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EDITORIAL

The Role of Mitochondria in Aging

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Summary

Aging is a process drawing attention of many researchers, and at present many theories exists, which try to explain this chain of inevitable events leading to death of organism. In this article we focused our attention on a theory explaining the degenerative changes occurring during aging by the effect of oxygen free radicals. These highly reactive radicals are produced during oxidative phosphorylation in mitochondria. All cellular components appear to be sensitive to oxygen-radical damage. Lipids, proteins and nucleic acids are probably the most susceptible to this injury. Lipoperoxidation of lipids together with cross-linking of proteins with phospholipids and nucleic acids caused changes in membrane fluidity. Mitochondrial DNA coding several subunits of respiratory chain enzymes can be also damaged by these radicals. All these changes together have negative impact on mitochondrial metabolism resulting progressive decrease of the efficiency of oxidative phosphorylation and thus of the whole organism.

Key words

Mitochondrial genome – Energetic metabolism – Free-oxygen radicals – Aging

Introduction

Aging can be defined as a sum of functional and structural changes accumulating in an organism during life. These changes are associated with or responsible for the decreasing physiological performance and increasing susceptibility to disease and death. Studies from many laboratories suggest that the cause of aging is a result of long-term toxic side-effects of normal metabolism and development. Thus, aging is probably not the result of a genetic program expressing specific genes for aging. One may conclude that the rate of aging of an organism must be controlled by some protective mechanisms acting against the unfavourable side-effects of normal biological processes.

According to Medvedev (1990) who reviewed the various theories of aging, there are now more than 300 of them and their number continues to increase. In this review we focused our attention on a theory which belongs to the group of theories related to primary damage. It explains the origin and causes of inevitable injury of cells and final death of the organism under the influence of possible external or internal deleterious factors.

Free-Oxygen Radical Theory of Aging

Since aging results in a great variety of changes at all levels of biological organization, it is logical that the first and main attempt was focused on assigning the key senescence-triggering factors. A theory which provides a logical explanation for the senescent involution observed in the tissues of multicellular organisms, ranging from loss of genetic information to a decline in physiological performance is the oxygen toxicity – free radical theory of aging (Miquel *et al.* 1979, 1984). The molecular mechanism of free radical-induced disorganization was first proposed by Harman (1956, 1972, 1981) and

Gerschman (1962, 1981). According to this theory, it was postulated that free radicals play a key role in starting the chain of age-related disorganization in target cells which contain mitochondria using high levels of oxygen and thereby releasing large amounts of oxygen radicals exceeding the homeostatic protection of cells (Fig. 1).

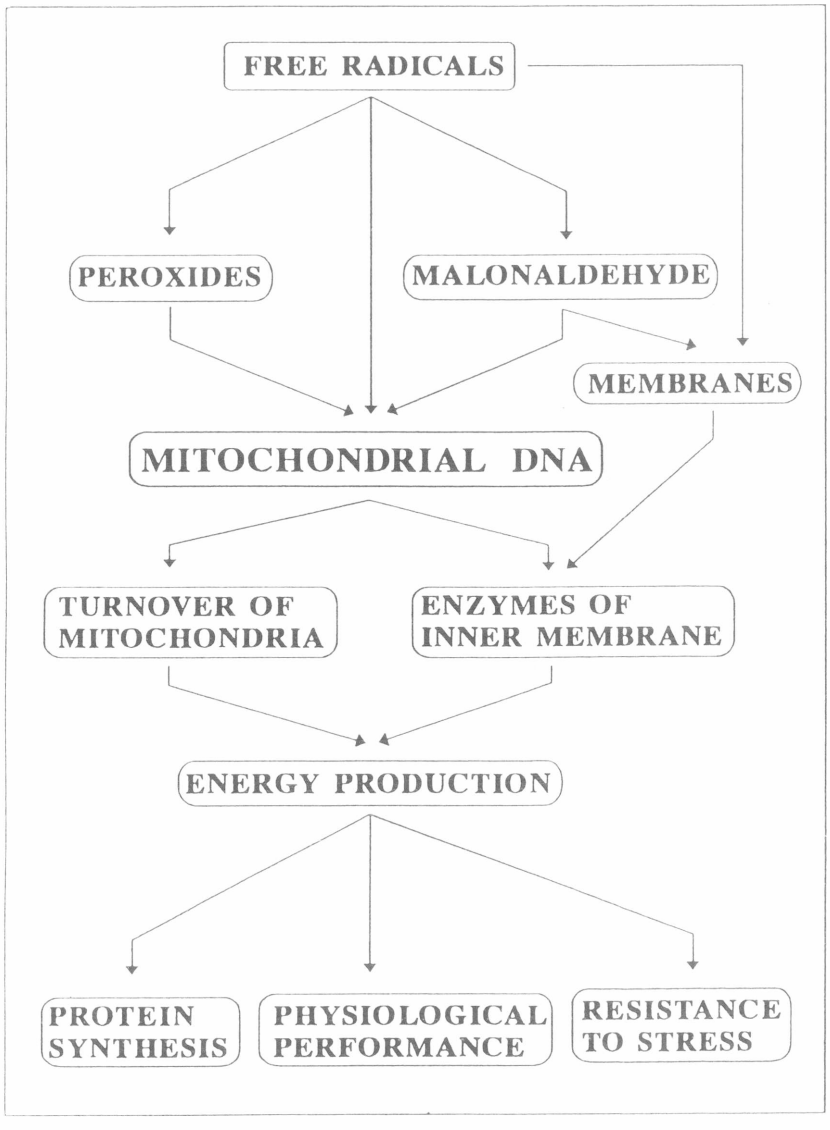

Fig. 1
The proposed mechanism of oxygen free radicals theory of aging. Free radicals generated in the mitochondrial inner membrane together with related substances (peroxides and malonaldehyde) injure mtDNA as well as mitochondrial membrane. Damage of mitochondrial components results in a decline of energy production and in concomitant senescent changes at the physiological level.

Oxygen plays a very important but paradoxical role in the life of aerobic organisms. It has a vital function as the terminal electron acceptor during respiration but, on the other hand, oxygen and its metabolites are potentially cytotoxic (Davies 1987). Univalent reduction of oxygen produces the superoxide radical ($^{\cdot}O_2$) whereas divalent reduction generates hydrogen peroxide (H_2O_2). Homolytic fission of the O–O bond in H_2O_2 produces two highly reactive hydroxyl radicals ($^{\cdot}OH$) (Halliwell and Gutteridge 1984). As was shown by many authors, all cellular components appear to be sensitive to oxygen-radical damage (Davies 1987, Fridovich 1978, Sevanian and Hochstein 1985, Adelman *et al.* 1988, Hunt *et al.* 1988). Free radicals originate during the reduction of oxygen in the respiratory chain of the inner mitochondrial membrane. Most of them are generated at the level of ubiquinone (Boveris *et al.* 1972, Loschen *et al.* 1971) or of cytochrome b-566 (Chance *et al.* 1979) and are almost completely destroyed by the action of Mn-superoxide dismutase. The product of superoxide dismutation, i.e. H_2O_2, accumulates in mitochondria due to the incomplete elimination by intramitochondrial catalase and peroxidase (Fridovich 1975). Moreover, hydrogen peroxide can react with the superoxide to produce an extremely active hydroxyl radical. The following step in the processes connected with aging is lipid peroxidation, triggered by these

radicals. One of the most injurious chemicals resulting from the free radicals-peroxidation reactions is malonaldehyde which may react with the β-amino group of lysine, inducing the cross-linking of proteins with the amino groups of phospholipids and nucleic acids. Nohl and Hegner (1978) observed that more superoxides escape quenching in the mitochondria in older tissues, because the ability of mitochondria-protecting mechanisms against disorganizing effects of free radicals decreases during senescence. It was shown the production of lipoperoxides is decreased by carnitine and this can protect various tissues against their destructive effects (Koudelová *et al.* 1994).

Lipoperoxidation induced by oxygen radicals has a great effect on the properties and function of mitochondria. It decreases the amount of polyunsaturated fatty acids in mitochondrial membranes and this effect together with cross-linking of proteins with the amino groups of phospholipids and nucleic acids induced by malonaldehyde causes changes in membrane fluidity, i.e. the increase of rigidity. Most of these negative changes are repaired by the action of phospholipases and the gaps in membranes are filled by freshly synthesized phospholipids. Membrane regeneration can be impaired by the production of membrane complexes by cross-linking of phospholipids and neighbouring proteins. These fragments are indigestible by proteolytic enzymes and they are stored in fluorescent granules called "age pigment" or lipofuscin (Miquel *et al.* 1977, 1980). The change in membrane fluidity is a very important factor affecting the characteristics of membrane-associated enzymes and it decreases the physiological efficiency of the organism against senescence. A decrease in membrane fluidity by about 25 % can lead to the loss of mitochondrial respiratory control (Vladimirov *et al.* 1981). Moreover, the deficiency of polyunsaturated acids caused by lipoperoxidation results in a loss of mitochondrial ATPase activity because their presence in the enzyme lipidic microenvironment is necessary for its proper function (Miquel and Fleming 1986).

The so-called calcium theory of aging is closely coupled with the oxygen free radical theory of aging. Calcium is responsible for the control of a large number of biochemical processes in cells of all organisms (Carafoli 1987, Campbell 1983). But in the course of senescence, and also in ischaemic cells, the ability to maintain calcium homeostasis decreases (Farber 1981, Khachaturian 1987) resulting in either an excess or a lack of intracellular calcium. It was shown that cellular and physiological changes in aging are linked to altered calcium homeostasis (Michaelis 1987). At this point, it is possible to link two of the above mentioned hypotheses of aging because lipids peroxidized by oxygen free radicals may act as calcium ionophores (Serhan *et al.* 1981) inducing changes in the ratios between inner and outer compartments of organelles and cells. Such a change means a serious threat to calcium-dependent metabolic pathways which can endanger the survival of organelles and cells. Moreover, the activation of phospholipases by calcium may cause further membrane damage and the generation of more free radicals (Seisjo 1981). At this point it is worth mentioning the role of nifedipine which is a well-known calcium channel blocker (Hess *et al.* 1984). Beside this, nifedipine reduces the lipid peroxidation level in microsomes (Engineer and Sridhar 1989) and in liposomes (Ondrias *et al.* 1989). This means that nifedipine is an effective free radical scavenger. This opinion is supported by the finding that application of nifedipine prolonged the life span of *Drosophila* (Massie *et al.* 1989) and of rotifers (McTavish *et al.*, 1990).

Age-Dependent Changes of Mitochondrial Genome

As it has already been mentioned above, aging is accompanied by degenerative changes at all levels of the biological organization of cells. That is why we can also predict changes of nucleic acids which could play a considerable role during senescence. Miquel and Fleming (1986) postulated the key role of oxygen radical damage of mitochondrial DNA (mtDNA) in the age-dependent disorganization. This hypothesis is based on the fact that production of all free radicals is compartmentalized inside intracellular organelles, especially in mitochondria. Mitochondrial DNA is synthesized on the inner mitochondrial membrane (Shearman and Kalf 1977) so that the free radicals can easily interact with the mitochondrial genome. Moreover, mtDNA is not protected either by histones as the nuclear DNA or by other adequate repairing mechanisms (Fukanaga and Yielding 1979).

The mammalian mitochondrial genome is relatively very small, (only 16.5 kbp in human) in comparison with the nuclear genome of about 10^9 bp (Anderson *et al.* 1981). Mitochondrial DNA is a closed circular molecule and each mitochondrion contains 2 to 5 copies. This means that depending on the type of tissue, each cell has 1 000 to 10 000 apparently identical copies of mtDNA (Bogenhagen and Clayton 1974). However, while a small part of chromosomal DNA is expressed in a certain tissue and at a particular stage of differentiation (about 7 %), the whole mitochondrial genome must always be expressed to ensure the main mitochondrial function in the cell, i.e. energy production. Except for short non-coding regions involved in the regulation of replication and transcription, mtDNA consists almost entirely of coding sequences (in fact several of the protein-coding genes overlap). Mitochondrial DNA contains 13 genes for respiratory chain polypeptides: subunits I, II and III of cytochrome oxidase, subunits 6 and 8 of the F_0 subunit of H^+-ATPase, the apoprotein of cytochrome b and 7 subunits of NADH dehydrogenase (Attardi

1985, Cantatore and Saccone 1987). Beside these genes, mitochondrial genome contains two ribosomal (12S and 16S) RNA genes and 22 tRNA genes necessary for the expression of protein-coding genes. As all genes in the mitochondrial genome are organized continuously, each mutation in mitochondrial DNA influences a functionally important part of the genome. On the other hand, the mutation in the nuclear DNA will very probably affect a non-expressed region of the genome. It has been estimated that under physiological condition the extent of oxidative damage to nuclear DNA is one base modification per 130 000 bases. The damage of mitochondrial DNA under the same conditions could be as high as one base modification per 8 000 bases (Richter *et al.* 1988). The main mutagen in mitochondria is probably malonaldehyde which is the product of lipid peroxidation and reacts easily with the amino groups of nucleic acids and forming cross-links with their bases. Hydrogen peroxide, which is normally present in mitochondria (Massie *et al.* 1972), could be another mutagenic agent. As has already been shown, the amount of mitochondrial DNA really decreases with age. Massie *et al.* (1975) found that *Drosophila* aging may be accompanied by a total loss of mitochondrial DNA while nuclear DNA seems to be unaffected. In the mitochondria isolated from rat liver senescent decrease in extractable mtDNA was also found (Stocco and Hutson 1978). Recently, Gadaleta *et al.* (1990) have shown that the steady-state concentration of the messenger RNA for the mtDNA coded subunit I of cytochrome oxidase, undergoes an age-dependent decrease in the rat brain and heart. Changes in mitochondrial protein synthesis are in agreement with those at the level of mtDNA. As was reported by Marcus *et al.* (1982a,b), the rate of protein synthesis by liver mitochondria isolated from very old rats is about 40 % of that found in young rats. Mitochondria from the liver and kidneys of mice and from *Drosophila* exhibited a similar age-dependent decrease in protein synthesis (Bailey and Webster 1984).

Energetic Metabolism During Aging

The process of aging is coupled with changes in fluidity of biological membranes, especially of the mitochondrial inner membrane. This phenomenon has a great impact on cellular bioenergetics for several reasons. Mitochondria are the main site for the production of ATP and under normal conditions they cover more than 80 % of the energy demand of the cell (Mitchell and Moyle 1967). Moreover, the energy transduction is impossible without membranes (Mitchell and Moyle 1967, Mitchell 1979) and mitochondrial metabolism is compartmentalized inside membranes of highly restrictive permeability properties. It is clear that changes in the membrane induced by free radicals can affect the properties of membrane-associated enzymes which are known to depend on composition of lipid microenvironment (Amler *et al.* 1986, Brown and Cunningham 1982).

Since 1959 when Weinbach and Garbus reported for the first time a decreased ability of liver mitochondria of old rats to phosphorylate ADP with 3-hydroxybutyrate as substrate, many articles concerning the influence of aging on different enzymes or enzymatic systems have been published. Mitochondrial respiratory functions of senescent tissues have been widely studied in many laboratories but the results are often controversial due to the variability of experimental conditions, animal species and tissues used as the source of mitochondria.

One of the crucial aspects in the regulation of mitochondrial energetic metabolism concerns the transport of substrates across the mitochondrial membrane. An important enzyme which could contribute to the control of respiration by modulating its activity is adenine nucleotide translocase (AdNT) (Groen *et al.* 1982). This translocator is embedded in the inner mitochondrial membrane and catalyzes a one:one exchange of cytosolic ADP for mitochondrial ATP generated in oxidative phosphorylation. Nohl and Kramer (1980) found a 40 % decrease in AdNT as a consequence of senescence. Similarly, Kim *et al.* (1988a) found a 32 % decrease in V_{max} of AdNT in rat liver mitochondria and a 20 % decrease of activity of the same enzyme in cardiac mitochondria (Kim *et al.* 1988b). The authors speculated that the decreased enzymatic activity is due to changes in lipid microenvironment (increased biomembrane rigidity) and diminished pool of exchangeable adenine nucleotides. These results are supported by recent work of Paradies and Ruggiero (1991) who found that the rate of phosphate transport in liver mitochondria from aged rats is significantly reduced (40 %) compared to that obtained in mitochondria from young control rats. But these authors did not find any decrease in the amount of endogenous phosphate in mitochondria from old animals and suggested that the main reason for a lower phosphate content are changes in membrane composition. It had been reported that cardiolipin is specifically required for the reconstitution of the isolated phosphate carrier (Kadenbach *et al.* 1982) and the content of this phospholipid is markedly reduced (by about 30 %) in the mitochondrial membrane of aged rats (Paradies and Ruggiero 1991). Considerable age-linked decrements (30–40 %) were also found for other transport systems in rat heart mitochondria. These include acylcarnitine-carnitine translocation (Hansford 1978), transport of Ca^{2+} (Hansford and Castro 1982) and pyruvate transport (Paradies and Ruggiero 1990). In the case of acylcarnitine-carnitine translocator, differences in the activity were attributed to a decreased intramitochondrial pool of carnitine and acylcarnitine

species in old age. On the other hand, the reduced activity of pyruvate translocator may be ascribed either to a general modification of membrane lipid composition leading to increased rigidity and/or to more localized changes in the lipid microenvironment.

At the beginning, the level of energetic processes in mitochondria during aging was measured mainly as State 3 oxidation. While a decrease of this oxidation was found during aging, State 4 rates of oxygen uptake were shown to be unchanged with aging in the brain (Chiu and Richardson 1980, Weindruch *et al.* 1980), the liver (Weindruch *et al.* 1980) and the heart (Chen *et al.* 1972, Chiu and Richardson 1980, Starnes *et al.* 1981). The mitochondrial respiratory rates were followed with the help of a broad spectrum of substrates, but again the results are often controversial. In the case of pyruvate, several authors found no changes in oxygen uptake with senescence (Inamdar *et al.* 1974, Chen *et al.* 1972, Starnes *et al.* 1981, Hansford 1978) but for the same substrate Chiu and Richardson (1980) showed 49 % decrement with age. In the case of succinate, all authors agree that State 3 rates of succinate oxidation are not diminished with age in mitochondria from most tissues (Chen *et al.* 1972, Inamdar *et al.* 1974, Chiu and Richardson 1980, Weindruch *et al.* 1980). Evidence also exists for both unchanged (Inamdar *et al.* 1974) and decreased (Chen *et al.* 1972) mitochondrial malate/glutamate oxidation with age. As far as fatty acids and acylcarnitine are concerned, there is little doubt that their oxidation is negatively affected by aging (Chen *et al.* 1972, Hansford 1978). The reason for this is not due to a single enzyme but probably to a common decline of activity of several enzymes involved in fatty acid oxidation. The above mentioned decrease in carnitin-acylcarnitin translocase activity is another reason. The effect of aging on the electron transport chain, especially on its individual components, is very important. Abu-Erreish and Sanadi (1978) found a lowered cytochrome content in heart homogenates in senescence but a constant proportion between cytochromes. In connection with these findings, the activity of cytochrome oxidase shows a senescent decline in the liver (Weindruch *et al.* 1980), the heart (Abu-Erreish and Sanadi 1978) and the brain (Benzi *et al.* 1990, Curti *et al.* 1990, Curti and Benzi 1991). The content of coenzyme Q also shows a striking decline with senescence in the heart, kidney and skeletal muscle (Beyer *et al.* 1985). This could have important functional consequences since ubiquinone plays a key role in mitochondria, supporting the mechanism of lipid antioxidation, electron transfer and regulation of neighbouring components in the respiratory chain (Rauchová *et al.* 1995).

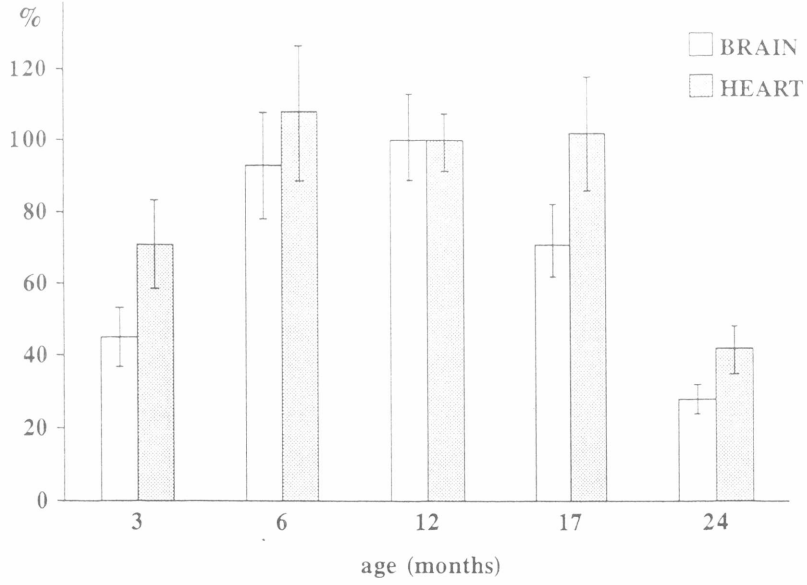

Fig. 2
Age-dependence of V_{max} of mitochondrial ATP hydrolase activity in the rat brain and heart. Enzyme activity was determined spectrophotometrically at 25 °C using a regenerating system containing an excess of phosphoenolpyruvate and pyruvate kinase as described in Guerrieri et al. (1989). Values are expressed in percentage of enzyme activity found in 12-month-old animals. Data are the means ± S.E.M. of 5 experiments.

Mitochondrial H^+-ATPase (complex V) plays a key role in cell energetic metabolism by synthesizing more than 80 % of total production of ATP under physiological conditions. As it is known that aging animals tend to be less "energetic", it is not surprising that Lakata and Yin (1982) found low levels of ATP and a decrease of the ATP/ADP ratio in cardiac muscles of old rats. Nohl and Kramer (1980) reported a decline of ATP hydrolysis catalyzed by heart mitochondria in 23-month-old rats compared with mitochondria in the heart of 3-month-old rats. An age-related decrease in the efficiency of oxidative

phosphorylation has been observed in synaptosomes (Benzi *et al.* 1990) and related to a possible alteration in the mitochondrial membrane-bound F_0F_1 ATP synthase and/or synaptosomal adenine nucleotide pool. The age-dependence of ATP hydrolase activity and oligomycin-sensitive passive proton conduction in submitochondrial particles were recently followed by Guerrieri *et al.* (1992). They showed an age-dependent variation of V_{max}, but no significant change in K_M, during the period from 3 to 24 months of age (Fig. 2). In the rat brain and heart, the ATP hydrolase activity increased from 3 to 6 months of age and was associated with an increase in the immunodetected β subunit of F_1 and a decrease of oligomycin-sensitive proton conduction. The depression of ATPase activity, observed in rats from the age of 12 to 24 months is accompanied by an increase of oligomycin-sensitive proton conduction. This is associated with a decrease of the F_1 subunit content (Fig. 3). This results in a dissipation of the electrochemical proton gradient set up by respiration. Thus the energy metabolism of brain and heart of aged rats, similar to that of foetal tissues, should be more dependent on the glycolytic activity for the production of cellular ATP.

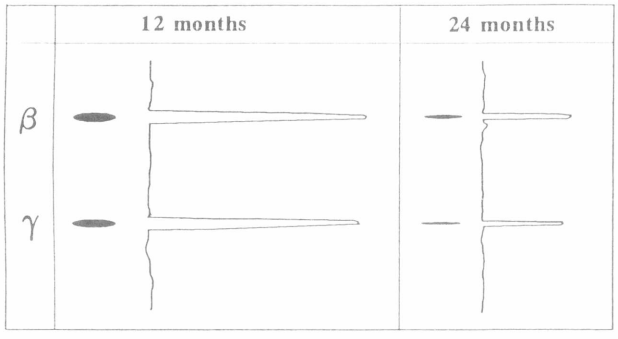

Fig. 3
Immunoblot analysis of β and γ subunits of mitochondrial ATPase F_1 sector in rat heart mitochondria during aging. The content of subunits was analyzed by immunoblotting with specific antiserum against bovine F_1 as described in Guerrieri et al. (1989). The amount of subunits was estimated by scanning nitrocellulose sheets at 590 nm.

Conclusions

From all the above mentioned data it is possible to conclude that aging is a very complex process which takes place at all levels of the organism. Degenerative changes are predominantly expressed in mitochondria. As the only place where energetically rich substrates are finally oxidized in the system of the electron-transport chain, they are also the main place of the production of oxygen free radicals. These radicals are considered by many authors to be the leading if not the sole factor causing degenerative changes of all kinds of biomolecules during aging. The structure which is most sensitive in the living organism during aging to the action of oxygen radicals is probably the mitochondrial genome. In comparison with the nuclear genome, this genome is not sufficiently protected against the damage induced by oxygen radicals. Changes in the mitochondrial genome can lead to lower efficiency of energetic metabolism and generally to a lower energetic turnover of the whole organism.

The second important area concerns the age-dependent changes in composition of the inner mitochondrial membrane. Generally, the inner mitochondrial membrane tends to be more rigid during senescence due to the decreasing content of polyunsaturated fatty acids induced by free-oxygen radicals. The other factor is malonaldehyde which, by cross-linking of integral membrane proteins, changes the physical as well as the chemical properties of the inner mitochondrial membrane. As a consequence of these changes, especially in membrane rigidity, the kinetic characteristics of integral membrane enzymes are changed, i.e. enzyme activity decreases.

According to the hypothesis of Linnane *et al.* (1989), the accumulation of somatic mitochondrial gene mutations during life leads to a mosaic of cells ranging in their bioenergetic capacity from normal to partially or grossly defective. From this point of view the key factor in the process of aging is the accumulation of bioenergetically defective cells. This also implies a progressive decrease in the efficiency of oxidative phosphorylation to which the organism must react and adjust its physical activities to compensate this loss.

The investigation of principles of cellular aging may have a great impact in human medicine. Some pathological states are known (e.g. Alzheimer's disease) which are classified as age-related diseases, i.e. those which are based on pathological expression of structural changes related to the particular period of life. Certain cytochemical and biochemical characteristics, e.g. of neurones and their mitochondria from patients with these diseases, are very similar to those from physiologically aged brains. Thus it is possible that detailed knowledge of the process of physiological aging could help in looking for ways of arresting or retarding the progress of these pathological processes.

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