Mitochondrial Free Radical Production and Aging in Mammals and Birds[a]

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ABSTRACT: The mitochondrial rate of oxygen radical (ROS) production is negatively correlated with maximum life span potential (MLSP) in mammals following the rate of living theory. In order to know if this relationship is more than circumstantial, homeothermic vertebrates with MLSP different from that predicted by the body size and metabolic rate of the majority of mammals (like birds and primates) must be studied. Birds are unique because they combine a high rate of basal oxygen consumption with a high MLSP. Heart, brain, and lung mitochondrial ROS production and free radical leak (percent of total electron flow directed to ROS production) are lower in three species of birds of different orders than in mammals of similar body size and metabolic rate. This suggests that the capacity to show a low rate of ROS production is a general characteristic of birds. Using substrates and inhibitors specific for different segments of the respiratory chain, the main ROS generator site (responsible for those bird–mammalian differences) in state 4 has been localized at complexes I and III in heart mitochondria and only at complex I in nonsynaptic brain mitochondria. In state 3, complex I is the only generator in both tissues. The results also suggest that the iron–sulphur centers are the ROS generators of complex I. A general mechanism that allows pigeon mitochondria to show a low rate of ROS production can be the capacity to maintain a low degree of reduction of the ROS generator site. In heart mitochondria, this is supplemented with a low rate of oxygen consumption physiologically compensated with a comparatively higher heart size. A low rate of free radical production near DNA, together with a high rate of DNA repair, can be responsible for the slow rate of accumulation of DNA damage and thus the slow aging rate of longevous animals.

Many theories have been proposed during the last decades trying to explain aging. Among them, the free radical theory of aging[1] and its special relationship to mitochondria first signaled by D. Harman in 1972,[2] is gaining increasing support from available data.

The majority of previous work regarding that theory has concentrated on the possible role of antioxidants. Experiments in large samples of animals have studied the effect of increasing the tissue antioxidant levels on long-term survival. These manipulations have been performed in different ways, including dietary supplementation,[3–5] antioxidant induction,[6,7] or gene transfection.[8–10] The outcome in the majority of these studies is the same: the increases in tissue antioxidants can increase survival and mean life span, especially when the experimental conditions are not fully optimum for survival, but the maximum life span potential (MLSP) is not increased. The observed improvement in mean life span is in agreement with the increasingly held notion that antioxidants can unspecifically

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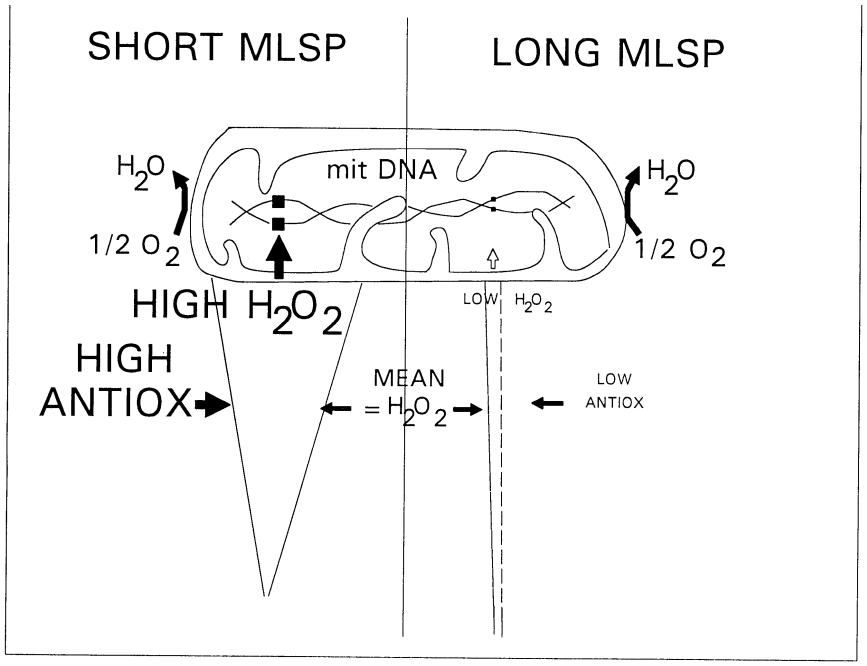


FIGURE 1. Animals with long MLSP show both low levels of tissue antioxidants and of mitochondrial free radical production, whereas the contrary occurs in short-lived species. Similar levels of *mean* cellular H_2O_2 in short- and long-lived species are expected, but the local concentration of O_2 radicals near the places of free radical production would be much lower in long- than in short-lived animals. This will decrease oxidative damage at critical targets situated near the sites of free radical production, like the mitochondrial DNA. Modified from Barja *et al.*[23]

protect against the development of degenerative diseases, including cardiovascular diseases[11] and cancers.[12]

On the other hand, comparative studies in mammalian[13–20] or vertebrate[21–23] species with widely different MLSPs have shown that endogenous constitutive levels of tissue antioxidants are negatively correlated with MLSP. This led us to propose[21,23] that the reason for this is that the rate of free radical production near DNA (*e.g.*, at the inner mitochondrial membrane) is negatively correlated with MLSP (FIG.1). Longevous species would produce radicals slowly at specific sites located near DNA, and this would be partially responsible for their slow rate of accumulation of DNA damage and slow aging rate. Available data supporting this notion in mammals and birds, accumulated in recent years, are the subject of this review.

THE RATE OF LIVING THEORY

Classic studies have shown that the basal metabolic rate is generally related to aging and MLSP in the majority of animal species studied. This relationship is known as the "rate of living" theory of aging.[24] Even though the first global approach to this problem

can be attributed to Pearl, the constancy of the total metabolic output (LEP = life energy potential; the number of total calories transformed per kg during the whole life span) in some mammalian species had already been described much earlier by Max Rubner.[25]

The basal specific rate of oxygen consumption (per gram weight) negatively correlates with body weight, with an exponent of –0.25 in mammals, whereas MLSP positively correlates with body weight, with an exponent of around +0.20, also in mammals. Thus, the LEP,[25,26] which is equal to the specific metabolic rate multiplied by the MLSP, is essentially a size-independent parameter in the majority of mammals inasmuch as both 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 because MLSP also increases as a function of body weight in birds, with an exponent of +0.19 (FIG. 2). In other animals there are not enough reliable data, but present information suggests that the rate of living theory will also hold essentially true inasmuch as 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.[27,28] Furthermore, in poikilothermic animals the MLSP increases in proportion to the decrease in metabolic rate brought about by a decrease in the temperature of maintenance. The specific metabolic rate corresponds to the number of calories transformed per unit time and weight. Nevertheless, because it is closely related to the rate of oxygen consumption 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 the mitochondrial level (to sustain a high basal metabolic rate), it would also produce a large number 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 and if the tissues consume more O_2 per

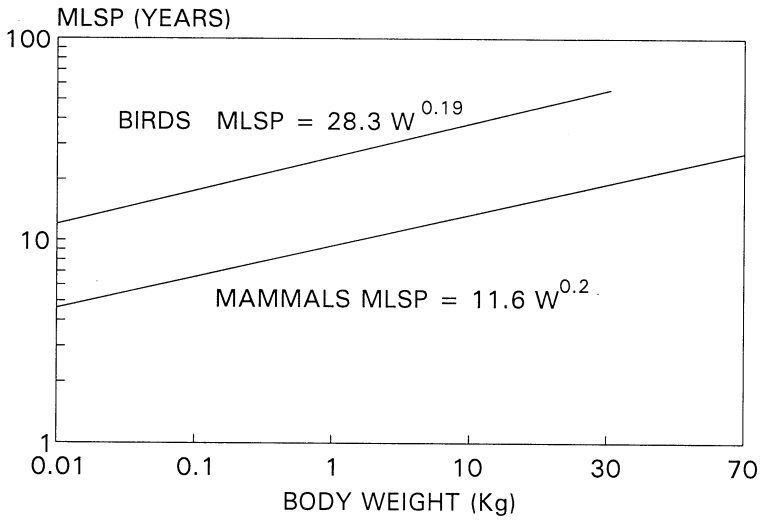


FIGURE 2. Relationship between body weight (W) and maximum life span potential (MLSP) in mammals and birds. Based on Calder[29] and Prinzinger.[28]

time unit, they will also synthesize and degrade many kinds of molecules faster, and a myriad of biochemical processes will 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 correspond to many other factors related to the metabolic rate.

In clarifying this problem, an interesting approach is to study animal species with different LEPs. When this occurs inside a phylogenetic group, the species implicated are considered as exceptions to the rate of living theory. Well-known examples are primates and humans, who have an LEP 2–4 times greater than that of the majority of mammals.[26] Humans show the longest MLSP and LEP among primates, and the human MLSP is four times higher than expected from the rate of living theory. The only two groups of animals in which the relationship between metabolic rate, body weight, and MLSP has been extensively studied, mammals and birds, also show different LEPs. As it is apparent in FIGURE 2, the slope of the line relating body weight and MLSP is similar in birds (exponent +0.19) and mammals (exponent +0.20), but the line is shifted upwards in birds in relation to mammals (the "a" coefficient is higher in birds). At a given size (and oxygen consumption), birds live around 3–4 times longer[29,30] than mammals (the LEP is higher in birds than in mammals). The causes of this extraordinary longevity are not known. The rate of living theory holds true inside both animal groups, but something occurred during evolutionary divergence from reptiles that allowed birds to show simultaneously high oxygen consumption values and high MLSPs, whereas from reptiles to mammals the "price" paid for an increased oxygen consumption was a decrease in MLSP (mammals of the same size as poikilothermic vertebrates usually show a much higher O_2 oxygen consumption and a shorter MLSP). Birds would represent a problem for the free radical theory of aging if their high basal rate of oxygen consumption would lead (as it is commonly believed) to a high rate of free radical generation in their mitochondria. If birds were to generate large numbers of free radicals in their tissues, the free radical theory would lack universal applicability, even for homeothermic vertebrates. On the contrary, if the free radical theory is correct, bird mitochondria must produce a small number of radicals per time unit, in spite of the high oxygen consumption and metabolic rate of these animals.

MITOCHONDRIAL FREE RADICAL PRODUCTION AND MLSP

Recent data show that mitochondrial free radical production is lower in the white-footed mouse (MLSP = 8 years) than in the house mouse (MLSP = 4 years) and that it inversely correlates with MLSP in five species of flies.[31] Mitochondrial oxygen radical production has also been compared among mammalian species following the rate of living theory and having different maximum longevities.[32,33] The results obtained showed a strong negative exponential correlation between liver mitochondrial O_2^- or H_2O_2 production ($r = -0.91$, FIG. 3) and MLSP. A similar negative relationship in mitochondria from kidney and heart of the same species was found afterwards by the same authors.[34] Nevertheless, because the species included 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. A positive correlation between mitochondrial oxygen consumption and oxygen radical production and between mitochondrial oxygen radical production and

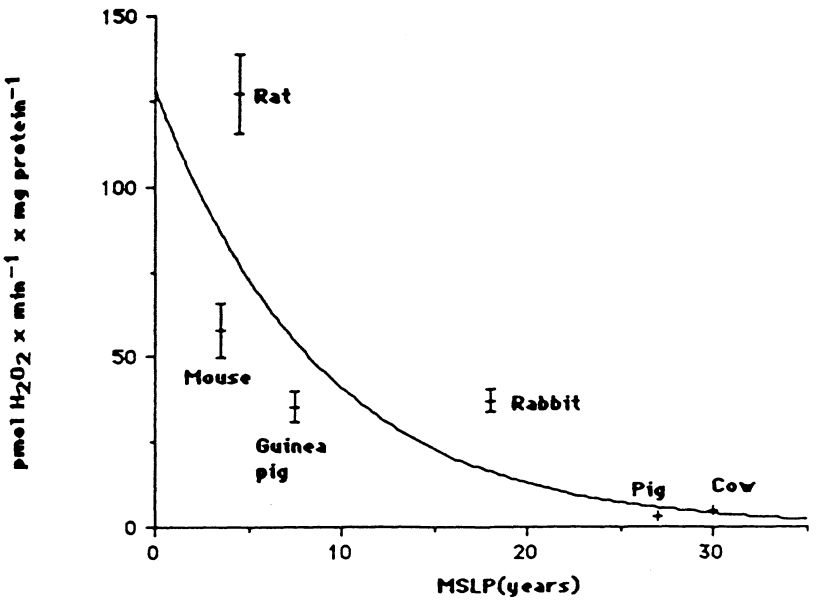


FIGURE 3. Negative correlation between liver mitochondrial H_2O_2 production and maximum life span (MLSP) in mammalian species following the rate of living theory. A similar relationship has been obtained in the same animal species for heart and kidney mitochondrial O_2^- and H_2O_2 production[34] and for liver mitochondrial O_2^- production.[32] $r = -0.91$. (Sohal *et al.*[33] With permission from *Mechanisms of Ageing and Development.*)

basal metabolic rate were indeed found in that work.[34] Thus, as explained above, these studies cannot discard the possibility that the correlation observed between mitochondrial oxygen radical production and MLSP were due to the correlation of mitochondrial oxygen radical production with the basal metabolic rate, this last parameter hypothetically correlating with other unknown factors causing aging. This is why the mitochondrial H_2O_2 production in birds, animals with both high oxygen consumption and high MLSP, was studied. If a low rate of free radical production contributes to slow aging rate in birds, their mitochondria should show a low rate of H_2O_2 production, in spite of their high respiratory activity. In an initial study, we have indeed shown that crude brain mitochondria and lung mitochondria show a rate of oxygen radical production substantially lower in pigeons (MLSP = 35 years) than in rats (MLSP = 4 years).[35] These two vertebrate homeotherms have a similar body size and basal metabolic rate (oxygen consumption). A lower mitochondrial H_2O_2 production in the pigeon than in the rat has been also independently reported for brain, heart, and kidney.[36] We also found that the percent of electrons out of sequence, which reduce oxygen to oxygen radicals at the respiratory chain (the percent free radical leak), instead of reducing oxygen to water at the terminal cytochrome oxidase, was lower in pigeon than in rat in crude brain mitochondria and lung mitochondria.[35] The simple idea that a high oxygen consumption necessarily leads to a high rate of oxygen radical production at mitochondria was not true, at least in the pigeon.

The above results obtained in crude pigeon mitochondria encouraged us to perform more detailed additional studies of free radical production in bird mitochondria of the same or different species, using a specific and sensitive fluorometric kinetic H_2O_2 detection method that does not alter the respiratory control index. This method instantaneously reacts to variations in H_2O_2 levels and does not show interference from mitochondrial antioxidants.[37] FIGURE 4 shows some of the results obtained, using substrates and inhibitors specific for different segments of the respiratory chain, in rat and pigeon mitochondria from a postmitotic tissue highly relevant for aging and having the richest mitochondrial density among vital organs, the heart. In both species, free radical production with pyruvate/malate was strongly increased to similar levels by addition of either rotenone or antimycin A, and further addition of myxothiazol decreased free radical production to the levels observed with pyruvate/malate alone (FIG. 4).[38] All the rates of free radical production shown in FIGURE 4 were higher in rat than in pigeon mitochondria. Other observations of that study were (1) free radical production was higher with pyruvate/malate than with succinate; (2) thenoyltrifluoroacetone did not increase succinate-supported free radical production; (3) both ethoxyformic anhydride and chloromercuribenzoate strongly depressed the rotenone-stimulated pyruvate/malate-supplemented rates of free radical production; and (4) the basal (substrate alone) and maximum (with pyruvate/malate plus rotenone or antimycin A) free radical leaks were higher in rat than in pigeon heart mitochondria. These observations localized the main free radical generator of both rat and pigeon heart mitochondria at complexes I and III of the respiratory chain, in agreement with previous studies, mainly performed in rat or cow heart mitochondria.[39–45] The results also suggest that the complex I free radical generator is situated between the sites of ferri-

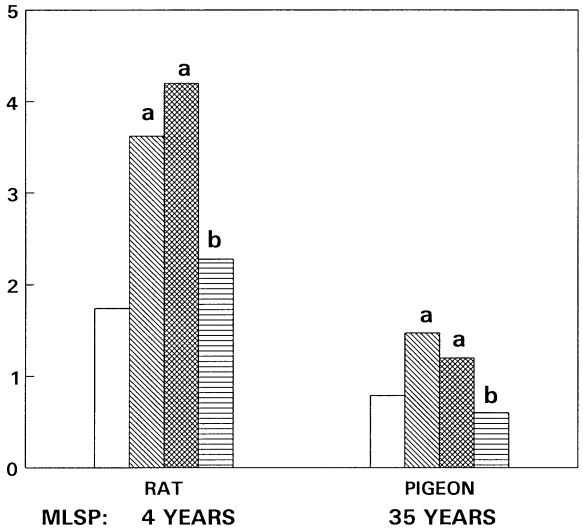


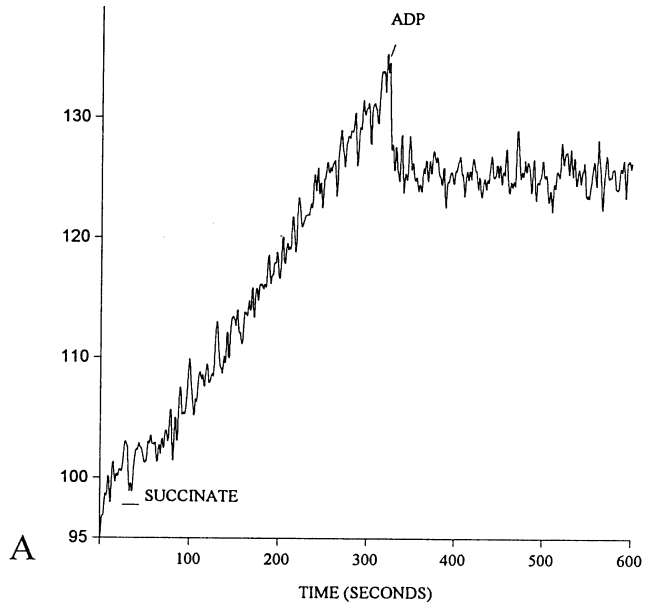
FIGURE 4. Heart mitochondrial H_2O_2 production in rats and pigeons with pyruvate/malate (PYR) as substrates (nmol H_2O_2/min/mg protein). ▭, PYR; ▨, PYR+AA; ▩, PYR+ROT; ▤, PYR+AA+MYX. Inhibitors: AA (antimycin A); ROT (rotenone); MYX (myxothiazol). MLSP = maximum life span potential. a: significantly different from pyruvate/malate alone; b: significantly different from PYR+AA. All analogous values were significantly higher in rats than in pigeons. Data from Herrero and Barja.[38]

cyanide and ubiquinone reduction and possibly corresponds to the iron-sulfur centers of this complex. In the case of heart mitochondria, both complex I and complex III would be responsible for the lower rate of free radical generation observed in the pigeon in relation to the rat.

Our initial rat–pigeon studies in crude brain mitochondria were also followed by more detailed studies in nonsynaptic brain mitochondria of these two animal species isolated with ficoll gradients.[46] Similar to what was obtained in heart mitochondria, rotenone and antimycin A strongly stimulated free radical production with pyruvate/malate, ethoxyformic anhydride and chloromercuribenzoate strongly depressed the rotenone-stimulated pyruvate/malate-supplemented rates of free radical production, thenoyltrifluoroacetone did not increase succinate-supported free radical production, and free radical production and leaks were higher again in the rat than in the pigeon. Nevertheless, this time myxothiazol did not decrease the antimycin-stimulated rate of free radical production with pyruvate/malate, and free radical production with succinate was not only lower than with pyruvate/malate, but it was almost undetectable. In addition, using ferrocytochrome c as substrate in hypotonically treated mitochondria, direct evidence of the lack of involvement of complex IV in free radical generation was obtained. All those results showed that the only free radical generator of nonsynaptic brain mitochondria is located at complex I in both rats and pigeons and probably corresponds again to the complex I iron–sulfur centers. This generator is thus exclusively responsible for the lower rate of free radical generation of pigeon versus rat nonsynaptic brain mitochondria.

The studies described above offered also some clues to the mechanism that allows pigeon mitochondria to show lower rates of free radical production than rat mitochondria. State 4 oxygen consumption was similar in rat and pigeon nonsynaptic brain mitochondria, whereas the free radical leak was lower in the pigeon (see above), and the addition of rotenone to pyruvate/malate-supplemented nonsynaptic brain mitochondria eliminated the differences in free radical production between both species.[46] In the case of heart mitochondria, however, state 4 oxygen consumption and free radical leak were lower in the pigeon than in the rat, and the difference in free radical generation between both species persisted after addition of rotenone to pyruvate/malate-supplemented mitochondria.[38] Addition of ADP caused a larger decrease in free radical leak in rat than in pigeon pyruvate/malate-supplemented mitochondria obtained from both tissues.[47] Thus, the relatively lower free radical production of nonsynaptic pigeon brain mitochondria seems to be due to a capacity of these mitochondria to maintain a lower degree of reduction of the complex I generator in the steady state. In the case of heart mitochondria, this mechanism is supplemented with a relatively low oxygen consumption and electron flow in the pigeon. This lower mitochondrial oxygen consumption (and thus probably lower rate of ATP generation) is compensated for with the much larger heart size (and stroke volume) of birds, in

FIGURE 5. Tracings showing the effect of ADP on free radical production of rat heart mitochondria with complex II- (A) or complex I-linked (B) substrates during the stimulation of oxygen consumption from state 4 to state 3. H_2O_2 increases the fluorescence at 312 nm excitation and 420 nm emission. PYR/MAL = pyruvate/malate; ADP (500 μM). Addition of ADP during the kinetic run stopped free radical production with succinate (A) but not with pyruvate/malate (B). Transient perturbation of the tracings at the moment of ADP addition are due to a slight opening of the sample compartment to add ADP. (Herrero and Barja.[47] With permission from the *Journal of Bioenergetics and Biomembranes.*)


ADP
130
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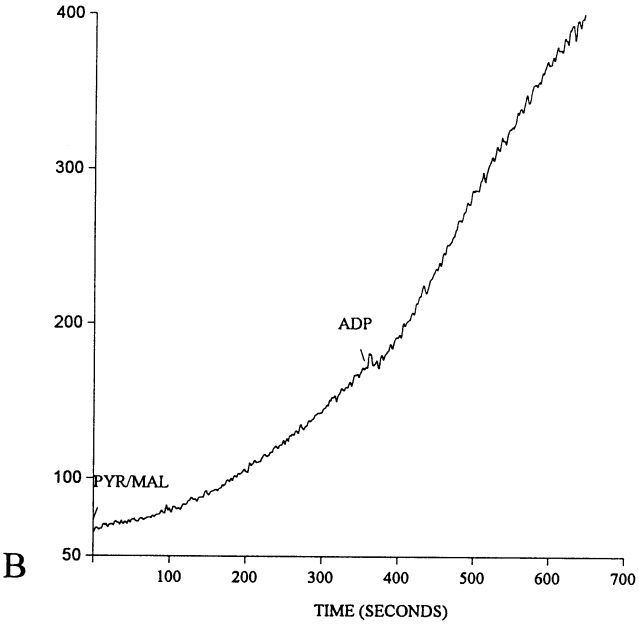
TIME (SECONDS)

A


400
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B

general (including pigeons), in relation to mammals, allowing a similar cardiac output per unit body mass in both groups.

The effect of ADP on the rate of oxygen radical generation of heart and nonsynaptic brain rat and pigeon mitochondria has also been recently studied.[47] In agreement with classic studies,[48,49] succinate-supplemented rat and pigeon heart mitochondrial free radical production was stopped by ADP additions causing the stimulation of respiration from state 4 to state 3 (FIG. 5A). Nevertheless, with complex I–linked substrates, mitochondria produce free radicals in state 3 at rates similar or somewhat higher than during resting respiration (FIG. 5B). The absence of sharp increases in free radical production in spite of a strong increase in oxygen consumption during oxidative phosphorylation (state 3) was possible due to strong decreases of free radical leak in that state. This shows again (like in the rat–pigeon comparison) that oxygen radical generation does not necessarily increase in proportion to increases in mitochondrial oxygen consumption. In fact, the decrease in free radical leakage during the state 4 to state 3 transition can help to explain two apparent paradoxes: (1) the lack of massive muscle oxidative damage and shortening of life span due to exercise, in spite of up to 23-fold increases of oxygen consumption, together with the very low levels of antioxidants present in muscles (and heart and brain); (2) the presence of some degree of oxidative stress during exercise, due to continuation or slight stimulation of complex I free radical production in state 3, in spite of the stop of mitochondrial free radical production by ADP with succinate as substrate. These results also showed that, whereas the complex III generator stops producing free radicals in state 3, this does not happen to the complex I generator. Taken together, the data point to a main role of complex I in free radical generation because, at variance with complex III, it is active in both nonsynaptic brain mitochondria and in heart mitochondria, and it is also active in the two main physiologically relevant states, state 3 and state 4.

The lower rates of free radical generation of pigeon versus rat mitochondria,[35,36,38,46,47] observed in at least four tissues (heart, brain, lung, and kidney) and occurring both in state 4 and state 3,[47] are in agreement with the free radical theory of aging, taking into account the MLSPs of the two species. Nevertheless, the lower free radical production of the

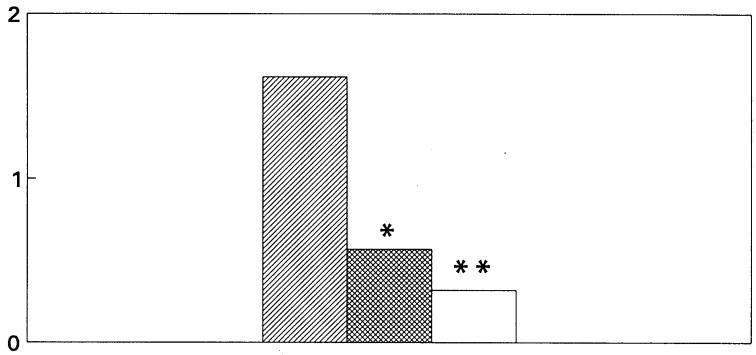

FIGURE 6. Heart mitochondrial H_2O_2 production in mice, parakeets (*Melopsittacus undulatus*), and canaries (*Serinus canarius*) with pyruvate/malate as substrates (nmol H_2O_2/min/mg protein). MLSP = maximum life span potential. * ($p < 0.05$) and ** ($p < 0.01$): significantly different from mouse H_2O_2 production. ▨, mouse (MLSP, 3.5 yr); ▩, parakeet (MLSP, 21 yr); ▭, canary (MLSP, 24 yr).

pigeon could also be due in principle to other characteristics of this particular animal species not related to its high MLSP. In order to extrapolate the low free radical production of the pigeon to birds, in general, we considered it important to study the same problem in other birds species appertaining to different orders than pigeons (Columbiformes). With this purpose, heart mitochondria were isolated from mice (MLSP = 3.5 years), canaries (*Serinus canarius*; MLSP = 24 years; order Passeriformes) and parakeets (*Melopsittacus undulatus*; MLSP = 21 years; order Psittaciformes). The three homeotherm species also show, like in the rat–pigeon comparison, similar values of basal-specific metabolic rate and body size. As is shown in FIGURE 6, oxygen radical production of heart mitochondria is also significantly lower in canaries and parakeets than in mice. This suggests that the capacity to show a low rate of ROS production is a general characteristic of bird mitochondria.

FREE RADICAL PRODUCTION, DNA, AND AGING

The lower free radical production of bird versus mammalian mitochondria strongly supports the free radical theory of aging. It also supports the notion that the inverse correlation observed between mitochondrial free radical production and MLSP in mammals following the rate of living theory is mechanistically related to their MLSPs, instead of being a simple correlate of their metabolic rates. The rate of living theory of aging has many exceptions, but the negative relationship between mitochondrial free radical production and MLSP occurs in all the animal species studied up to date, either mammals or birds. Longevous animals always show relatively low rates of mitochondrial free radical production, no matter if their metabolic rate is low (mammals of large body size) or high (birds). Our results also show that the rate of oxygen radical production of heart mitochondria is similar in rats and mice (see FIGURES 4 and 6), in agreement with their similar longevities (3.5 and 4 years), even though the basal-specific metabolic rate is two-fold higher in the mouse than in the rat. Here again the rate of free radical production, not the rate of oxygen consumption, correlates with MLSP and with the rate of aging. Available data indicate that a parameter relating oxidative stress to MLSP is the (mitochondrial) rate of free radical production.

If mitochondrial free radical production is important for aging, this will have a reflection in the mitochondria themselves. The possible relevance of these organelles for aging was first signaled by Denham Harman in 1972.[2] Recently, much evidence of the implication of mitochondria in the aging process has accumulated. More than 90% of the oxygen is consumed by mitochondria in healthy tissues. Thus, it is expected that the main free radical source in healthy tissues, not subjected to a higher than normal oxidative stress, will be the mitochondria. The mitochondrial DNA has a very high information density, lacks protective histones and polyamines, and shows a much lower repair than nuclear DNA. Furthermore, the mitochondrial DNA is situated in close vicinity to the main free radical generator of the healthy organism, the inner mitochondrial membrane, and occasionally even contacting it.[50,51] In agreement with the above, oxidative damage in mitochondrial DNA (oxo^{8}dG: 8-oxo-2′-deoxyguanosine, referred to dG) is around 15-fold higher than in nuclear DNA in rats.[51] The same is true for mitochondrial versus nuclear DNA deletions. Oxidative damage and deletions in mitochondrial DNA exponentially increase with age in the human heart.[52] Caloric restriction, the only experimental manipulation capable of

slowing the rate of aging and of increasing the MLSP, decreases mitochondrial free radical production in various mice tissues without consistently changing antioxidant levels.[53] We have also found recently that liver mitochondria from longevous species constitutively have a lower degree of membrane fatty acid unsaturation than those of short-lived species. This is not due to a low polyunsaturated fatty acid (PUFA) content; instead, it is mainly due to a redistribution between types of PUFAs, from the highly unsaturated docosahexaenoic (22:6n-3) and arachidonic (20:4n-6) acids to the less unsaturated linoleic acid (18:2n-6) in longevous animals.[54] This leads to a much higher resistance to lipid peroxidation in mitochondria from longevous animals.[54]

Short-lived species, like laboratory rodents, have a large rate of generation of oxygen radicals and high levels of cellular antioxidants. These two characteristics would be compensatory in many parts of the cell. Longevous species have small rates of free radical generation, evolutionarily compensated for with low constitutive antioxidant levels. The mean cellular H_2O_2 concentration will thus be similar in both kinds of species. At the places of free radical generation (*e.g.*, at the inner mitochondrial membrane), however, the local concentration of free radicals will be much higher in short-lived than in long-lived animals (FIG. 1), possibly leading to a much higher oxidative damage to mitochondrial DNA at a given age in animals with short MLSPs.[21,23,55]

The majority of cellular genes are situated in the nucleus, not at the mitochondria. Even though all reports are not in agreement,[56] it has been described that oxo^{8}dG increases with age in rat tissues.[57] Most importantly, liver and urine oxo^{8}dG levels are inversely related to MLSP in mammals, humans showing the minimum levels and the largest longevity among studied species.[14,58] Recent data from our laboratory show that heart oxo^{8}dG is also threefold lower in pigeons than in rats.[59] This is consistent with the lower rates of mitochondrial free radical generation of longevous species. How the mitochondrial-derived oxidative damage is transmitted from mitochondria to the nucleus is not known at present. Various possible explanations have been proposed,[60,61] but up to now, none of them has reached experimental demonstration. A complementary possibility is that differences among animal species in the rate of free radical production at critical sites (near DNA) occur not only at mitochondria but also at the nuclear membrane or at sites of binding of metals to nuclear DNA. Although much weaker in activity than the mitochondrial respiratory chain, the nuclear membrane is known to contain an electron transport chain capable of free radical generation. Clarification of these possibilities must wait for the overcoming of methodological difficulties concerning measurements of free radical production at those nuclear sites in species with different longevities.

It is generally believed that DNA damage is of paramount importance for aging. Thus, the rate of production of free radicals and other damaging 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 indeed shown the existence of strong positive correlations between DNA repair systems and mammalian MLSP across species, including humans.[62–65] The combination of high levels of DNA repair with low rates of free radical production near DNA in longevous animals can contribute to explain their very small levels of steady-state oxidative DNA damage[14,58] and slow aging rate (FIG. 7), whereas the quantitative differences in these two factors between animals are not enough, when independently considered, to explain their differences in maximum longevity.

Much previous work has been centered on testing the possibility that antioxidants decrease with age and, more scarcely, on possible increases of free radical production with

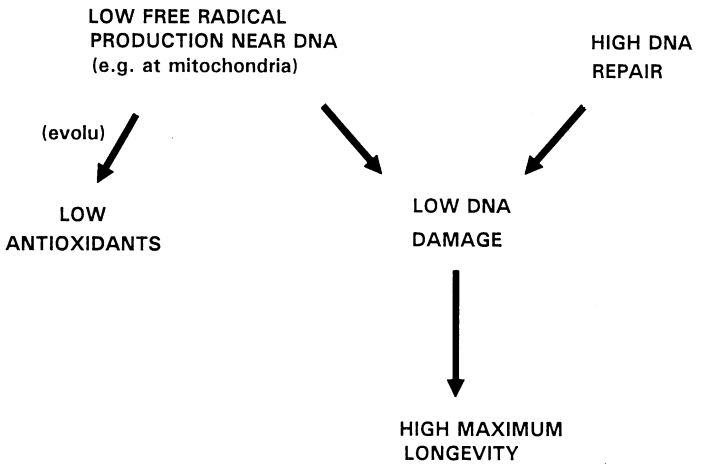

FIGURE 7. Model linking characteristics found in longevous animals (evolu: evolutionary relationship). The rest of the relationships linked by arrows are mechanistic.

age. Available data do not consistently support either the first[66–69] or the second[45,70–72] possibility, but neither of them are needed for the free radical theory of aging to be true. A constant rate of free radical production would cause an accumulation of oxidative DNA damage, because 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 intensity of these last two processes did not change during aging. It is the difference between animal species in the rate of these two processes in the adult animal (both young and old) that matters in explaining their differences in the rate of accumulation of DNA damage and rate of aging.

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