistically significant inverse correlation between both the rate of autoxidation and the amount of peroxidizable substrate with maximum lifespan potential. Kinetic analysis of the data indicates that the amount of peroxidizable substrate was the major factor determining the rate of autoxidation. For human tissues, antioxidants also appear to contribute to their unusually low sensitivity to peroxidation. These results support the hypothesis that aging may be caused in part by oxygen radicals initiating peroxidation reactions and that peroxidation defense processes are involved in governing the longevity of mammalian species.

Much evidence now supports oxygen radicals as a causative factor in a number of pathologies (1–4), but their importance to aging has largely been based on circumstantial evidence (5–8). Past work in our laboratory has shown a positive correlation in the concentration of a number of different antioxidants per specific metabolic rate with the maximum lifespan potential of mammalian species. These results suggested the potential importance of antioxidants in governing aging rate. However, negative or no correlations with some antioxidants were also found (7, 9, 10). Because of the difficulties in determining the total defense these antioxidants were contributing, a method was needed to estimate the net sensitivity a tissue has against oxidative damage.

Aerobically incubated tissue homogenates spontaneously undergo autoxidation (11–15). The rate and extent of this reaction depends upon factors affecting its initiation, propagation, and termination (16–19). Much evidence also indicates that the sensitivity of tissue homogenates to autoxidation in vitro reflects their normal in vivo sensitivity to similar oxidative processes (20–26). Thus, a measure of the autoxidation of aerobically incubated tissue homogenates appears to represent one means of assessing a complex array of tissue defenses against oxygen radicals that may be important in governing the aging rate of different mammalian species.

MATERIALS AND METHODS

Source of Tissues. Tissues were from Yerkes Regional Primate Center, Duke University Primate Facility, the Wash-ington Regional Primate Center, and Pel-Freez Biologicals. *Peromyscus maniculatus* (deer mouse), *Peromyscus leucopus* (white-footed mouse), and *Mus musculus* (field mouse) were from our own animal colony.

Estimation of Species Longevity. Maximum lifespan potentials (MLSPs) are from a literature survey covering over 100 zoos world-wide (27). From these data, mean ± SD values were calculated. Lifespan energy potential (LEP, kcal/g) is the product of MLSP (in yr) and specific metabolic rate (SMR, cal/g·day) and reflects the total lifespan energy expenditure of specific tissues on a per unit weight basis (9). This conclusion is based on the evidence that individual organs of an animal have SMR values proportional to total body SMR (28) and basal SMR is proportional to a daily average SMR (29–31).

Assay of Peroxidation Potential. Peroxidation potentials of tissue homogenates were determined by the thiobarbituric acid (TBA) assay as described by Stocks et al. (15) and Boehme et al. (32). Tissues were thawed, minced, and washed in ice-cold 0.15 M KCl solution to remove all traces of blood. Variable amounts of blood were found to have no significant effect on the rate of peroxidation. One gram of tissue (wet weight) was added to 6 ml of 0.1 M Tris·HCl/0.135 M KCl, pH 7.4, buffer and homogenized with a Polytron (Brinkmann) for 15 sec at a setting of 8. The homogenate was centrifuged at 750 × g for 10 min at 4°C. A 4-ml sample of supernatant was removed and added to 16 ml of cold Tris/KCl buffer in a 100-ml beaker placed in ice. Crude homogenates without centrifugation also gave similar relative TBA values. Fifty microliters of the antibiotic gentamycin sulfate at 50 mg/ml was added to each beaker to prevent bacterial growth. No difference was found in TBA values with or without use of the antibiotic for up to 4 hr of incubation. After the zero-time sample had been taken, the beakers were incubated at 37°C in a rotary water bath oscillating at 200 rpm. The water bath was covered with a lid and fresh water-saturated air was circulated within the incubator to maintain a constant concentration of oxygen in the incubator and prevent sample evaporation.

Two-milliliter samples were withdrawn from the beakers at timed intervals and the TBA assay was carried out as previously described (15, 32). Concentration of the tissue homogenate (33 mg/ml) and pH of the TBA assay mixture (7.6) were adjusted to ensure optimal sensitivity and linear concentration dependence (32). The uncorrected absorbance measurements at 532 nm indicate total TBA-reacting material (TBARM). Although the TBA assay does not measure total peroxides, it does represent a reasonable approximation of the relative peroxide concentration produced during the incubation (13, 15, 33). TBARM correlates well with production of chemiluminescence, oxygen consumption, the loss of

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Abbreviations: MLSP, maximum lifespan potential; LEP, lifespan energy potential; SMR, specific metabolic rate; TBA, thiobarbituric acid.

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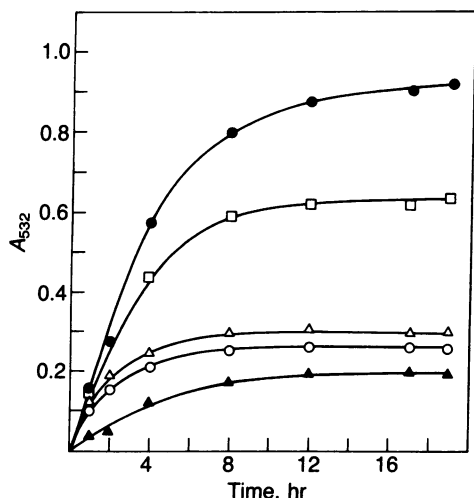
Proc. Natl. Acad. Sci. USA 82 (1985)   4799

FIG. 1. Kinetics of autoxidation of brain homogenate. Species and MLSPs are ●, field mouse, 3.5 yr; □, white-footed mouse, 8 yr; △, squirrel monkey, 18 yr; ○, rhesus monkey, 34 yr; ▲, human, 90 yr.

unsaturated fatty acids (15), and the concentration of peroxides determined by other methods (34).

RESULTS

Kinetics of Autoxidation of Brain Tissue Homogenates.
Typical data of the kinetics of autoxidation of brain tissue homogenates for representative mammalian species are shown in Fig. 1. The rate of reaction is found to be approximately linear for the first few hours, and the plateau value is reached after 18 hr of incubation. Both the rates of reaction and the plateau values are seen to be proportional to one another and to decrease as the MLSP of the species increases.

The autoxidation reaction was found to follow the equation

$$P = P_0(1 - e^{-kt}),$$

in which P represents the concentration of peroxides (A_{532}/g) as a function of time (t), P_0 is the initial concentration of peroxidizable material at zero time, and k is the reaction rate constant. At small values of time:

$$dP/dt = P_0k \text{ or } (dP/dt)/P_0 = k.$$

P_0 represents an estimation of the total amount of peroxidizable substrate in the tissue, and k is related to the tissue levels of antioxidants and factors other than substrate that govern autoxidation rate. Oxygen is maintained at a constant concentration during the reaction and does not enter as a variable. The amount of peroxides detected at zero time was always found to be close or equal to zero. Thus, the amount of peroxides measured is dependent only on the time the homogenates are incubated and therefore does not include peroxides that could have formed prior to the incubation of the tissue.

Brain Autoxidation as a Function of Lifespan Potential.
Tabulation of the peroxidation data for brains of 24 different mammalian species as a function of MLSP, LEP, and SMR is presented in Table 1 and illustrated in Figs. 2–4. In Fig. 2, an excellent linear inverse correlation is shown for both the

Table 1. Autoxidation of brain tissue from primate and nonprimate species

Ident. no.	Species (common name)	MLSP, yr	SMR, cal/g·day	LEP, kcal/g	dP/dt,* $(\Delta A_{532}/\text{min·g}) \times 10^4$	P_0,† $(A_{532}/g) \times 10^1$	k,‡ $\text{min}^{-1} \times 10^3$
				Primates			
1	Human	90	24.8	815	2.08 ± 1.89 (44)	1.54 ± 0.950 (22)	1.35 ± 1.22
2	Orangutan	50	24.5	447	6.85 (1)	—	—
3	Chimpanzee	48	26.8	470	6.62 ± 1.16 (1)	—	—
4	Gibbon	35	44.6	570	7.24 (1)	—	—
5	Baboon	35	36.7	469	7.57 ± 3.51 (26)	2.82 ± 0.995 (17)	2.68 ± 1.55
6	Rhesus monkey	34	41.3	512	7.39 ± 2.78 (36)	2.48 ± 0.680 (18)	2.68 ± 1.24
7	Pig-tailed macaque	34	41.3	512	7.17 ± 4.62 (20)	2.68 ± 1.62 (15)	2.67 ± 2.35
8	African green monkey	34	41.3	512	6.91 (1)	2.75 (1)	2.51
9	Mangabey	33	40.3	485	7.57 ± 3.62 (5)	3.45 ± 1.91 (2)	2.19 ± 1.60
10	Marmoset	20	88.4	645	6.94 (1)	—	—
11	Squirrel monkey	18	73.9	485	8.32 ± 0.58 (4)	3.03 ± 0.245 (3)	2.74 ± 0.293
12	Galago	17	67.6	419	5.03 ± 1.2 (2)	—	—
13	Tree shrew	13	99.6	473	16.9 (1)	—	—
				Nonprimate mammals			
14	Cow	30	15	164	6.49 (4)	0.950 (4)	6.81
15	Pig	30	20	219	8.59 ± 1.27 (2)	2.75 ± 0.560 (2)	3.12 ± 0.785
16	Dog	20	35	255	8.24 (4)	2.57 (4)	3.20
17	Sheep	20	25.6	186	6.80 (4)	1.82 (4)	3.73
18	Rabbit	12	58.7	257	9.60 (4)	3.65 (4)	2.65
19	White-footed mouse	8	150	438	15.5 ± 3.34 (15)	6.44 ± 0.700 (9)	2.40 ± 0.579
20	Deer mouse	6	150	320	19.1 ± 6.14 (11)	8.26 ± 3.30 (4)	2.31 ± 1.18
21	Rat	4	104	151	15.5 (4)	5.48 (4)	2.83
22	Field mouse	3.5	182	232	23.1 ± 6.64 (23)	8.49 ± 3.02	2.72 ± 1.24
23	C57BL/6J mouse	3.5	182	232	24.1 ± 7.38 (8)	8.63 ± 0.512 (4)	2.79 ± 0.870
24	Mouse strains§	3.5	182	232	24.0 ± 3.85 (46)	—	—

Experimental data are presented as mean ± SD, with the number of different individuals used in a determination given in parentheses. Ident. no., identification number.
*Rate of autoxidation.
†Saturation plateau of autoxidation reaction.
‡Rate constant of autoxidation reaction.
§Summation of 23 different laboratory mouse strains, two animals of each strain.
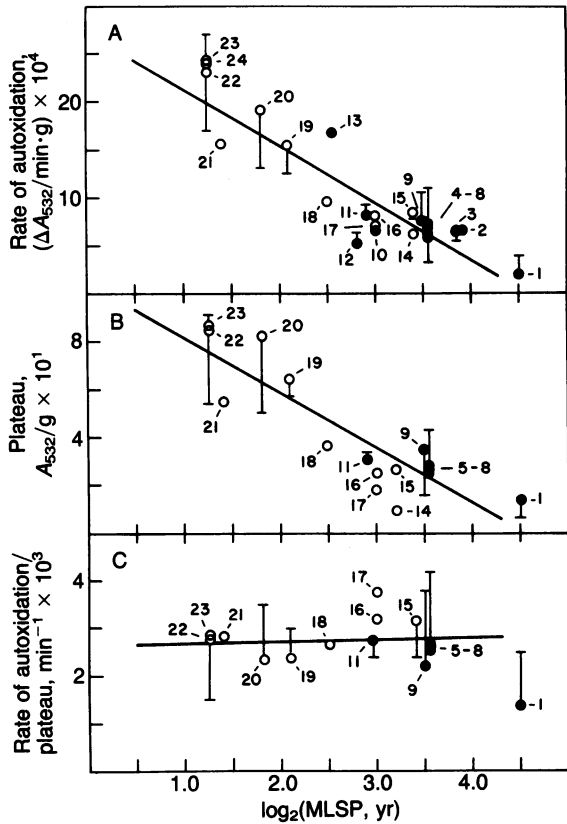
FIG. 2. Characteristics of autoxidation of brain homogenate as a function of MLSP. ●, Primates; ○, nonprimate mammalian species. Identification numbers of species are listed in Table 1. Error bars represent SD. The same symbols are used in Figs. 3 and 4. Linear correlation coefficients of lines drawn and probabilities that there is no correlation are as follows: (A) $r = -0.891$, $P < 0.001$; (B) $r = -0.884$, $P < 0.001$; (C) $r = 0.0779$, $P > 0.1$.

rate of peroxidation (dP/dt) and the plateau value (P_0) as a function of log₂(MLSP). In contrast, the reaction rate constant (k) is remarkably constant for most species, the outstanding exception being humans, for which the k value is unusually low. These results indicate that the major determinant of peroxidation potential for most species (except human) is the P_0 value of the tissue. This conclusion is further supported by Fig. 3, showing that dP/dt is linearly correlated with P_0, but again humans appear out of line, having a lower than normal dP/dt value related to P_0.
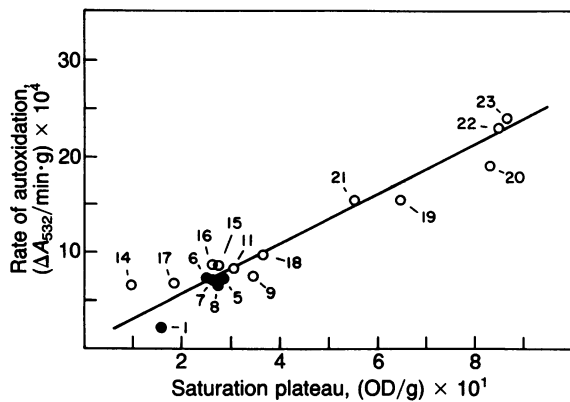
Autoxidation of Brain as a Function of LEP. Superoxide

FIG. 3. Rate of autoxidation of brain homogenate as a function of saturation plateau value. Linear correlation coefficient $r = 0.967$, $P < 0.001$.
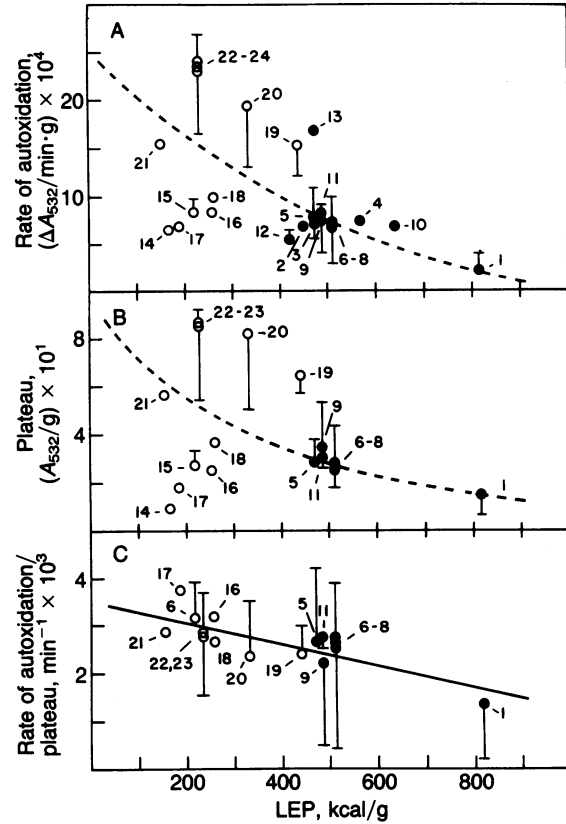

FIG. 4. Rate of autoxidation of brain homogenate as a function of LEP. The linear correlation coefficient of the line drawn in C is $r = -0.765$; $P < 0.001$. The broken lines in A and B were drawn by eye.

dismutase levels in brain have been found to be proportional to a species' LEP value (34, 35). Thus, LEP value may reflect a tissue's total antioxidant capacity or ability to resist the toxic effects of a life-long utilization of oxygen. The peroxidation potential of a tissue in relation to a species' LEP value is given in Fig. 4 and Tables 1 and 2. No significant correlation is found of their dP/dt and P_0 values with LEP. However, the reaction rate constant, k, shows a significant inverse correlation. These results indicate that factors determining the reaction rate constant of tissue peroxidation may be important in determining a species' LEP value.

Autoxidation of Brain as a Function of Age. It was necessary to determine if peroxidation potential was dependent upon age or sex. Typical results of dP/dt as a function of age are illustrated in Fig. 5 for rhesus monkeys and humans. No significant sex- or age-dependent correlation was found.
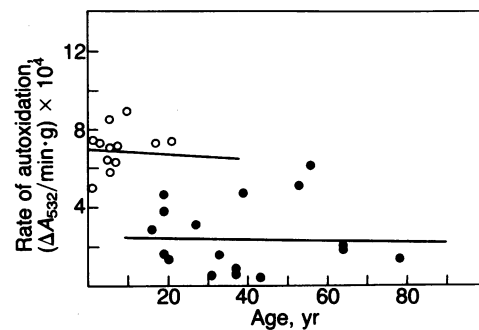

FIG. 5. Rate of autoxidation of brain homogenate as a function of age. ●, Humans; ○, rhesus monkeys. Each point represents one individual.

Medical Sciences: Cutler

Proc. Natl. Acad. Sci. USA 82 (1985)   4801

Table 2. Autoxidation of kidney tissue from primate and nonprimate species

Ident. no.	Species	MLSP, yr	SMR, cal/g·day	LEP, kcal/g	dP/dt, $(\Delta A_{532}/\text{min·g}) \times 10^4$	P_0, $(A_{532}/g) \times 10^1$	k, $\text{min}^{-1} \times 10^3$
1	Human	90	24.8	815	1.50 ± 0.82 (10)	0.492 ± 0.268 (9)	3.04 ± 2.34
2	Orangutan	50	24.5	447	3.04 ± 2.22 (2)	1.15 ± 0.778 (2)	2.64 ± 2.62
3	Chimpanzee	48	26.8	470	1.93 ± 1.27 (6)	0.683 ± 0.458 (6)	2.82 ± 2.64
4	Gorilla	43	19.6	307	2.72 (1)	0.720 (1)	3.77
5	Rhesus monkey	34	41.3	512	4.74 ± 2.99 (5)	1.26 ± 0.699 (5)	3.76 ± 3.15
6	Tree shrew	13	99.6	473	7.39 (4)	1.30 (4)	5.68
7	White-footed mouse	8	150	438	6.43 (4)	1.41 (4)	4.56
8	Deer mouse	6	150	328	8.22 ± 0.45 (2)	2.33 ± 1.07	3.52 ± 1.62
9	Field mouse	3.5	182	232	13.8 (4)	2.71 (4)	5.09

Similar results were also found for baboon, pig-tailed macaque, deer mouse, and field mouse (data not shown).

Autoxidation of Kidney. Although brain was the major tissue of interest in these studies, it was considered important to examine another tissue as a control against the possibility that brain was unique in some manner. Kidney tissue was used for this purpose, and results are tabulated in Table 2 and illustrated in Fig. 6. Less tissues and fewer different species were available for these studies, and so the results are more variable. Nevertheless, as is apparent on comparing the figures for brain and kidney, the results for both tissues are remarkably similar.

DISCUSSION

The rate of autoxidation of aerobically incubated brain and kidney homogenates was found to be inversely related to the MLSP of mammalian species. Most importantly, human brain and kidney tissue homogenates are found to be most resistant to autoxidation, in agreement with humans having the longest MLSP. Because the rate of autoxidation of tissues *in vivo* appears to correlate well with the rate of peroxidation (20–26) *in vitro*, these findings suggest that (*i*) aging of mammalian species is caused in part by oxygen radical-initiated peroxidation of tissues and (*ii*) longevity of different mammalian species is determined in part by intrinsic differences in tissue peroxidation potential.

Although the factors determining peroxidation potential of a tissue are complex, a simple kinetic analysis of data as carried out in this study suggests that for most species, the major factor is the concentration of peroxidizable substrate. However, humans and possibly the *Peromyscus* rodent species (deer mouse and white-footed mouse) are clearly outstanding exceptions: their tissues may contain unusually high concentrations of antioxidants and possibly other defenses against peroxidation reactions.

The different P_0 values suggest that a change in the composition of lipid membranes occurred during the evolution of increased MLSP for the mammalian species, resulting in the tissues being less susceptible to oxygen radical-initiated peroxidation reactions. During the recent hominid evolution of MLSP leading to humans (36), an increased concentration of some antioxidants in tissues also appears to have occurred, contributing importantly to the unusually high MLSP and LEP values for *Homo sapiens* (7, 9, 10). The possibility that differences in membrane composition as well as antioxidant concentration play a role in determining the sensitivity of cells to oxygen radicals and thus the duration of healthy human lifespan would appear to warrant further study of this area, aimed towards our understanding the biological basis of human longevity.
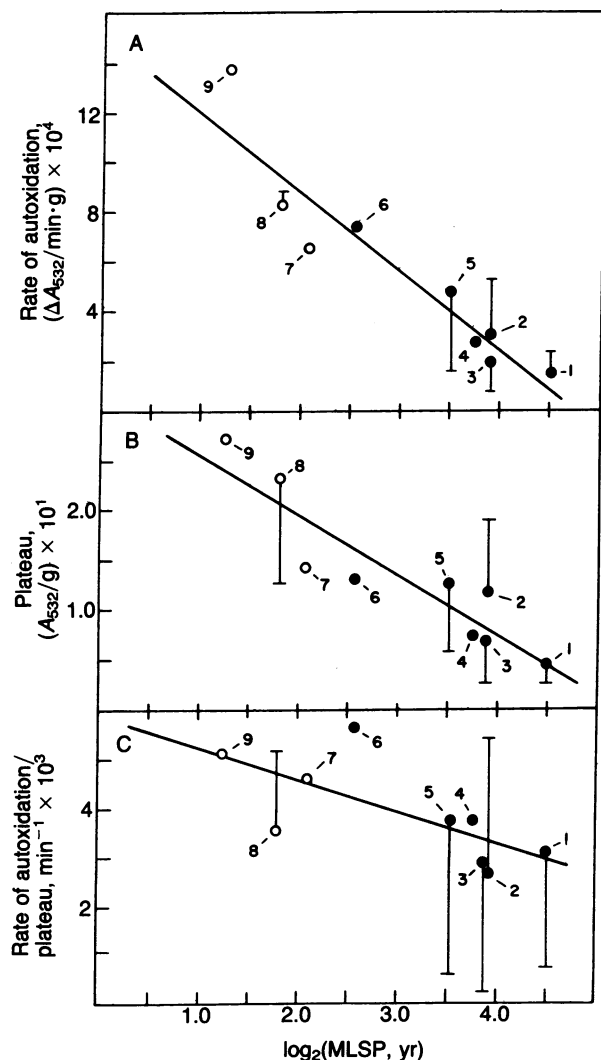
FIG. 6. Characteristics of autoxidation of kidney homogenate as a function of MLSP. ●, Primates; ○, nonprimate mammals. Identification numbers of species are listed in Table 2. Error bars represent SD. Linear correlation coefficients of lines drawn are as follows: (*A*) $r = -0.935$, $P < 0.001$; (*B*) $r = -0.914$, $P < 0.001$; (*C*) $r = -0.876$, $P > 0.01$.

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