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MINI REVIEW

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The rate of free radical production as a determinant of the rate of aging: evidence from the comparative approach

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Abstract The relationship of oxidative stress with maximum life span (MLSP) in different vertebrate species is reviewed. In all animal groups the endogenous levels of enzymatic and non-enzymatic antioxidants in tissues negatively correlate with MLSP and the most longevous animals studied in each group, pigeon or man, show the minimum levels of antioxidants. A possible evolutionary reason for this is that longevous animals produce oxygen radicals at a low rate. This has been analysed at the place where more than 90% of oxygen is consumed in the cell, the mitochondria. All available work agrees that, across species, the longer the life span, the lower the rate of mitochondrial oxygen radical production. This is true even in animal groups that do not conform to the rate of living theory of aging, such as birds. Birds have low rates of mitochondrial oxygen radical production, frequently due to a low free radical leak in their respiratory chain. Possibly the low rate of mitochondrial oxygen radical production of longevous species can decrease oxidative damage at targets important for aging (like mitochondrial DNA) that are situated near the places of free radical generation. A low rate of free radical production can contribute to a low aging rate both in animals that conform to the rate of living (metabolic) theory of aging and in animals with exceptional longevities, like birds and primates. Available research indicates there are at least two main characteristics of longevous species: a high rate of DNA repair together with a low rate of free radical production near DNA. Simultaneous consideration of these two characteristics can explain part of the quantitative differences in longevity between animal species.

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Key words Aging · Maximum longevity · Mitochondria · Free radical production · Antioxidants

Abbreviations *GSH* reduced glutathione · *GSSG* oxidized glutathione · *LEP* life energy potential · *MLSP* maximum life span · *mtDNA* mitochondrial DNA · *SOD* superoxide dismutase · VO_2 oxygen consumption

Introduction

Many theories have been proposed to explain the basis of aging. These have been classified into organ theories (immune or neuroendocrine), physiological theories (free radical, cross-linking and waste-product accumulation) and genome-based theories (somatic mutation, error theory and program theory) of aging (Hayflick 1985). Organic theories lack universal applicability and the changes observed can be due to more basic phenomena in the cellular or genomic components of the organ. Some theories, like the error catastrophe theory relating to protein synthesis, are now in general disfavour. Although the basic causes of aging continue to be unknown, the theory more frequently being tested nowadays is the free radical theory of aging (Harman 1968). It can partially explain the accumulation of deleterious changes in macromolecules during aging, since potentially damaging free radicals are continuously produced during normal cellular respiration. It can explain, at least in part, aging changes central to other basic theories: cross-linking, waste-product and lipofuscin accumulation or somatic mutations. Nevertheless, it must be emphasized that aging is a complex process and that most probably there are multiple mechanisms of aging (Jazwinski 1996). On the other hand, classification of aging theories can obscure obvious overlaps. Even though the free radical theory is classified above as "physiological" and is often considered a "stochastic" theory, this is not contradictory to a genetic determi-

nation of aging. Rates of free radical production and elimination in a healthy cell depend on multiple polypeptides and enzymes that are encoded in the genome. Important reviews about the possible causes of aging have been published (Kirkwood 1996; Martin et al. 1996).

Many papers have appeared in recent years concerning variations in free radical related parameters in animal tissues as a function of age, and some studies have shown experimentally and longitudinally the effect of antioxidants on mean life span. Papers have also been published on the possible relationship between free radical-related parameters and MLSP, a genetically determined parameter not affected greatly by the environmental conditions of animal maintenance. Different animal species can have very different MLSPs. The comparative approach can supply insights into the fundamental processes determining aging (the longer the MLSP the slower the rate of aging), whereas mean life span is also related to environmental conditions of animal maintenance. Much previous work showed that increasing the tissue levels of antioxidants increases mean life span, leading to more rectangular survival curves by reducing early deaths, but this did not increase MLSP or reduce the rate of aging. In this paper we review available evidence relating antioxidants and free radicals to the MLSP of different vertebrate species.

Among the different possible causes of aging, the intensity of the basal metabolic rate stands as the parameter most clearly related to aging and MLSP in the majority of animal species studied. This relationship is known as the "rate of living" theory of aging (Pearl 1928). Even though the first global approach to this problem can be attributed to Pearl (1928), the constancy of the total metabolic output (LEP = life energy potential, the number of total joules transformed per kilogram during the whole life span) in some mammalian species had already been described much earlier (Rubner 1908; Table 1).

The specific oxygen consumption (per gram weight) is negatively correlated with body weight with an exponent of −0.25 in mammals, whereas MLSP is positively correlated with body weight with an exponent of around +0.20, also in mammals. Thus, LEP (Rubner 1908; Cutler 1984a), which is equal to the specific metabolic rate multiplied by the MLSP, is essentially a size-independent parameter in the majority of mammals, since the two exponents (−0.25 and about +0.20) tend to cancel each other.

The constancy of LEP in the majority of mammals is not restricted to this particular animal group, since MLSP also increases as a function of body weight in birds with an exponent of +0.19 (Fig. 1). Although there are not enough reliable data, present information suggests that the rate of living theory also basically holds true for other vertebrates and invertebrates, since large animals in each phylogenetic group also tend to live longer and the negative relationship between specific metabolic rate and body weight with an exponent of −0.25 is a universal characteristic in all vertebrate and invertebrate animal groups so far studied (Schmidt-Nielsen 1984; Prinzinger 1993). Furthermore, in ectothermic animals the MLSP increases in proportion to the decrease in metabolic rate brought about by a decrease in the maintenance temperature. The metabolic rate corresponds to the number of joules transformed per unit time and weight. Nevertheless, since it is closely related to the rate of oxygen consumption (VO_2) in aerobic animals, it is tempting to suggest that the rate of living theory arises from the possibility that if a given animal species consumes a large amount of O_2 at mitochondria (to sustain a high basal metabolic rate) it would also produce a large amount of oxygen radicals per unit time at these organelles. The basic process underlying the rate of living theory could then be the rate of production of O_2 radicals. However, we must critically consider that if the basal metabolic rate is faster, the tissues not only consume more O_2 per unit time but they also synthesize and degrade many kinds of molecules faster and a myriad of biochemical processes also run at a quicker pace. Thus, the basic phenomenon underlying the rate of living theory could be the rate of O_2 radical production but, in principle, it could also be many other factors related to the rate of many cellular processes.

An interesting approach to clarify this problem is to study animal species with different LEPs. When this

Table 1 Constancy of life energy potential (*LEP*) in mammals as described by Rubner (1908) (*MLSP* maximum lifespan)

	Body weight (kg)	MLSP (years)	LEP (kjoule/kg)
Horse	450	30	711
Cow	450	26	590
Dog	22	9	686
Cat	3	8	937
Guinea pig	0.6	6	1,113

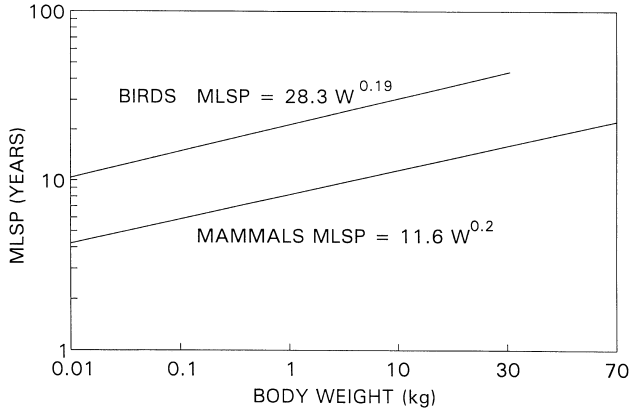

Fig. 1 Relationship between body weight (*W*) and maximum life span (*MLSP*) in mammals and birds. Based on Prinzinger (1993) and Calder (1985)

occurs inside a phylogenetic group, outlying species implicated are considered as exceptions to the rate of living theory. A well-known example are primates (including humans) which have an LEP 2–4 times greater than that of the majority of other mammals (Cutler 1984a). Humans show the longest MLSP and LEP among primates; our MLSP is 4 times longer than expected from the rate of living theory. The only two groups of animals in which the relationships between metabolic rate, body weight and MLSP have been extensively studied, mammals and birds, also show different LEPs. As it is apparent in Fig. 1, the slope of the line relating body weight and MLSP is similar in birds (exponent +0.19) and mammals (exponent +0.20), but the intercept is shifted upwards in birds in relation to mammals. At a given size (and VO_2), birds live 3–4 times longer (Calder 1985; Holmes and Austad 1995) than mammals (the LEP is higher in birds than in mammals). The causes of this different longevity are not known. The rate of living theory holds true inside both animal groups, but something occurred during their evolutionary divergence from reptiles that allowed birds to show simultaneously high VO_2 values and high MLSPs, whereas from reptiles to mammals the "price" paid for an increased VO_2 was a decrease in MLSP (mammals of the same size as ectothermic vertebrates usually show a much higher VO_2 and a shorter MLSP).

Antioxidants and longevity in mammals

What are the causes of the extraordinary longevity in primates and birds? If it is due in part to free radicals, we will have an explanation for aging and longevity that covers species following the rate of living theory in mammals, as well as those species considered as "exceptions" or showing a different LEP. The first important attempt to clarify this issue was the study of various antioxidants, including a comprehensive gathering of data from other authors, in tissues from mammals with different MLSPs, including primates (Tolmasoff et al. 1980; Cutler 1984a, 1986). The main conclusion reached in these works was that antioxidants could be longevity determinants. This was based on the existence of positive correlations between MLSP and various tissue antioxidants, but only when they were divided by the basal metabolic rate (VO_2) of the whole animal. Thus, the positive correlations obtained could be due to the negative correlation of the denominator (VO_2) with MLSP, not to a putative positive correlation of the numerator (the antioxidant concentration) with MLSP.

A solution to this problem would be to study directly the relationship between tissue antioxidants (without referring to basal metabolic rate) and MLSP. This was also done in the above mentioned studies, using the same data, and two types of results appeared: either there was no correlation at all between antioxidants and MLSP in mammals (this was true for superoxide dismutase (SOD) in liver, brain and heart; Tolmasoff et al. 1980), or the correlation obtained between antioxidants and MLSP was strongly negative (Figs. 2, 3). In addition to the negative relationship between antioxidants and MLSP, humans, the most longevous species included, showed minimum levels of the antioxidant studied. Table 2 summarizes all previous studies of this kind known to us in which mammals with exceptionally long MLSP (primates) are included. The correlation between antioxidants and MLSP in mammals and primates is not positive but negative, with the exception of SOD which did not correlate with MLSP. The absence of any correlation between SOD and MLSP was stated in the original publication (Tolmasoff et al. 1980), but later reviews from the same laboratory emphasized positive correlations with MLSP which were obtained only when

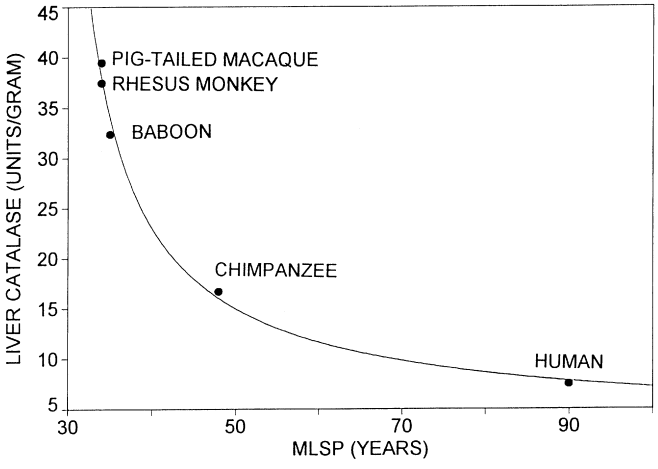

Fig. 2 Relationship between *MLSP* and liver catalase activity. The figure was drawn from Cutler (1986). The curve fit and equation were calculated with Table-Curve software ($y^{-1} = a + b\ lnx; r^2 = 0.9925$). *Points* are the mean values obtained in each species

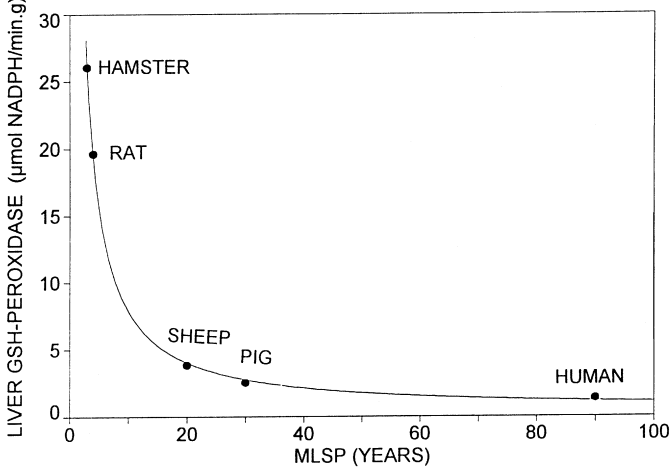

Fig. 3 Relationship between *MLSP* and liver selenium-dependent GSH-peroxidase activity. The figure was drawn from Lawrence and Burk (1978). The curve fit and equation were calculated with Table-Curve software ($y^2 = a + b/x^2; r^2 = 0.9998$). *Points* are the mean values obtained in each species

Table 2 Summary of correlations between tissue antioxidants or other detoxifying systems and *MLSP* in mammals, including species showing a MLSP higher than that predicted by the rate of living theory (primates) [*SOD* superoxide dismutase, *CAT* catalase, *GPx* GHS-peroxidase, *GST* GSH-transferase, *ASC* ascorbate, *r* linear Pearson correlation coefficient (*r* for CAT was calculated from CAT/VO_2 values for reference 3), *Minimum* species showing minimum antioxidant values in each study, *NS* non-significant, *L* liver, *B* brain, *H* heart, *(1)* Lawrence and Burk 1978, *(2)* Cutler 1985, *(3)* Cutler 1986, *(4)* Cutler 1984b, *(5)* Summer and Greim 1981, *(6)* Pashko and Schwartz 1982, *(7)* Tolmasoff et al. 1980, *(8)* De Marchena et al. 1974]

Species	Parameter	Organ	*r*	Minimum	Reference
5 Mammals + 1 bird	GPx	Liver	−0.68	Human	(1)
5 Mammals	CAT	Liver	−0.87	Human	(2)
6 Mammals	CAT	Kidney	−0.89	Human	(2)
5 Mammals	GSH	Brain	−0.79	Human	(3)
3 Mammals	GSH	Liver	−0.98	Human	(3)
7 Mammals	ASC	Liver	−0.55	Pig-Human	(3)
9 Mammals	ASC	Brain	−0.43	Rhesus-Human	(4)
4 Mammals	GST	Liver	−0.95	Human	(5)
6 Mammals	cit.P-448	Fibroblasts	−0.94	Human	(6)
6 Mammals	cit.P-450	Fibroblasts	−0.85	Human	(6)
13 Mammals	SOD	L, B, H	NS		(7)
5 Mammals + 1 fish	GPx	Liver	−0.68	Calf	(8)
5 Mammals + 1 fish	GPx	Brain	−0.84	Calf	(8)

the antioxidants were divided by the basal metabolic rate (e.g., SOD/VO_2) (Cutler 1984a, 1986). In our opinion, this leads to confusion rather than clarification of the issue, as explained above. Negative correlations between antioxidants and MLSP have been observed for many different enzymatic and non-enzymatic antioxidants in different types of cells and tissues, and occur with rather high linear correlation coefficients. It is interesting also that, whenever humans were included, they were the species showing the lowest (or the second lowest) levels of the antioxidant considered [studies by De Marchena et al. (1974) show calves had the minimum levels of GSH-peroxidase and were also the longest-lived species included in the studies; Table 2]. Table 2 also shows negative correlations with MLSP for glutathione (GSH)-transferase, cytochrome P450 and cytochrome P448, molecules also implicated in detoxification pathways. Antioxidant vitamins (such as vitamin E or carotenes) were not included in this summary since we considered that a diet-dependent factor cannot be a main determinant of species-specific MLSP, as this parameter is controlled, in a direct or indirect way, by the genotype of the species. In any case, positive correlations of these diet-derived antioxidants were obtained again only after dividing them by the basal metabolic rate (VO_2; Cutler 1986).

Antioxidants and longevity in vertebrates

The relationship between antioxidants and MLSP was also examined in vertebrate species in studies that incorporated two variations. The first was to include birds, which have higher MLSPs than mammals of the same basal metabolic rate or body weight. The second was to measure the major endogenous antioxidants simultaneously in different tissues of species from various vertebrate classes and using the same laboratory methods (López-Torres et al. 1993a; Barja et al. 1994a; Pérez-Campo et al. 1994), instead of comparing data from different sources (Cutler 1984a,b, 1986). The particular bird species included showed an exceptional MLSP (35 years in the pigeon and 24 years in the canary) in contrast to that of the two mammals of equivalent body weight included in the study (4 years in the rat and 3.5 years in the mouse; Table 3). This difference in MLSP cannot be explained by the difference in basal metabolic rate. On the contrary, total VO_2 at rest was 2 times higher in the pigeon than in the rat, and was also higher in the canary than in the mouse (Table 3; López-Torres et al. 1993a; Barja et al. 1994a; Pérez-Campo et al. 1994). The result was a LEP 16–20 times higher in these two birds than in the two mammals of similar body weight.

Only vertebrate species with well-known MLSP were used. The range in both MLSP and LEP among species were large (Table 3), and reached one (MLSP) or two (LEP) orders of magnitude between extreme values. MLSP was low in mammals, intermediate in ectothermic

Table 3 *MLSP*, oxygen consumption at rest (VO_2), and *LEP* in vertebrate species selected for a study on the relationship between antioxidants and MLSP (López-Torres et al. 1993a; Barja et al. 1994a; Pérez-Campo et al. 1994). LEP = MLSP (years) × VO_2 ($l\ O_2\ g^{-1}\cdot year^{-1}$). VO_2 values were measured in 6–8 animals except for trout and mouse data which come from the literature. The VO_2 values were obtained at rest except in the trout, in which a minimum level of routine activity is unavoidable. Thus, the VO_2 and LEP values shown for the trout must be overestimations

Species	MLSP (years)	VO_2 ($\mu l\ O_2/g\cdot h$)	LEP ($l\ O_2/g$)
Canary (*S canarius*)	24	5,812 ± 21	1,222
Pigeon (*C livia*)	35	1,518 ± 97	465
Toad (*X laevis*)	15	47 ± 4	6.2
Trout (*S trutta*)	13	226	26
Frog (*R perezi*)	7	87 ± 5	5.3
Guinea pig	8	681 ± 27	48
Rat (Wistar)	4	790 ± 45	28
Mouse (OF1)	3.5	2,000	62

animals, and high in birds, whereas the LEP was low in ectotherms, higher in mammals, and even higher in birds. The large differences in MLSPs allowed a statistical analysis, and the variation in LEP was necessary to study species showing differences in MLSP which could not be explained on the basis of their basal metabolic rates. The animals used were young adults at around one fourth of their MLSP.

When the levels of tissue antioxidants in these vertebrate species were compared, strongly negative correlations were obtained (López-Torres et al. 1993a; Barja et al. 1994a; Pérez-Campo et al. 1994), and pigeons, the species with the longest MLSP included in the correlation, showed the lowest levels of all antioxidants considered. Figures 4 and 5 show this for brain GSH-peroxidase and lung GSH-reductase. Table 4 summarizes all the data obtained by us for vertebrate species covering fish, amphibia, birds and mammals (López-Torres et al. 1993a; Barja et al. 1994a; Pérez-Campo et al. 1994). The six antioxidants considered, studied in three animal tissues, resulted in 18 correlations, 14 of which were negative. In the other four cases (liver SOD, GSH-reductase and ascorbate, and brain GSH) no correlation with MLSP was obtained, and a positive correlation with MLSP was not found in any case. In the same table, the previous works performed in mammals including primates, already mentioned and included in Table 2, are given inside parentheses for comparison. In six out of nine cases, results obtained for vertebrates in general (including birds) coincide with those obtained for mammals (including primates). Tissue antioxidants seem significantly and negatively correlated with MLSP: 21 significantly negative correlations and 6 cases of no significant correlation, but no positive correlations at all. This agreement occurs with a wide range of species and using data from six different laboratories. The longer the MLSP the lower are the endogenous levels of tissue antioxidants. The only two well-described cases of animals showing an extraordinarily high MLSP, not explainable by their basal metabolic rate when compared to the majority of mammals, primates and birds, seem to share a common characteristic: they have very low levels of tissue antioxidants.

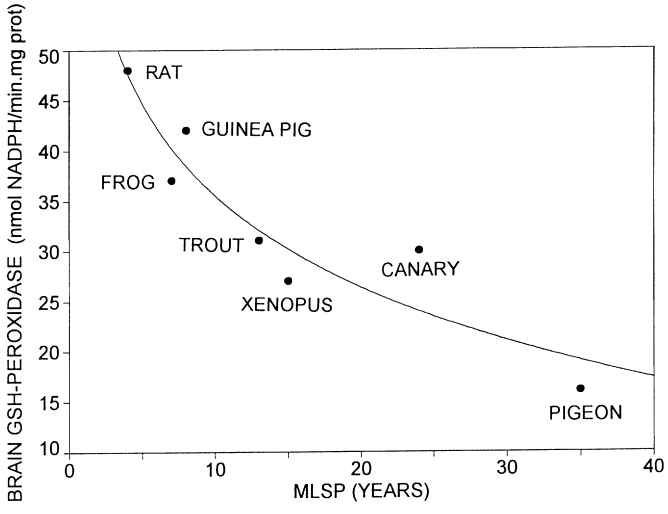

Fig. 4 Relationship between *MLSP* and brain selenium-dependent GSH-peroxidase in vertebrate species including birds. The figure was drawn from Barja et al. (1994a). The curve fit and equation were calculated with Table-Curve software ($y = a - b\ lnx; r^2 = 0.889$). *Points* are the mean values obtained in each species

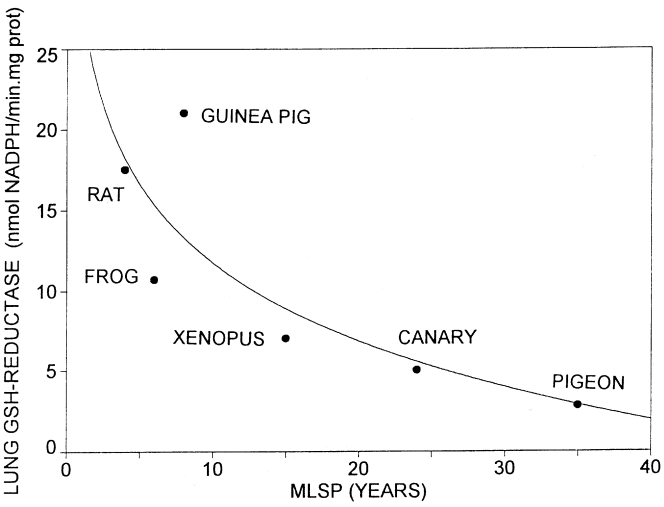

Fig. 5 Relationship between *MLSP* and lung GSH-reductase activity in vertebrate species including birds. The figure was drawn from Pérez et al. (1994). The curve fit and equation were calculated with Table-Curve software ($y = a - b\ lnx; r^2 = 0.77$). *Points* are the mean values obtained in each species

Table 4 Summary of published correlations between antioxidants and MLSP obtained in vertebrates including birds (not in parentheses), or in mammals including primates (in parentheses). For references see Tables 2, 3 and text. [*n* no correlation, – statistically significant negative correlation ($P < 0.05$ or lower) *GR* GSH-reductase]

	Liver	Lung	Brain
SOD	(n) n	–	(n) –
CAT	(–) –	–	–
GPX	(–) –	–	(–) –
GR	n	–	–
GSH	(–) –	–	(–) n
ASC	(–) n	–	(–) –

Rate of free radical production, DNA damage and aging

The general result obtained in the previous section may seem paradoxical at first glance. Nevertheless, the effect of supplementation with natural (vitamins E or C) or synthetic antioxidants throughout the life span of a single animal species has been studied many times in different laboratories. The result of these studies has been on some occasions a moderate or strong increase in mean life span (possibly when conditions of maintenance were not totally optimum), but MLSP was essentially not changed. This kind of result was obtained

in a range of animals including mice (Harman 1968), frogs (López-Torres et al. 1993b,c) and insects (Allen et al. 1983; Seto et al. 1990; Staveley et al. 1990; Fleming et al. 1992; Orr and Sohal 1992; Viña et al. 1992). The increases in antioxidants in these studies were obtained in three different ways, namely, by dietary supplementation, antioxidant induction or an increase in gene dosage in transgenic animals, but the outcome was usually the same: an increase in mean life span but not in MLSP. Simultaneous overexpression with SOD and catalase has been recently obtained in 15 transgenic lines of *Drosophila melanogaster* flies. Eight of these showed some increase in MLSP (30% or less), six showed no effect and one showed a decrease (Orr and Sohal 1994). Since only 50% of normal SOD and 14% of normal catalase are necessary for normal life, some authors suggest that the MLSP increase observed in some lines in that experiment can be related to other changes due to interventions that are not related to excess enzymatic activity (Jazwinski 1996).

In the comparative studies in vertebrates mentioned above, some indicators of oxidative stress, such as in vitro lipid peroxidation and oxidised glutathione (GSSG)/GSH ratio, were also measured and in the majority of cases, no significant correlations between these parameters and MLSP were obtained (López-Torres et al. 1993a; Barja et al. 1994a; Pérez-Campo et al. 1994). A possible reason why antioxidant levels are low in longevous species, whereas mean cellular oxidative stress does not correlate with MLSP, could be that oxygen radical production is negatively correlated with MLSP (López et al. 1993a; Barja et al. 1994a; Pérez et al. 1994). It is unlikely that very longevous species such as pigeons and humans could stand high levels of tissue free radical production with very low levels of tissue antioxidants. It was therefore proposed that the rate of oxygen radical production in vivo helps to determine the aging rate across species (López-Torres et al. 1993a). Short-lived species should show high levels of tissue antioxidants in order to face their high rates of oxygen radical production, whereas the opposite would be the case in very longevous species. Both factors would cancel each other and a similar mean cellular oxidative stress would be expected. Nevertheless, gradients of H_2O_2 would be found in vivo surrounding places of oxygen radical production. The result would be that the local concentration of active O_2 species near places of free radical production would be lower in long- than in short-lived animals (Barja et al. 1994a). Short-lived species would suffer higher oxidative damage at relevant targets, such as mitochondrial DNA (mt-DNA), situated near places of oxygen radical production. Our working hypothesis, ''free radical production near DNA targets as a cause of aging'', proposes that the rate of free radical production is among the determinants of aging rate and maximum longevity. Various observations consistent with the implication of oxidative DNA damage and mitochondria in aging are listed in Table 5.

Table 5 Oxidative DNA damage, mitochondria, aging and longevity [*MLSP* maximum life span, *ROS* reactive oxygen species. *oxo^{8}dG* 8-oxo-7,8-dihydro-2′-deoxyguanosine, (the most frequently measured marker of oxidative DNA damage), *mt-DNA* mitochondrial DNA, *(1)* Eimon et al. 1996, *(2)* Fraga et al. 1990, *(3)* Sai et al. 1992, *(4)* Ozawa et al. 1995, *(5)* Asunción et al. 1996, *(6)* Richter et al. 1988, *(7)* Chung et al. 1992, *(8)* Shibutani et al. 1991, *(9)* Cheng et al. 1992, *(10)* Wallace 1992, *(11)* Adelman et al. 1988, *(12)* Cutler 1991, *(13)* Hruszkewycz and Bergtold 1990, *(14)* Agarwal and Draper 1992, *(15)* Shearman and Kalf 1977, *(16)* Richter et al. 1995 *(17)* Kristal et al. 1994

	References
1. More than 90% of oxygen is consumed by mitochondria	
2. mt-DNA is situated near the mitochondrial ROS generator	
3. mt-DNA has a high information density	
4. Low protection by proteins and low repair of mit-DNA	(1)
5. oxo^{8}dG increases with age in mt-DNA and nuclear DNA	(2–5)
6. oxo^{8}dG levels are higher in mt-DNA than in nuclear DNA	(6, 7)
7. oxo^{8}dG is mutagenic	(8, 9)
8. Deletions and mutations increase with age in mt-DNA	(9, 10)
9. Oxidative DNA damage negatively correlates with MLSP across species	(11, 12)
10. Unsaturated fatty acids are most sensitive to ROS	
11. The inner mitochondrial membrane is rich in unsaturated fatty acids	
12. Lipid peroxidation products oxidatively damage DNA	(13, 14)
13. Transient attachment of mt-DNA to the inner mitochondrial membrane	(15, 16)
14. ROS inhibit mitochondrial transcription	(17)

The free radical production theory of aging is also compatible with the observation that increases in tissue levels of antioxidants in a given species can increase mean life span but do not change MLSP. The added antioxidants would increase defense against oxidative damage in many parts of the cell, and this could confer protection against various diseases that lead to early death. However, the antioxidants would not be capable of significantly reducing the very high local concentrations of oxygen radicals present at sites of intense production (e.g., mitochondrial membranes), which are located near targets possibly relevant for maximum longevity [e.g., mitochondrial DNA (mt-DNA)]. Many oxygen radicals produced at the inner mitochondrial membrane could react with mt-DNA before antioxidants could intercept them. The free radical production theory of aging agrees with previous emphasis on the possible importance of mitochondria in the aging process (Harman 1972), and with the recent observation that caloric restriction, the only widely recognized manipulation that increases MLSP, decreases mitochondrial free radical production in mice (Sohal et al. 1994). Improvements in oxidative stress have been observed after caloric restriction in various studies (Weindruch

and Walford 1982; Koizumi et al. 1987; Sohal and Weindruch 1996). Also, there is a growing consensus that the current two most tested hypotheses of aging, based on free radicals and on glycation, could be interconnected (Yu 1993).

Mitochondrial H_2O_2 production and animal longevity

Some studies have compared mitochondrial oxygen radical production between species showing different longevities. This problem was studied by one laboratory (Sohal et al. 1989, 1990) in mammalian species which "follow" the rate of living theory. The results showed a strong negative exponential correlation between liver mitochondrial O_2^- or H_2O_2 production ($r = -0.91$; Fig. 6) and MLSP. Similar negative relationships in kidney and heart of the same species were found in the same laboratory (Ku et al. 1993). Nevertheless, since the included species followed the rate of living theory (decrease in MLSP as body size decreases and basal metabolic rate increases), the results obtained could also be interpreted as a correlate of that theory: the species with short MLSP could show high mitochondrial H_2O_2 production simply because their rates of mitochondrial O_2 consumption were also higher. Positive correlations between mitochondrial oxygen consumption and oxygen radical production, and between mitochondrial oxygen radical production and basal metabolic rate, were indeed found in this study (Ku et al. 1993). Thus, as explained in the first section of this review, these studies cannot discard the possibility that the correlations observed between mitochondrial oxygen radical production and MLSP were due to the correlation of mitochondrial oxygen radical production with the basal metabolic rate. Basal metabolic rate could also correlate, in principle, with other unknown factors causing aging. This is why

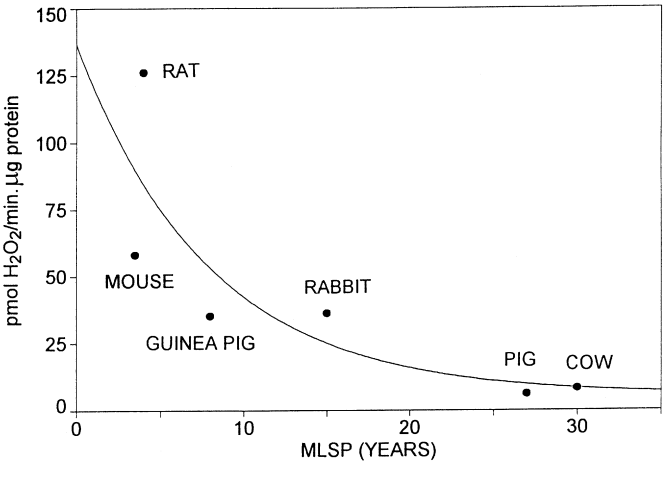

Fig. 6 Relationship between H_2O_2 production by liver mitochondria and *MLSP* in mammalian species following the rate of living theory ($y = a.10^{-b}x; r = 0.91$). Redrawn from Sohal et al. (1990). *Points* are the mean values obtained in each species

mitochondrial H_2O_2 production in birds, animals with both high VO_2 and high MLSP, was studied. If a low rate of free radical production contributes to a slow aging rate in birds, their mitochondria should show a low rate of H_2O_2 production in spite of their high respiratory activity. It was indeed found (Barja et al. 1994b) that the rate of H_2O_2 production by crude brain mitochondria was about fourfold lower in pigeons (MLSP = 35 years) than in rats (MLSP = 4 years). A similar situation was found for lung and liver mitochondria (Barja et al. 1994b). A lower H_2O_2 production in the pigeon than in the rat has also been reported for kidney and crude brain mitochondria (Ku and Sohal 1993), heart mitochondria (Herrero and Barja 1997a) and non-synaptic brain mitochondria (Herrero and Barja 1997b). This was possible because the proportion of O_2 reduced to H_2O_2 at the mitochondria (the "free radical leak") was lower in the pigeon than in the rat (Barja et al. 1994b; Herrero and Barja 1997a,b). The simple idea that a high oxygen consumption necessarily leads to a high rate of oxygen radical production at mitochondria is not true, at least in the pigeon. The mitochondrial free radical production is also lower in the white-footed mouse (MLSP = 8 years) than in the house mouse (MLSP = 4 years) and it correlates inversely with MLSP in five species of flies (Sohal and Weindruch 1996). Recent data from our laboratory (unpublished) also show that free radical production of heart mitochondria is lower in two more birds, canaries (MLSP = 24 years) and parakeets (MLSP = 21 years), than in mice (MLSP = 3.5 years).

Perspectives and summary

The results described above suggest the possibility that birds developed their extraordinarily high longevity (not explainable by their high rates of oxygen consumption) during evolution due in part to their capacity to reduce free radical production and free radical leak in the mitochondrial electron transport chain. It is possible that other species like primates used the same mechanism to increase their MLSP beyond that predicted from their basal metabolic rate. This way of increasing MLSP is specially interesting as it would allow animals to live longer without the need to reduce metabolic rate and general activity levels.

The low mitochondrial free radical production of birds, and perhaps also of primates, supports the idea that the variation in MLSP among animals following the "rate of living theory" is due in part to variations in the rate of oxygen radical production, rather than being due to other factors related to basal metabolic rate. The bird case is especially demonstrative, since, by dissociating high MLSPs from low basal metabolic rates, it suggests that the factor related to MLSP in all species studied to date may be the rate of free radical production, not metabolic rate. Present information is consistent with the idea that O_2 radical production can be a factor

contributing to aging rate in all species, including mammals that follow the rate of living theory and species with exceptional longevities (like birds). The variation of total O_2 consumption, and then in O_2 radical production, as a function of body weight would be the reason in the first case, and a specific decrease in O_2 radical production per unit oxygen consumption would be responsible in the second case. In summary, available data show that increasing the tissue antioxidant levels in a single species generally increases the mean life span but not the MLSP, whereas a decrease in the rate of free radical production may have resulted in increases in maximum longevity during evolution.

Two final points may help to form a more integrative view of probable causes of aging and longevity. Other processes capable of damaging DNA should also be taken into account in addition to free radical production. The negative correlations with MLSP found by various authors for detoxifying systems such as GSH transferases and cytochromes P450 and P448 (Table 2) suggest that production and/or threat by toxic chemicals is generally lower in longevous species. Finally, many data support the concept that DNA damage is of paramount importance for aging (Fraga et al. 1990; Ames and Shigenaga 1992; Richter 1995). If this is true, the rate of production of free radicals and toxic chemicals would be an important determinant of aging and MLSP but not the only one. The rate of DNA repair must also be important. Various works have shown the existence of strong positive correlations between DNA repair systems and mammalian MLSP across species including humans (Hart and Setlow 1974; Francis et al. 1981; Hall et al. 1984; Bürkle et al. 1992). The combination of high levels of DNA repair with low rates of free radical production near DNA in longevous animals can help to explain their very low levels of steady-state oxidative DNA damage (Adelman et al. 1988; Cutler 1991) and their slow aging rate, even when the quantitative differences in these two factors between animals are not enough, when considered independently, to explain differences in maximum longevity.

Much previous work has centred on testing the possibility that antioxidants decrease with age and, more rarely, on possible increases in free radical production with age. Available data do not consistently support either the first (Barja et al. 1990, 1992; Pérez et al. 1991; Benzi and Moretti 1995) or the second (Muscari et al. 1990; Zhan et al. 1992; Yu 1995; Hansford et al. 1997) possibility. However, neither is needed for the free radical theory of aging to be true. A constant rate of free radical attack can cause accumulation of oxidative DNA damage even if antioxidant concentrations do not change during aging, because antioxidant defenses and DNA repair cannot be 100% effective. The accumulation of oxidative DNA damage would be quicker in animals with a high rate of free radical production and/or lower DNA repair, even if the velocity of these last two processes does not change during aging. It is the difference between animal species in the rate of these two processes in the adult animal (young or old) that seems to explain their differences in rates of accumulation of oxidative DNA damage. The consequences of this damage in relation to aging and longevity should be considered within the idea that multiple mechanisms cause aging.

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