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The Role of Free-radical Oxidation Reactions in the Molecular Mechanisms of the Ageing of Living Organisms

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The review describes the present state of knowledge about the free radical oxidation reaction of biomacromolecules as one of the ageing factors and considers the possible causes of the changes in the rate of ageing and of the prolongation of life in experiments involving exposure to chemical geroprotectants.
The bibliography includes 157 references.

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I. INTRODUCTION

Regardless of the complexity and wide variety of theoretical ideas about the mechanisms of ageing, the majority of gerontologists are unanimous in their view that it can be a consequence of the accumulation of lesions and defects at all levels of the living organisms.

One can point to at least two fundamentally different sources of the breakdown of the functions and of the structural damage to macromolecules. Firstly, errors may arise in the course of the normal functioning of the cell, for example, in the copying of the DNA matrix or in the synthesis of protein as a consequence of the imperfection of the transmission of information from one macromolecule to another. The reliability of the replication of DNA and of the components of the protein synthesising mechanism depends on their molecular structure and is genetically determined.

In this review attention is concentrated mainly on the second source of the impairment of functions as a result of ageing, associated with the random changes in various components of the cell caused by processes involving the oxidative degradation of the macromolecule. The "free radical" hypothesis, which was initially based on the similarity of the radiation-induced and natural ageing processes, was at one time received with reserve by some gerontologists, but it nevertheless proved fruitful because it led to the possibility of influencing the rate of ageing by chemical agents. Radioprotectants and antioxidants are at the head of the list (fairly extensive nowadays) of chemical geroprotectants.

Chemical geroprotectants are widely used as instruments for the study of the mechanisms of the ageing of various objects of experimental gerontology: cell cultures, unicellular organisms, nematodes, insects, mammals, etc. Recognition of the usefulness of antioxidants was reflected, in

particular, in the fact that, during the last decade, the study of the so-called "membrane component" of the mechanism of ageing has become widely popular.

On the basis of an enormous amount of empirical data, the view has been established in the gerontological literature that there exist many independent ways in which age-induced changes accumulate even at the molecular level. This view is difficult to reconcile with the attempts to create a simple and universal theory of ageing and to suggest some way of altering the rate of ageing and the species dependent limits of the lifespan established in the course of evolution.

In the present review we attempted, using our own and literature data concerning the nature of the stochastic damage to biostructures, to justify the view that free-radical oxidation processes constitute a special and all-permeating background against which the metabolic activity of the cell takes place.

II. FREE-RADICAL SPECIES (O_2^-, OH·, $RO_2^·$) AS ENDOGENIC DAMAGING AGENTS

The free radical states of organic coenzymes, mainly semioxidised flavins and the semiquinone forms of ubiquinones localised in mitochondria, cytochrome P-450 functioning in the electron-transport chain of microsomes, as well as haemoproteins, copper-containing proteins, and manganese, molybdenum, etc. complexes, can be relatively readily detected in biological objects by radiospectroscopic methods. There is a direct quantitative relation between the metabolic activity of the tissue and the intensity of the EPR signals[1-3]. Attention has been devoted in a number of studies[4-6] to the changes in the concentrations of these metabolic free radicals during ageing. The involvement in the ageing mechanism of free radicals whose source is non-enzymatic reduction of oxygen, resulting in the formation of paramagnetic species such as the hydroxy-radical, the superoxide radical-ion, and the peroxy-radical (OH·, O_2^-, $RO_2^·$), appears to be more appreciable. The O_2 reduction reactions are presented below:

$$O_2 + e^- \rightarrow O_2^{\cdot-}$$
$$O_2 + 2e^- + 2H^+ \rightarrow H_2O_2$$
$$O_2 + 3e^- + 3H^+ \rightarrow H_2O + OH^·$$
$$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$$

The complete reduction of oxygen to water requires the participation of four electrons, while in the remaining cases partial reduction products are formed: hydrogen peroxide and the radical species O_2^- and OH·, whose high reactivity is the main cause of the toxic properties of pure oxygen.

Attention has been drawn to the fact[7] that the prebiotic atmosphere of the Earth did not contain free oxygen, which appeared in it as the product of the vital activity of photosynthesising blue-green algae. The origin, propagation, and certain initial stages of the development of life took place in an atmosphere lacking oxygen, whose appearance could not fail to influence evolution.

Oxygen accumulating in the atmosphere and the world ocean led to new possibilities for its utilisation by living organisms in biosynthetic processes and energetic reactions but at the same time it constituted a definite threat to the very existence of life. Obligatory anaerobes, which live in the absence of oxygen and have no means of protection against the free radical toxic products of its reduction, are destroyed in air[8]. For all respiring organisms, oxygen, which maintains their life, is at the same time toxic; they exist in its presence solely as a result of definite protecting systems which arose during evolution and function so effectively that the toxic properties of O_2 have attracted the serious attention of scientists only comparatively recently in view of the analogy between oxygen poisoning and radiation damage[9].

It is possible to formulate briefly the general postulates reflecting the fundamental features of the "free radical danger" in the following manner:

(1) Non-enzymatic O_2 reduction reactions constitute the background against which all processes involved in vital activity take place;

(2) oxygen-containing free radicals are the cause of the oxidative degradation of biological macromolecules;

(3) by virtue of their high reactivity (the rate constants for the interactions of the OH· radical are close to the diffusional values), the free radicals do not require a specific substrate for their reactions, the site which they attack is random, and they can damage any unit in the molecular structure of the cell;

(4) transition metals (Fe, Cu) play the role of catalysts of peroxide oxidation.

It can now be claimed that the "free radical" hypothesis of ageing proposed in the 1950's[10] refers to a small number of ideas in gerontology which have withstood experimental tests. Indeed, following self-evident logic, one may expect the acceleration of ageing with increase of the concentration of free radicals, which can be readily achieved by chronic irradiation or by an increase of O_2 pressure. Exposure to ionising radiation is one of the most effective methods for the experimental acceleration of ageing. On irradiation by sublethal doses, explicit signs of radiation damage do not arise in animals (with the exception of the early greying of hair) but their lifespan is shortened in proportion to the size of the dose. An example of the acceleration of ageing in Drosophila males irradiated at the age of one day by γ-ray doses of 80 and 40 krad has been quoted in a monograph[11]. Doubling of the dose shortens the average lifespan of the flies by a factor of two. Analogous data are available for laboratory mice and rats[12]. There is no complete identity between natural ageing and the reduction of the lifespan induced by radiation[13], but analysis of the causes of the death of the irradiated animals showed that they die due to the same illnesses as the non-irradiated animals, although the relative frequencies of the different illnesses may not be the same. Thus, presumably at least some of the damage due to radiation is similar to age-induced changes.

An increase of the O_2 concentration in air also leads to an appreciable decrease of the lifespan of Drosophila, which is accompanied by an intense accumulation of the "age pigment" (lipofuscin) in the tissues of the flies, which indicates the activation of the free-radical oxidation[14]. In this case the average lifespan of the flies fell from 78.1 to 34.6 days and their viability decreased much faster than in the control. The study of their tissues under light and electron microscopes revealed the presence of large accumulations of ceroid lipofuscin in the internal organs, consisting of dense lamellar bodies, and the breakdown of cytoplasmatic membranes in the nerve tissues.

The ageing process in cultured cells also proved sensitive to changes in the oxygen concentration. When human diploid fibroblasts were cultured under a high O_2 pressure up to 50 atm, a retardation of the rate of cell division and of capacity for the formation of colonies, particularly in later stages, was observed. On the other hand, if the same pressure was established with an excess of nitrogen, there was no effect on the viability of the cells, i.e. the observed decrease of the capacity for proliferation is a consequence of the toxicity of the O_2 and not the increased pressure[15].

Another method of accelerating free-radical oxidation, by enriching the diet in unsaturated readily oxidisable fats, was used in other studies. An increase in the amount and degree

of unsaturation of fats reduced the average lifespan of mice and increased the frequency of malignant tumours[16]. When two strains of Drosophila were maintained on a medium in which glucose was replaced by palmitic acid, vegetable oil, margarine, etc., keeping the calorific value of the feed at a constant level, a decrease of the average lifespan proportional to the amount of added fat was observed. If the flies were transferred from the fat-enriched diet to the standard diet, their mortality diminished but not all the changes induced by the introduction of excess fat into the diet were reversible[17].

On the other hand, a decrease of the intensity of the free-radical reactions in the organism retards ageing and increases the lifespan. The results of these studies will be presented in the concluding section of this review in connection with the discussion of the mechanism of the action of geroprotectants.

In a more careful examination of the reaction of oxygen-containing free radicals, account must be taken of specific features of their interaction with the cell components. The interaction of the superoxide radical-ion $O_2^{\cdot-}$ with hydrogen peroxide leads to the formation of the radicals OH· and singlet oxygen whose molecule carries excess energy[18]:

$$O_2^{\cdot-} + H_2O_2 + H^+ \rightarrow OH^{\cdot} + H_2O + O_2^*$$

$$O_2^{\cdot-} + H_2O_2 \rightarrow O_2 + OH^- + OH^{\cdot}$$

The spontaneous dismutation (disproportionation) of $O_2^{\cdot-}$ leads to the reformation of hydrogen peroxide[19]:

$$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \rightarrow O_2 + H_2O_2$$

the rate constant for this reaction at pH 7.4 amounting to 10^5 s^{-1}.

Numerous spontaneous and enzymatic oxidation reactions, generating considerable amounts of $O_2^{\cdot-}$, are known: autoxidation of reduced flavins, hydroquinones, catecholamines, ferredoxins, and haemoproteins, enzymatic processes with participation of xanthine oxidase and many flavin hydrogenases, etc.[20,21] The superoxide radical-ion reacts as both a reductant and an oxidant. It participates in reactions involving the reduction of cytochrome c, Nitro Blue Tetrazolium, tetranitromethane, etc. and also oxidises sulphydryl compounds, sulphites, ascorbic acid, etc. The reduced NADH when linked to lactate dehydrogenase is readily oxidised by the $O_2^{\cdot-}$ radical-ion, while free NADH is oxidised more slowly[22].

The enzymatic dismutation of $O_2^{\cdot-}$ to hydrogen peroxide and oxygen is effected by the enzyme superoxide dismutase (SOD); the cytoplasm of the cells of eukaryotes contains SOD incorporating copper and zinc and its participation in the disproportionation reaction of the $O_2^{\cdot-}$ radical-ions makes the latter take place up to the theoretical diffusion limit, the rate of spontaneous dismutation being exceeded by a factor of approximately 20000. SOD protects the cell from the toxic action of the $O_2^{\cdot-}$ radical-ion and the OH· radical, which is even more reactive and arises on interaction of hydrogen peroxide with $O_2^{\cdot-}$ or bivalent iron salts[23,24].

The oxidative decomposition of the lysosomal membrane has been observed[25] and it has been established that the cause of the decomposition is free-radical reactions initiated by the superoxide radical-ion, formed on oxidation with reduced flavoprotein of liver microsomes. The authors showed that the $O_2^{\cdot-}$ radical-ion does not itself react with the membrane and that the direct participant in the chain oxidation reactions of the membrane lipids is OH· formed in the presence of ADP and Fe^{3+}. The presence of SOD prevents the breakdown of lysosomes.

Ascorbic acid can be used as a reductant for the ADP–Fe^{3+} complex which is more effective than superoxide, which apparently accounts for its role as the promoter of peroxide oxidation in those cases where vitamin C is used in doses known to be many times greater than the physiological dose[26].

Using the oxidation of xanthine by xanthine oxidase as the source of superoxide radical-ions, measurements were made of the rate of oxidation of vitamin E; the rate constant for the interaction of $O_2^{\cdot-}$ with α-tocopherol proved to be fairly large (1.7×10^{-4} mol^{-1} s^{-1}), which enabled the authors to postulate that the α-tocopherol built into the membrane can trap the $O_2^{\cdot-}$ radical-ions and prevent the initiation of the lipid oxidation[27]. The coefficient of the permeability of the two-layer lipid membrane to oxygen radical-anions is close to the coefficients of permeability to other ions[28]. On irradiation, stimulation of peroxide oxidation, and in inflammatory processes, the depolymerisation of the synovial liquid and of purified preparations of hyaluronic acid, occurring with participation of the $O_2^{\cdot-}$ and OH· radical species, was observed. SOD and catalase have a protecting effect[29,30].

Many aspects of the role of superoxide dismutase in the ageing process still remain obscure. Some information can be derived from data concerning the age-induced changes in the activity and properties of SOD. Much work in this field has been done by Reiss and Gershon[31], who were able to observe differences between the properties of the purified enzyme isolated from the livers of young and old rats. The "old" enzyme was 1.5 times less active than the "young" enzyme, although their kinetic properties were identical. The inhomogeneity of the "old" enzyme, which included a thermolabile fraction absent from the specimen of the "young" enzyme, was discovered in the study of thermal inactivation. Thus SOD supplemented the list of "inactive" enzymes (it includes enzymes such as aldolase, glucose-6-phosphate dehydrogenase, isocitrate lyase, etc.[32]).

Few data are available concerning the changes during ageing of the activity of SOD in the tissues of laboratory animals. Sometimes the age differences are quite distinct, but instances are known where they are absent. The activity of cytoplasmatic SOD in the hearts and livers of 34-month old mice was reduced by 50% compared with 3-month old mice. The same pattern was observed in 40-day old and 4-day old nematodes[33]; in the mouse brain tissues the activity of this enzyme decreases by 32–36% (relative to the protein or DNA content) in the range 50–900 days[34]. No changes were found[35] in the activity of SOD in 3-month and 2-year old rats, but an appreciable increase of the activity of catalase and glutathione peroxidase was noted.

The simultaneous decrease of the efficiency of the generation of $O_2^{\cdot-}$ and of the activity of SOD in the livers of old rats has been demonstrated[36], which enabled the authors to suggest the retention in the cells of a balance between free radical generation processes and the protecting reactions tending to inhibit the stochastic oxidation.

Opposed views have been expressed concerning the question whether or not superoxide dismutase can be regarded as an adaptive enzyme. An increase of the activity of this enzyme has been observed under the conditions of hyperoxia in human lung tissues[37] and also under the influence of the dye methylviologen, which is able to increase the intracellular concentration of the $O_2^{\cdot-}$ radical-ion[38]. After prolonged irradiation with moderate doses, no decrease in

the SOD activity in the tissues of Drosophila and rats was observed[39]. When more severe conditions reproducing the peroxidation syndrome were generated experimentally by maintaining rabbits on a diet lacking exogenic antioxidants, an increase in the activity of SOD was observed[40,41]. The latter study established a monotonic decrease of its activity with age; assuming that the SOD activity in the livers of sexually immature rats is 100%, the following data were obtained: 50% activity in 8-month old rats, 18% activity in 23-month old rats, and 15% activity in 30-month old rats.

In the course of the preimago development of Drosophila, the SOD activity increases by a factor of 3.5, but in the imago it hardly changes with age (in relation to the entire body weight)[42]. The activity of the mitochondrial enzyme reaches a maximum in 8-day old Drosophilas and then decreases by 21% in the course of the next 50 days. One cannot rule out the possibility of detecting age-induced changes in the SOD activity in various organs and tissues of the adult insect. In a mutant strain of Drosophila with an appreciably reduced lifespan the activity of cytoplasmic SOD was always lower than in the wild flies[43].

Interesting data were published in a communication[44] dealing with the study of the life cycle of a mutant of *Neurospora crassa* with a short lifespan. In the ageing stage an EPR signal due to the superoxide radical-anion with $g = 2.02$ was detected. In the presence of nordihydroguaiaretic acid as the antioxidant, the intensity of this signal diminished, but at the same time there was an increase in the intensity of the signal of high-spin Fe with $g = 5.45$. According to the author, the antioxidant accelerates the conversion of $O_2^{\cdot-}$ into hydrogen peroxide, inducing the synthesis of catalase with which the $g = 5.45$ signal is associated.

In cultured strains of diploid human cells, which have only a limited lifespan and are usually regarded as a model for cell ageing[45], the observed changes in the SOD activity do not as yet permit an unambiguous interpretation. In cells whose source was embryonic lung tissue the SOD activity did not change in the course of the last 30 passages, the life of the culture[46]. According to other data[47], the greatest increase in the activity of superoxide dismutase occurs in the early stages of the growth of the culture, from the tenth to the twentieth passage. In the above study the specific activity of SOD was measured in the cells of diploid cultures obtained from adult donors; a significant increase of activity with increase of the number of times the population doubled was noted for strains derived from cells of embryos or the newborn but the maximum (and constant) activity of SOD proved to be characteristic of cells in cultures derived from the tissues of adult humans. Simultaneously with the increase of the SOD activity in the cells of the skin of the newborn, the rate of protein metabolism diminished and the fraction of thermolabile enzymes and the rate of reparative synthesis of DNA increased against the background of the accumulation of oxidation products. The authors are inclined to assume that the SOD activity in the old cells increases in response to the activation of the free radical oxidation processes.

Thus at the present time there are numerous facts showing that the enzymes superoxide dismutase, catalase, and glutathione peroxidase, which eliminate $O_2^{\cdot-}$, H_2O_2, and organic peroxides, play the role of the most important cell protecting systems together with natural antioxidants. The factors having a negative or positive influence on the lifespan of mammals have been arranged systematically[48]. Among the negative factors (there are 10 in all), the second place is taken by the accumulation of the age pigment lipofuscin, which is formed in the free radical oxidation of the lipid and protein components of biomembranes according to the majority of gerontologists. In the list of the positive factors (there are 18) the first place is occupied by DNA reparation processes and is followed in order of importance by the antiradical (enzymatic and antioxidant) protecting systems.

The possible role of SOD in determining the longevity of mammals has been investigated[49]. Its specific activity was measured in the cytoplasm of the liver, brain, and heart of two species of rodents, eleven species of primates, and man; the range of the changes in the maximum lifespan was from 3.5 to 95 years. The SOD activity in the tissues of animals of one species was the same but in different species its level differed by a factor greater than two and was a maximum in man. The age-induced changes in activity are not clearly expressed in all the species. The ratio of the SOD activity to the specific metabolic rate in a given tissue or the whole organism (the metabolic rate was determined from the absorption of O_2) increases with the maximum lifespan in all the species. This correlation suggests that in the long-lived species there is a more effective protection against the side products of the O_2 metabolism. Here it is appropriate to mention the results of a search for a relation between the lifespan and the anatomic-physiological parameters of the organism[50]. It has been shown by the method of multiple regression for 85 mammalian species that the lifespan is determined by only two independent parameters: the "cephalisation index", i.e. the ratio of the weight of the brain to the body weight, and the intensity of the metabolism (calculated from the absorption of O_2 at 37 °C). The equation obtained shows that the lifespan is longer the higher the cephalisation index and the lower the metabolic rate (the longer the period of development associated with it). According to Cutler[48], the increase of lifespan in the evolution of mammals occurred as a result of the selection and improvement of the protecting systems and "life support processes" (the opposite of ageing), which led, in particular, to the increase of the period of development in the long-lived species.

It is not possible to consider within the framework of this review another aspect of the biological role of H_2O_2 and $O_2^{\cdot-}$, whose high reactivity is used by the cells for protection against unfavourable factors. It is known, for example, that phagocyte-producing leukoctyes contain 2–4 times more H_2O_2 and generate $O_2^{\cdot-}$ more actively than the non-phagocyte-producing polymorphic-nuclear cells[51]. We shall confine ourselves to listing the literature references on this question[20–22,24,52].

The enzymatic dismutation of $O_2^{\cdot-}$ radical-ions results in the formation of water and oxygen in the triplet state. The spontaneous disproportionation reaction leads to the formation of a hydrogen peroxide molecule, which reacts in its turn with another $O_2^{\cdot-}$ radical-ion, giving rise to singlet oxygen and the OH· radical. The molecule of singlet oxygen reacts with the biosubstrate by virtue of its excess energy and initiates new free radical oxidation centres. The damage to the DNA molecule by singlet O_2 has been investigated[53]; a review has been devoted to the role of singlet oxygen in the pathology of cell membranes[52]. The known producers of singlet oxygen, namely NaOCl and the 3-AT herbicide, the latter inactivating catalase with the consequent accumulation of excess H_2O_2 in the tissues, were added to the Drosophila feed. The average lifespan then decreased by 25–29%. The simultaneous addition of large doses of $O_2^{\cdot-}$ quenching agents, namely β-carotene and 1,4-diazabicyclo[2.2.2]octane, had some protecting effect but it could not fully prevent the negative influence of the sources of $O_2^{\cdot-}$. The quenching agents did not exhibit the properties of geroprotectors, i.e. they did not retard the ageing of intact flies. On this basis, it has been concluded[54] that the role of singlet oxygen in natural ageing is insignificant and becomes appreciable only in those cases where its high concentrations are created artificially.

III. THE CONTRIBUTION OF PEROXIDE OXIDATION PROCESSES TO STOCHASTIC MECHANISMS OF AGEING

There exists an extensive literature on the relation between ageing processes and the peroxide oxidation of lipids, which also have a definite place in the normal vital activity of the cell[6,55-59]. In contrast to mitochondrial oxidation, which serves mainly as a source of energy, the role of peroxide oxidation is associated primarily with the formation of active metabolic products involved in the reactions of lipids—the main substrates of peroxide oxidation. The rate of peroxide oxidation is regulated by many factors, which can both initiate the process (pro-oxidants) and inhibit it. The structure of the protecting mechanisms, which protect the cell against the excess accumulation of toxic oxidation products, is usually believed to comprise two stages[60]. The first involves antioxidants, which react directly with free radicals and the second involves enzymes and non-enzymatic components, which reduce hydroperoxides and which were partly examined in the previous Section. In another study[61] an additional antioxygen protecting stage was introduced. The relevant function is fulfilled mainly by compounds of the type of carotenoids, which absorb the excess of free oxygen and maintain the O_2 pressure in the cell within the physiological limits.

The concentration of endogenic lipid peroxides in tissues is determined by the ratio of their formation and decomposition. In the cell membrane there is a constant content of lipid peroxides on the concentration of which the permeability of the membrane depends. In rat liver, for example, the normal concentration of lipid peroxides does not exceed 10^{-7} mole. The biosynthesis of hormones such as the prostaglandins proceeds via a stage involving the formation of unsaturated acid peroxides[62]; in the synthesis of cholesterol and steroid hormones, squalene oxide is formed as an intermediate[63].

Studies relating the lipid metabolism to the rate of cell division are of great interest. Analysis of extensive factual data led the authors of a book[64] to the conclusion that the decrease of the proliferative activity of cells is accompanied by a decrease of the amount of their natural antioxidants and an increase of the intensity of free radical reactions. The role of cell division inhibitors is usually attributed to lipid peroxides. These observations serve as a basis for the hypothesis of a free radical mechanism of the regulation of cell division which is very labile and sensitive to a wide variety of external influences which alter the concentration of natural antioxidants, the lipid composition, the rate of initiation of free radical reactions, etc. The presence of antioxidants in the lipid phase of the membrane is nowadays regarded as an obligatory condition for the maintenance of its structure, while peroxide oxidation reactions of lipids lead to its breakdown. One cannot rule out the possibility that vitamin E also plays a structural role[65-67].

An important place in the biochemistry of peroxides has been assigned[68] to their interaction with proteins, assuming that the NH_2 and sulphydryl groups (the latter are oxidised to disulphide groups) are the most vulnerable; not only peroxides but also the product of their further reactions, for example, epoxides, form insoluble complexes by combining with proteins. The above communication lists 15 enzymes whose activities are inhibited by lipid peroxides, including the enzymes involved in glycolysis, the tricarboxylic acid cycle, and the respiratory chain. Ribonuclease A, which contains no SH groups, is nevertheless inactivated by the products of the oxidation of unsaturated aliphatic acids, the inactivated enzyme containing high-molecular-weight polymers, whose fluorescence spectrum is identical with that of lipofuscin accumulating in senile heart muscle[69]. Interaction with lipid peroxides causes the degradation of catalase, γ-globulin, haemoglobin, egg and serum albumins, methionine, histidine, and other aminoacids[70].

Structures most vulnerable to oxidative breakdown are the microsomal and mitochondrial membranes in which are located enzymes such as monoamino-oxidase or microsomal cytochrome oxidase, whose normal function is catalysis of the oxidation by oxygen. The destabilisation of membranes as a result of the local interaction of lipids with metabolic or exogenic free radicals converts them into a substrate readily susceptible to oxidation. When suspensions of rat liver mitochondria were treated with a solution of bivalent iron salts, the activation of free-radical oxidation was observed, which resulted in the loss of approximately 65% of mitochondrial protein and of 40% of lipids. Induction of peroxide oxidation leads to the complete inactivation of isocitrate dehydrogenase, 3-hydroxybutyrate dehydrogenase is 80% inactivated, and malate and succinate dehydrogenases are 36 and 23% inactivated. Oxidative phosphorylation breaks down[71]. A secondary consequence of the partial oxidation of the unsaturated aliphatic acids of the membrane is their replacement by saturated lipids, which alters the permeability and functional properties of the membranes; the affinity of the membranes of microsomes for cholesterol diminishes and a deficiency of the latter arises[72,73].

The breakdown of the biological mechanism of the inhibition of free radical oxidation may be the factor initiating the development of pathological conditions such as radiation sickness, atherosclerosis, and tumours[2,4,74]. Ageing, regarded as the experimental syndrome of lipid peroxidation, is characterised, according to Voskresenskii's definition[75], by a "tetrad" of features—damage of the membranes, inactivation of the enzymes, breakdown of cell division, and the accumulation of ballast polymers. Experimentally recorded deviations at the physiological, cellular, and subcellular levels, whose origin is damage of the membrane during the ageing process, have been listed in a review[72]. The most significant of these are the breakdown of the permeability of the cell membranes, the appearance of microsomes and mitochondria with heterogeneous structures and properties, a decrease of the amount of cholesterol in the liver microsomes of old animals, a decrease of the efficiency in relation to endocrine effectors such as insulin and catecholamines, whose physiological activity depends on the stereospecificity of the interaction between the hormone and the receptor, and the accumulation of the transoxidation products in the cells and in the blood.

The search for a criterion of the ageing of human diploid fibroblasts, cultured routinely outside the organism, has led to the conclusion[76] that the "imprint of age" is most appreciably reflected in the properties of the cell membrane. By fractionating a normal cell population which has lived through 20 passages *in vitro*, the authors obtained several subcultures consisting of cells of identical size and traced their transition to the ageing phase. It was found that ageing parameters frequently employed such as cell size, the volume of the nucleus, the intensity of the incorporation of thymidine, the DNA content, etc. are poorly correlated with the age of the culture. As the cell ages, the adsorption properties of its surface change; concanavalin A, bound to erythrocytes, is weakly adsorbed by cells of young cultures; the adsorption intensity increases linearly with the number of passes and is independent of the cell cycle phase and the metabolic age.

Thus the main source of the structural and functional disorders arising in ageing may consist in the breakdown of the steric and cooperative interactions in the cytoplasm, in the matrix, in the membranes of organelles, and on the surfaces of cells. The activity of membrane enzymes, reception,

and permeability depend on these properties, which determine the specific organisation. If the properties undergo irreversible changes or are lost, then, even with a correct programme of synthesis, the functioning of the cell is difficult owing to the impaired efficiency of the endocrine receptors or modulators ensuring the reliability of the transport of metabolites.

The maintenance of the relative constancy of the internal medium within the organism is one of the obligatory conditions for existence. The constancy of the physiological level of free radical oxidation reactions is achieved mainly by antioxidants, among which the main role is played by vitamin E whose distribution and metabolism have been adequately studied mainly in connection with the E vitamin deficiency[64, 65,68,75,77-82]. The need for vitamin E and other antioxidants changes in the course of life. The content of vitamin E in the blood, livers, and skeletal muscles of sexually immature rabbits and rats progressively increases during growth, in the adult animals a relative stabilisation is observed, while in the old animals there is a decrease. The latter may be associated with the intense breakdown of antioxidants in oxidation reactions and their inadequate assimilation. Compared with 30-year old humans, in 60–70-year-old individuals the concentration of vitamin E in the blood plasma is reduced to 60%.[83] According to Leibovitz et al.,[84] during the ageing of stable strains of mice and New Zealand black mice with a hereditary defect of the immune system, a redistribution of ascorbic acid in the liver and spleen tissues is observed. In immunodeficient mice the level of vitamin C in the spleen tissue decreased during the period preceding the development of the autoimmune sickness. A steady decrease of the antioxidant activity of lipids with age has been recorded[85,86]; it is significant that the rate of decrease of this quantity was greater for short-lived mice. The change in the rate of formation of lipid peroxides in rat microsomes during ageing has been described[87]; in the presence of ascorbate the rate of oxidation increased non-uniformly with age: at the age of 600 days it was lower than at the age of 400 days but exceeded that in the young animals. The author explains this observation by the decrease of the efficiency of the monoxygenase microsomal system in old rats.

The data concerning the age-induced changes in the intensity of peroxide oxidation in the subcellular fractions of tissue homogenates are contradictory; a decrease of the accumulation of malonic dialdehyde under the conditions of the induced oxidation of the microsomal, mitochondrial, and postnuclear fractions of the livers of old rats has been reported recently[88,89]; oxidation was retarded with increase of the protein concentration.

Many facts indicate a functional relation between different units of the antioxidant protecting system in cells. The activity of the glutathione peroxidase of erythrocytes, which reduces lipid peroxides, depends on the vitamin E content of the blood[90]. The concentration of reduced glutathione in mouse erythrocytes decreases both on ageing of the red cells themselves and on ageing of the organism. In old mice (31 months) its amount in the liver, kidneys, and heart muscle is lower by 30, 34, and 24% than in the 17–23-month old animals. Involution of the thymus in mice is accompanied by a depletion of its tissues in coenzyme Q (ubiquinone), which is lost to the extent of 80% by the age of two years, and this leads in its turn to the almost complete inactivation of succinate dehydrogenase in this organ[91,92].

It has been concluded[75] that the biological inhibition of free radical reactions in the organisms, which takes place via a series of interacting oxidation-reduction reactions of tocopherols, glutathione, and ascorbate, is systemic in nature.

The SH-containing proteins, whose concentration in the human blood plasma decreases with age almost by 30%, belong to the class of natural antioxidants[93]. There is now no doubt that the status of vitamin E in the organism is associated with the biochemical function of selenium compounds; when there is a deficiency of selenium in food, the metabolism of tocopherols and the catabolism of the proteins in the liver microsomes are accelerated[94].

When there is a deficiency of vitamin E or after prolonged hyperoxia, distinct changes arise in the morphology of cells, particularly non-dividing cells. Lipofuscin is formed in the cytoplasm of the nerve and muscle cells and accumulates in the form of small yellow or brown granules with a characteristic weak fluorescence. Lipofuscin or the "age pigment" is regarded by many gerontologists as the best indicator of age; the linear relation between age and the content of lipofuscin in the human myocardium established by Strehler[95] is generally known. There is an extensive literature on the origin of the lipofuscin grains and their biochemical properties and biological role[96-98], which makes it unnecessary to describe the problem in detail. Numerous data on the structural and biochemical properties of lipofuscin have shown that free radical oxidation reactions of membrane lipids and proteins may be the source of its formation. It is known that on ageing and under unfavourable conditions (stress and poisoning), the membranes are depleted in natural antioxidants. On oxidation of the membranes of mitochondria, microsomes, and lysosomes under model conditions, phosphatidylethanolamine is "cross-linked" by malonic dialdehyde with formation of a fluorescent product; in animals fed a diet lacking vitamin E, lipofuscin accumulates in various organs faster by a factor of 1.5–6.[97] An increase in the number of lipofuscin grains towards the end of the life cycle of cultured normal cells has never been observed. Human diploid cells are in stage III of growth after 40–42 passages and contain approximately 80% of non-dividing cells; secondary lysosomes of the type of residual bodies accumulate in such cells; by analysing X-ray scattering, it has been found that the residual bodies contain large amounts of iron[99].

The way in which the accumulation of lipofuscin grains affects cell functions remains a controversial question. Large accumulations of lipofuscin can themselves hinder the functioning of the cell, particularly of neurones, in which the content of endogenic lysophosphoglycerides, exhibiting a detergent effect on the membranes, increases with age and also under the conditions of an incomplete diet or an irrational administration of drugs. Electron microscope studies on the tissues of various sections of the brains of patients suffering from neuronal ceroid lypofuscinosis who died at the age of 7.5–22 established a characteristic accumulation of large masses of lipofuscin in the nerve cells at a young age. The characteristics of premature ageing (loss of vision, extensive pigmentation of tissues, muscular atrophy) also accompany this hereditary disease[100]. Measurements have been made[101] of the average percentages of the volume of the large human neurone occupied by the nucleus, the cytoplasm, and lipofuscin at different ages. At the age of 40, these percentages are in the proportions 1:2:2, i.e. cytoplasm has already been half-absorbed by lipofuscin; at the age of 60 and 80 the volume of the nucleus does not change but the volume of the functioning cytoplasm decreases by factors of 1.3 and 2; in humans who have reached the age of 100 the volume of the nucleus diminishes by 60% (compared with 40-year old individuals) and lipofuscin occupies more than 80% of the overall cytoplasmic volume. The accumulation during ageing of lipofuscin and amyloid and the increase of the number of senile end-plates has been demonstrated by electron microscopy in 17 sections of human brain[102]. The authors of this study

believe that the nature of the age-induced changes and their consequences for the organism depend on the type of tissues and attribute the role of the main regulator of the rate of ageing—the "biological clock"— to the cells of the central nervous system. The non-uniform accumulation of lipofuscin in different sections of rat and mouse brains is indicated by the data of Reichel et al.[103] In the hippocampus of old rats and mice the accumulation of the pigment begins much sooner (from the age of 9–11 months) and is much faster than in the Purkinje cells and in the brain cortex. The pigment occupies less than 0.6% of the surface of the section in the layer of granular cells of the cerebellum in rats and mice of all age groups.

The ageing of insects is also accompanied by the accumulation of the ageing pigment. The low content of tocopherols and the absence of glutathione peroxidase are the cause of the relatively high rate of peroxide oxidation in the cells of the honey bee. The evolution of pentane, formed as a result of the cleavage of unsaturated aliphatic acid hydroperoxides and the age dependent increase of the concentration of lipofuscin in the cells of the thoracic segment in worker bees have been recorded[104]. Measurements have been made of the content of fluorescent substances in the tissues of house flies aged 1,4,11, and 15 days and an increase of their concentration with age was observed, the greatest amount being extracted from neurones. Analysis of the absorption and emission spectra of their complexes with europium made it possible to identify the fluorescent pigments as conjugated Schiff bases; their localisation in the lipofuscin grains was discovered by means of fluorescence microscopy[105]. New data, confirming the role of lipofuscin as the cellular marker of physiological age, have been obtained in studies where the rate of ageing was varied experimentally[106,107]. Flies belonging to a highly inbred strain were separated into two groups in one of which flying activity was restricted, which increased by a factor greater than two the average and maximum lifespans. The relative contents of lipofuscin in the giant neurones in the brains of flies of different ages were determined by electron microscopy and spectrofluorimetry. The rate of accumulation of the pigment in short-lived flies with a high flying activity was four times greater and its maximum content (the same for both groups) was attained sooner.

However, one cannot ignore studies whose authors adhere to the directly opposite point of view, regarding the accumulation of lipofuscin as adaptive and assuming that the age pigment is a normal structural component of the cell. There is a view[108] that the inclusions of lipofuscin are identical with the carotenoid-containing cytosomes in the neurones of molluscs, which function as an intracellular depot of oxygen in certain lower animals which exist permanently under the conditions of hypoxia. This view is shared by the authors of a book[109] who regard the accumulation of lipofuscin as an adaptive process partly balancing the hypoxic state of the cell on ageing. It is known that the oxygen tension in the tissues decreases in elderly and old individuals which is apparently associated with the age-dependent decrease of the level of energy metabolism. At the same time the activation of free radical oxidation reactions inhibits tissue respiration by damaging the mitochondria and the biological oxidation enzymes. The decrease of the consumption of oxygen in the late stages of ontogenesis by no means indicates a decrease of the need for O_2 and may represent the result of the breakdown of its utilisation. According to Voskresenskii and Levitskii[68], there exists an antagonistic interaction between the enzymatic oxidation and the peroxide oxidation involving a free radical mechanism. Under the optimum conditions of the functioning of the oxidative enzyme systems of the cell, the possibility of the formation of lipid peroxides diminishes and, conversely, on ageing the direct oxidation of lipids is enhanced as a result of the decrease of the utilisation of O_2 in energetic processes.

The possible protecting role of vitamin E in acute experimental hypoxia is indicated by a study[110] in which an increase of the concentration of tocopherols in the adrenal tissue was observed, which leads to release of the reserves of adrenalin, a decrease of the intensity of the stress response, and a decrease of the consumption of O_2 by the tissues.

Among the pathological states characteristic of elderly individuals, the most common is undoubtedly atherosclerosis, the development of which may be associated with a change in the antioxidant status of the organism. The results of studies employing experimental models of atherosclerosis are of interest. When animals are maintained for a long time on a fat-rich diet lacking antioxidants, a decrease of the parameters of the functional activity of the antioxidant system and the development of the biochemical and morphological signs of atherosclerosis are observed. As the severity of the disease increases there is a rise in the concentration of total lipids, lipid peroxides, and malonic dialdehyde in the blood, and the wall of the aorta is degraded. The employment of antioxidants makes it possible to regulate their supply to the organism and to retard the development of atherosclerosis. Tests on ionol, hexahydro-4-ubiquinol, quercetin, sodium selenite, and vitamins P, E, and C showed that the action of these compounds is associated with the inhibition of peroxide oxidation and protection against the oxidative degradation of the elastic fibres of the arteries[111–113].

IV. CHARACTERISTICS OF THE ACTION OF ANTIOXIDANT GEROPROTECTANTS

Several careful reviews[114–117] and also one of the chapters in Comfort's monograph[118] were devoted to the mechanisms of the action of chemical geroprotectants of different classes, which enables us to avoid a detailed description except for antioxidants which we believe to be the most promising geroprotectants. The compilation published in 1975[119] of the geroprotectants known at the time must be supplemented by several new compounds. Lactic and gluconic acids, which are inhibitors of the OH' radical, as well as sodium and magnesium salts of thiazolidinecarboxylic acid (antioxidants) retard the ageing of Drosophila and laboratory mice[120,121]. Succinic acid, which acts on the energy balance of the cell, proved to be active[122]. Diphenine (5,5-diphenylhydantoin-sodium), phenformin (1-phenylethylbiguanide hydrochloride), and the polypeptide extract from epiphysis are able to reduce the threshhold of the sensitivity of the hypothalamo-hypophyseal complex to regulating influences, to decrease the frequency of spontaneous tumours, and to increase the lifespan of the experimental animals[123,124]. Inhibitors of the biosynthesis of protein—olivomycin, actinomycin D, and morphocycline—constitute a new group of chemical geroprotectants with a specific mechanism of their action[125,126].

In most cases whether or not a substance is a geroprotectant is determined by the set of properties characterising its biological activity and not its chemical structure or some one property. In this respect antioxidants have been studied in greatest detail. The free-radical hypothesis involves the postulate that antioxidants retard the ageing process because they prevent the accumulation of lesions at the molecular level[127]. It is implied that the accumulation of lesions embraces all cell structures without exception, ranging from the genetic material to membrane lipids. Arguments in

support of this hypothesis are examined in detail in previous Sections of this review. It would appear that agreement with this thesis logically requires the recognition of the universality of the action of antioxidants, which, by reducing the background level of the free radical reactions in the cell, decrease the number of stochastic lesions in biostructures. However, this simple treatment conflicts with facts: not all the antioxidants tested experimentally proved to be geroprotectants and the latter are in their turn active only in relation to specific objects. Thus according to Harman[128], 2-mercaptoethylamine, which has been tested on four strains of mice, increased by 29% the average lifespan of LAF_1 mice, but was almost inactive in relation to the AKR, Swiss, and C3H strains. The antioxidant 2,6-di-t-butyl-4-methylphenol (ionol or BHT) showed no influence on the rate of ageing of C57BL mice, but greatly increased the average lifespan of the BALB/C strain of mice[129,130]; the authors of both studies emphasise that the animals were maintained under optimum conditions. The geroprotectant 2-ethyl-3-hydroxy-6-methylpyridine hydrochloride retarded the rate of ageing of SHK at C3HA mice by a factor of 1.5–1.8. SHK mice belong to a non-inbred population characterised by a high incidence (50–90%) of spontaneous cancer of the mammary gland; the administration of this compound to mice of the AKR strain affected by spontaneous leukaemia was unsuccessful[131]. No attention has been devoted to the specific causes of the non-reproducibility of the results in the gerontological literature, it being merely stated that the given compound is active or inactive. This reflects the predominantly empirical approach to the problem. As a rule, in communications dealing with the retardation of ageing by chemical agents no well-founded methods for the selection of the dose of the preparation used in chronic experiments on survival are described; at best a preliminary study is made of toxic properties. As regards the selection of the dose of synthetic antioxidants, account must be taken primarily of their interaction with natural antioxidants, which can lead to the inhibition of stochastic free radical processes and to an increase of the reliability of the protecting systems of the organism or, conversely, it can induce the activation of peroxide oxidation and rapid depletion of the tissues in antioxidants[64]. It has been shown[86] that the range of doses of 2-ethyl-3-hydroxy-6-methylpyridine suitable for chronic experiments on laboratory mice is from 75 to 300 mg per kilogram of live weight. The use of this synthetic antioxidant in such amounts during the life of the animal makes it possible to maintain the antioxidant activity of the lipids (it is proportional to the content of the antioxidants in the tissues) above the normal level and to compensate for the age-induced decrease in the amount of natural antioxidants. The effectiveness of 2-ethyl-3-hydroxy-6-methylpyridine as regards the retardation of the ageing of Drosophila depends not only on its concentration but also on the stage of development, the age of the imago, sex, and the particular strain of the insects. In particular, it has been found that males are more sensitive to the geroprotectant than females; in experiments with females an increase of only the average lifespan was observed, while in the experimental groups of males the maximum lifespan also increased. The effect of this geroprotectant on flies of the $R(1)2yv/y^+Y$ strain with a rearranged karyotype also differed significantly from its effect on the D-32 wild strain. The average and maximum lifespan of males of the $R(1)2yv/y^+Y$ strain increased by 20% at a concentration of the preparation of 0.1 wt.%, whereas for the males of the D-32 strain this concentration of the preparation had the opposite effect: the rate of ageing increased and the lifespan diminished by 30%.[132] The decrease of the lifespan of guinea pigs observed when vitamin C was used in doses exceeding severalfold the physiological dose can be accounted for by the activation of the free-radical oxidation of lipids[133].

It is known that constant addition of certain chemicals to the diet can influence the consumption of food. This important aspect of the action of geroprotectants has been very little investigated. The influence of the antioxidant ionol on the appetite and live weight of adult rats is ambiguous and depends both on the concentration of the additive and on the composition of the food. The weight of the animals increased with increase of the concentration of ionol in the food up to 0.5% and remained constant when its content was raised to 0.9%, after which its slow decrease was observed. If the food contained at least 13% of protein, then the permissible dose of the antioxidant was 300 mg per kilogram of live weight per day; an increase of the protein content by a factor of two correspondingly made it possible to increase the safe dose of ionol[134]. Thus the application of the same chemical agent in different doses and for a variable diet may lead to opposite results even in the same species of animals.

Biological systems of different degrees of complexity, ranging from cell cultures to mammals, have been used as models in experimental gerontology. The more complex the system, the more stringent the requirements imposed on the potential geroprotectants, which must not interfere grossly in the metabolism and must not affect the course of the normal physiological processes. Hydrocortisone, which is a cell division stimulator, maintains active proliferation in old cell cultures, i.e. is a geroprotectant of the ageing of cultured cells in the given instance[135]. The properties of stabilisers of biomembranes make it possible to employ corticosteroids to retard the ageing of insects[136], whose tissues consist mainly of populations of non-dividing cells, in which the specific activity of lysosomal enzymes increases by a factor of 4–23 with increase of age[137]. However, the results of Akif'ev et al.[138] justify the rejection of corticosteroids as geroprotectants for mammals. The authors showed that constant stimulation of liver cells by hydrocortisone has a negative effect and leads to the breakdown of genetic regulation. Only instances of substitutional therapy are exceptions when hormones are used: the lifespan of dwarf mice increases under the influence of the growth hormone[139]; the ageing of autoimmune mice is retarded by prednisolone and hydrocortisone[140].

Vitamin E has been used successfully to retard the ageing of cultured diploid human fibroblasts and also increased the lifespan of Drosophila and nematodes[141,142]; however, numerous attempts to retard the ageing of laboratory mice demonstrated either a very low effectiveness of tocopherol or the complete absence of its influence on the rate of ageing. Nevertheless, vitamin E has a set of properties similar to those of synthetic antioxidants, namely ionol and santoquin (there is also a structural similarity with the latter), which may determine the geroprotecting activity or may accompany it[119]. It is still unknown why synthetic antioxidants have a more effective influence on the ageing of mammals than natural antioxidants. Presumably, when the physiological level of tocopherol or ascorbate is exceeded, the equilibrium involving the vitamins in the organism is disturbed[143]. Furthermore, it has been suggested that the function of vitamin E does not reduce exclusively to the antiradical activity[144].

The results of a study which established a decrease of the age-induced mortality of mice of the Swiss strain born to mothers which received the following antioxidants in their diet are very interesting and difficult to explain[145]: 0.2% of santoquin; 0.5% of 2-mercaptoethylamine; 0.2% of vitamin

E; 1% of sodium hypophosphite; a mixture of 0.2% of santoquin and 1.0% of sodium hypophosphite. The offspring were maintained on a constant diet without extraneous additives. At the age of 26 months, the fraction of surviving males whose mothers consumed 2-mercaptoethylamine and hypophosphite exceeded the control by 26 and 8%; santoquin and 2-mercaptoethylamine decreased the mortality of males by 13 and 23%. Vitamin E was inactive. The interpretation of this result is difficult, because the communication gives no information concerning any physiological or biochemical peculiarities of the offspring. It may be that the phenomenon of enzymatic imprinting, in which the administration of xenobiotics or other gene inductors to newborn animals induces in the latter a stable (over a period of 8 months and more) enhancement of adaptive synthesis, may be reflected in this instance in an unusual manner. Thus there is a phenotype correction of the symptoms of certain hereditary diseases. In 1974 attention was drawn to the fact that effective geroprotectants are as a rule inductors of microsomal oxidases[116]. The induction of the enzyme detoxification system of microsomes constitutes the molecular mechanism of the mobilisation of the protecting forces of the organism against the harmful influence of the chemical components of the environment and is manifested particularly clearly in chemical carcinogenesis, in which antioxidant inductors decrease the number of tumours or prevent their formation[147,148].

The results of several studies of the influence of antioxidants on the ageing of Drosophila showed that the most important events determining the lifespan occur in early ontogenesis. Using 2-ethyl-3-hydroxy-6-methylpyridine hydrochloride as the geroprotectant, it has been established[132] that the retardation of the ageing of Drosophila observed in this instance is not a consequence of the increase in the period of development. In addition it was found that the greatest effect is attained on treatment in the preimago stage or of the young imago, i.e. during the period when the phenotype characteristics of ageing are still absent. The older the imago, the less effective the geroprotectant: treatment begun at the age of 20 days and later is virtually without results. It has been suggested that lesions leading subsequently to ageing accumulate in the tissues of the larva from which the organs of the adult flies are formed (nervous tissue, imaginal discs). By regulating the incidence of these lesions in the larval period by means of a geroprotectant or a mutagen, it is possible to influence the rate of ageing of the adult individuals. According to the authors, the ageing of Drosophila consists of two stages: the stage of the accumulation of lesions and the stage of their realisation; the onset of the second stage requires not only a definite threshhold level of lesions but also a period of time after the elapse of which phenotype signs of ageing appear. Once this time has elapsed, a further increase in the number of lesions no longer has an appreciable influence on the rate of ageing. Since these stages are separated in time, it has been suggested that only molecules capable of prolonged storage of information about the harmful influence can serve as the "ageing substrate", i.e. the most likely candidate for the role of the ageing substrate is the molecule of chromosomal DNA. Information about the injury to other chemical components of the cell cannot be directly transmitted from the larvae to the adult individuals, since all the remaining components are repeatedly renewed in the course of metamorphosis.

The existence of a critical age limit beyond which treatment ceases to be effective or has the opposite result has been noted in a number of studies. In the investigation of the influence of α-tocopherylquinone on the ageing of a nematode, it has been observed[49] that the antioxidant is active only in relation to young animals; at the age of 20 days it was not possible to retard ageing. It may be that in the presence of an antioxidant the number of errors in the synthesis of membranes, which occurs most actively during the first ten days of the life of the nematode, is reduced[150]. Similar results have been published recently. The addition of lactic and gluconic acids to the feed of Drosophila larvae increased the average lifespan by 22%, while treatment of the young imagos was less effective by a factor of 2. The authors suggest that the retardation of ageing is achieved by inhibiting the reactions of the $OH^{\cdot}$ radical[120].

The causes of the existence of threshhold phenomena in ageing apparently involve the ratio of the rates of accumulation and repair of lesions. A mathematical treatment of a generalised scheme for the ageing process, taking into account critical phenomena, has been described[127].

The protecting effect of antioxidants in the age-induced accumulation of lesions of the secondary DNA structure is indicated by the results of Khudolii et al.[151] They established that lesions, recognised by endonuclease S_1 as apparently single-stranded breaks, accumulate during ageing in the mouse liver nuclear DNA. The retardation of the ageing of mice receiving an antioxidant is accompanied by a decrease of the number of similar defects.

The action of the majority of geroprotectants lacks a distinct specificity and is varied. The non-specific activity of antioxidants can be partly accounted for by their involvement in the reactions of the general adaptation syndrome, whose development occurs against the background of decreasing antioxidant activity of the tissues. In the state of stress, peroxide oxidation processes are activated[64]. Antioxidants modify the rate of supply of corticosteroids to the blood and lower the overall stress response. The prophylactic administration of antioxidants promotes adaptation under the conditions of experimental stress[152].

Studies in recent years established that antioxidants belong to the category of immunoactive compounds. The maximum humoral response of mice whose diet was permanently treated with antioxidants (vitamin E, ionol, santoquin, 2-mercaptoethylamine, etc.) corresponded to the age of 50–65 weeks and exceeded the control values approximately by a factor of 2. The cell immune response was enhanced to a lesser extent. It is believed[153] that life is prolonged because the age-induced immune deficiency is made good with the aid of antioxidants. A more careful examination of the results of this study makes it impossible to agree with this conclusion, since stimulation is replaced by immunodepression, which actually occurs in the experimental groups before the age-induced decrease of the immune response. This group of long-lived mice, whose life is prolonged as a result of a decrease of their mortality rate, reach the normal lifespan when the immune status is reduced. The retardation by antioxidants of the development of amyloidosis, a typical senile disease of the autoimmune type, can be logically explained by immunodepression[154]. The decrease of the rate of migration and proliferation of blood-forming stem cells in mice has been noted in chronic treatment with a geroprotectant belonging to the class of 3-hydroxypyridines. An increase by a factor of 1.5 of the latent period in the formation of spontaneous tumours of the mammary gland has been observed against the background of the immunodepression induced by the preparation[155]. The same preparation normalised the level of adrenalin in the adrenal gland of tumour carriers, which is believed[156] to be extremely significant for the outcome of the relations between the tumour and the organism.

The experimental data presented throw some light on the question why the action of antioxidants lacks a distinct specificity, depends on the complexity of the object, and is

frequently manifested by effects which are not clearly interrelated at first sight. Indeed, it is difficult to explain by a single cause data obtained for the same antioxidant and showing a decrease of the fraction of "defective protein" in liver cells[157] and a change of the rate of migration of blood-forming stem cells[155]. Although the hypothesis that geroprotectants act in both cases on the "membrane component" of ageing may be regarded as likely, a more exact answer requires a pharmacokinetic study of the preparation. Interference with the free radical oxidation reactions, which we believe constitute a universal background for metabolic processes, can be realised in accordance with the "weak link" principle and can lead to changes in a wide variety of biochemical mechanisms responsible for age-induced deviations. The structure and functional inter-relations between cells, tissues, and organs ensure the similarity of the final results of the age-induced changes due to different causes.

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