



Antioxidant Biomedicine: From Chemistry of Free-Radicals to Reliability of Biological Systems



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Abstract

Natural and synthetic antioxidants are widely used in modern medicine. *In vitro*, antioxidants effectively scavenge active free radicals thereby inhibiting free-radical chain reactions of oxidation of fatty acids, edible fats, etc. *In vivo*, however, neither natural antioxidants, like vitamin E and flavonoids, nor synthetic antioxidants, like butylated hydroxytoluene (BHT), are able to serve as efficient scavengers of oxygen free radicals. The reason is that the rate constants of the antioxidants in the reactions with oxygen free radicals and the concentrations of the antioxidants in cells and tissues are negligible as compared with the natural antioxidant enzymes to compete with the antioxidant enzymes for reactive oxygen species (ROS). Actually, the so-called antioxidants provide prophylactic maintenance against ROS by preventing the formation of oxygen radicals and free radical oxidation in cells and tissues. The protection mechanisms are different for antioxidants of different types. For example, synthetic BHT prevents generation of the oxygen anion-radical ("superoxide", $O_2^{\bullet-}$) as the by-product of the mitochondrial electron transport, while natural flavonoids exert a preventive antioxidant action by inducing the expression of the antioxidant enzymes, superoxide dismutase and catalase. At this, the endocrine system, i.e. - prophylactic maintenance via the organism's neuro-hormonal system plays the significant role in the antioxidant therapy. Besides, there are more and more data indicating that the therapeutic effects of many pharmaceutical drugs, including antioxidants, are due to their beneficial action not only on cells and tissues of host organism but also on gastric and intestinal the organism's microbiota. Thus, true mechanisms of the antioxidant prophylaxis are to be studied on the ways of systems biology instead of free-radical chemistry. It might be said now that antioxidant biomedicine has passed a long way from "false knowledge to true ignorance".

Keywords: Antioxidants; Free radicals; Oxygen; Prophylaxis; Preventive maintenance; Biosystems; Reliability; robustness.

Introduction

The vigorous research of free radical processes and of the antioxidants in biology and medicine was stimulated by awarding the Nobel Prize in Chemistry in 1956 to Cyril Hinshelwood (University of Oxford, United Kingdom) and Nikolay Semenov (Institute of Chemical Physics of USSR Academy of Sciences, Moscow) for their studies of free radical mechanisms of chain radical reactions. By then, it was already known that free radicals, specifically the hydroxyl radical (OH^{\bullet}), arise in water under the action of ionizing radiation and that toxic products of the free radical oxidation of lipids, the peroxides, appear in animal tissues on exposure to ionizing radiation (see refs. in [1,2]). In 1954, the idea that free radicals of oxygen are responsible for the oxygen toxicity was stated by Rebecca Gershman and Daniel Gilbert (quoted from [2]). In 1954, the signals of electron spin resonance (ESR) of the free radicals were first detected in animal tissues and in yeast cells [3]. At the same time, the free radical hypothesis of aging was put forward and inhibitors of free radical chain oxidation, antioxidants, were proposed for prolongation of life of humans and animals [4-6]. Furthermore, it was proved that antioxidant 2,6-ditertbutyl-4-methylphenol (butylated hydroxytoluene, BHT, in English literature and dibunol or ionol in Russian literature) retards

the development of leucosis in mice [7]. Since then, the beneficial effects of antioxidant therapy were experimentally proved over and over again (see in refs. [8-12]).

Currently, however, the mechanisms of action of antioxidants in living systems (*In vivo*) do not seem as unambiguous as half a century ago. This review presents data according to which diverse physiological, in particular, therapeutic effects of synthetic and natural antioxidants are due not so much to direct radical trapping as to the ability of the antioxidants to provide preventive protection from free radicals and their chemically reactive products via the system of neuro-hormonal regulation along with the beneficial antioxidant effects on the organism's microbiota cells.

Antioxidants: Setting to Free-Radical Chemistry

By definition, antioxidants are the substances capable to terminate branching chain reactions of oxidation. The valence-saturated antioxidant molecule, containing an active hydrogen atom, reacts with an active free radical that runs the oxidative chain to give a relatively nonreactive free radical of the antioxidant molecule. The relatively nonreactive radical of the antioxidant cannot participate in chain propagation reactions and is destroyed

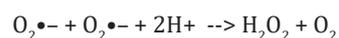
upon collision with another radical (or with the vessel wall). In modern chemistry, these are mainly derivatives of secondary aromatic amines and phenols and organic phosphites and sulfides. In biomedical literature, antioxidants represent a broad class of phenolic compounds, synthetic and natural (see refs. in [8-12]). The most common antioxidants are phenolic derivatives in which the OH group is shielded, i.e., the so-called sterically hindered phenols. For example, in the mid-20th century, the synthetic antioxidant BHT found wide use as stabilizer for industrial oils and edible fats. The synthetic antioxidants based on alkyl-substituted hydroxypyridines are water-soluble, unlike BHT. With appropriate dosage, antioxidants do exert various favorable therapeutic effects. Therefore they have been long successfully used in medical practice. For example, BHT is used to treat burns, gunshot wounds, trophic ulcers, and bladder cancer. Mexidol (2-ethyl-3-hydroxy-6-methylpyridine succinate) is used to treat brain circulation disorders. Emoxipine (2-ethyl-3-hydroxy-6-methylpyridine hydrochloride) is used in ophthalmology [8-12]. The same synthetic antioxidants proved to be effective geroprotectors, i.e., compounds that extend the life span of laboratory animals when added to food or drinking water on a regular basis. For example, by adding BHT to food, it was possible in some cases to extend the lives of fruit flies and of mice of some lines by 25-30%. The addition of the water-soluble antioxidant, 2-ethyl-3-hydroxy-6-methylpyridine hydrochloride, to drinking water extends the average life spans of flies and mice by 24 and 38%, respectively (see, for example, reviews [8,12]).

The class of natural antioxidants includes not less than twenty compounds starting from "classical" vitamin E (α -tocopherol) and vitamin C (ascorbic acid). Most of the natural antioxidants are substituted phenols or polyphenols, which, owing to their hydroxyl groups, are capable of inhibiting free-radical chain reactions of oxidation in model systems (*In vitro*), for example, oxidation of linolenic acid. Most of them are of plant origin. These are flavonoids, in particular, quercetin, flavones, and resveratrol which is especially abundant in grapes and red wine, simple catechols which are present in large amounts in green tea, and catechol oligomers present in high concentrations in grapes and cocoa beans, as well as carotenoids, tannins, anthocyanins, coumarins, and hydroxycinnamic acid derivatives (see refs. in [8-12]). Currently the highest geroprotector effect has been found for resveratrol: the extension of the maximum life span by 59% in experiments on fishes and extension of the average life span by 30% in experiments on mice (on a fatty diet) [13].

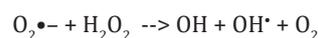
It is worthy to underline, however, that the classical natural antioxidant, vitamin E, has turned out to be inefficient in analogous biomedical testing [14]. Furthermore, the results of analysis of the rate constants and actual concentrations of the antioxidants, both natural and synthetic ones, in cells and tissues also raise doubts whether the antioxidants perform *In vivo* their antioxidant functions in as simple way as *In vitro*, i.e., as the free-radical inhibitors. Indeed, chemically reactive (toxic) ROS are produced in cells and tissues of aerobic organisms. It is also known that the oxygen radical anion ("superoxide radical", $O_2^{\bullet-}$) is the most

important source of ROS. Among the generators of $O_2^{\bullet-}$ in cells and tissues, there are NADPH-cytochrome-C reductase and cytochrome P-450 of endoplasmic reticulum, xanthine oxidase, catecholamine and other biogenic amines, mono- and diamine oxidases, aldehyde oxidases, oxidases of D-aminoacids, D-galactosidase, lipoxigenase, nitric oxide synthase, leukoflavines, hemoglobin and myoglobin, ascorbate, NADPH-oxidase of phagocytes and other so-called NOX enzymes (NADPH oxidases) [15]. However, the main bulk of $O_2^{\bullet-}$ is formed as a by-product of the electron transport in mitochondria, the organelles that use up to 99% of all oxygen consumed by cells for ATP synthesis. Normal functioning of the mitochondrial electron-transport lies in the transport of electrons from the oxidation substrates, NADH and succinate, to cytochrome oxidase and, then, to oxygen with reduction of oxygen molecules to water and synthesis of ATP [15]. However, the reliability characteristics of mitochondrial nanoreactors are not perfect. As a result, normal elementary acts of electron transfers alternate with accidental malfunctions which result in formation of $O_2^{\bullet-}$, similarly to "recurrent failures" in engineering [9].

Besides, hydrogen peroxide (H_2O_2) is formed as the product of the reaction of dismutation of the $O_2^{\bullet-}$ radicals:



Next, $O_2^{\bullet-}$ reacts with H_2O_2 with formation of the OH \cdot radical:



From chemistry, it is known that OH \cdot radical is a strong oxidant which initiates free-radical reactions of oxidation of lipids and proteins, oxidative degradation of DNA with formation of 8-oxo-7,8-dihydroguanine and 5,6-dihydroxy-5,6-dihydrothymine, and so on [15]. Besides, there are reasons to believe that $O_2^{\bullet-}$ can react with the nitric oxide (NO \cdot) radical with formation of peroxynitrite (ONOO $^-$), which is considered as a strong oxidant, and nitrating species that mediates biological effects of the superoxide and the nitric oxide [16].

From biochemistry, however, it is also known that there are specific enzymes, superoxide dismutases (SOD), which catalyze the dismutation of $O_2^{\bullet-}$ to H_2O_2 and oxygen [15,17]. These enzymes, mitochondrial (Mn-SOD) and cytosol (Cu,Zn-SOD) along with periplasmatic bacterial (Fe-SOD), react with the $O_2^{\bullet-}$ radical anion with the rate constant $k \approx 2 \cdot 10^9 \text{ L mol}^{-1} \text{ s}^{-1}$ (see refs. in [11,12]). Meanwhile, the rate constants for the reactions of ascorbic acid and 5,7,8-trimethyltolcol (water-soluble derivative of vitamin E) with the $O_2^{\bullet-}$ radical do not exceed $10^5 \text{ L mol}^{-1} \text{ s}^{-1}$, while those for hydroxypyridine antioxidants are no more than $10^2 \text{ L mol}^{-1} \text{ s}^{-1}$ [11,12].

Not long ago, the derivatives of vitamin E and ubiquinone were synthesized, Mito Vit-E and Mito Q, which contain, in addition to the hydrophobic antioxidant group, also a lipophilic cationic group [18]. Besides, the mitochondria-targeted plastoquinone compounds with rhodamine and triphenylphosphonium as cations were synthesized [19]. Owing to the electric charge of the cation, these molecules can use the mitochondrial transmembrane

potential to pass through the mitochondrial membranes and, therefore, they get into mitochondria, i.e., just those organelles that generate the highest amount of reactive oxygen species. The mitochondria-targeted antioxidants Mito Vit-E and Mito Q exert the beneficial effects for treating sepsis [18]. Similar antioxidants based on plastoquinone were applied profitably against the age pathologies in animals, in particular, for treating a number of cardiovascular and ocular diseases and even as geroprotectors in experiments on mice or other animal species [19]. For Mito Q (ubiquinone-based antioxidant), the rate constant for the reaction with $O_2^{\cdot-}$ in water can be as high as $10^8 \text{ L mol}^{-1} \text{ s}^{-1}$ according to pulse radiolysis data. However, in this case, too, it remains an order of magnitude lower than for SOD. In principle, mitochondria-targeted antioxidants can be accumulated in mitochondria. However, they can hardly be accumulated up to a concentration comparable with the amount of SOD (about $10^{-5} \text{ mol L}^{-1}$) without considerable disturbance of the operation of mitochondrial nanoreactors.

Of course, inasmuch as reliability ("robustness") of the SOD protection is limited, there exists a finite probability that the $O_2^{\cdot-}$ radical still penetrates the SOD-barrier and then reacts with H_2O_2 to give the hydroxyl radical (OH^{\cdot}) which is known as the strong oxidant in chemistry. However, there are other specialized antioxidant enzymes, catalase and glutathione peroxidase, which catalyze hydrogen peroxide decomposition to water and oxygen [15]. The rate constant for the reaction of vitamin E with OH^{\cdot} radical can be as high as $8 \cdot 10^{10} \text{ L mol}^{-1} \text{ s}^{-1}$. Meanwhile, the OH^{\cdot} radical is known to react with any organic molecules as a strong oxidant with the rate constants close to the diffusion limit, $>10^{10} - 10^{11} \text{ L mol}^{-1} \text{ s}^{-1}$ (see refs. in [11,12]). Therefore, *In vivo*, none of the antioxidants can compete for the hydroxyl radical with other organic molecules which are obviously always present around this radical in considerably greater numbers than the molecules of any antioxidants. It is known from chemistry, that peroxy radicals RO_2^{\cdot} can appear in reactions of OH^{\cdot} radicals with lipids, for example, with mitochondrial cardiolipin. The rate constants for the reactions of synthetic and natural antioxidants with RO_2^{\cdot} in model reactions may range up to about $10^6 \text{ L mol}^{-1} \text{ s}^{-1}$. *In vivo*, however, RO_2^{\cdot} and other products of peroxidation arise mainly as secondary products in the reactions that accompany cell death on apoptosis and auto phagocytosis during utilization of the cellular waste by lysosomes and peroxysomes. One can suggest that antioxidants are unlikely to be highly necessary for scavenging the active radicals in catabolism. The reports on *In vivo* yields of the DNA oxidation products for both mitochondrial and nuclear DNA are also overestimated due to various artifacts [20]. Thus, manifold beneficial effects of antioxidants *In vivo* can hardly be interpreted on the basis of simple chemical analogy with the action of the same antioxidants as radical scavengers *In vitro*.

Antioxidants: Setting to Bioreliability

From the general theory of reliability, it is well known that the most efficient way to increase the reliability (robustness) of any complex system is prophylaxis, i.e. - the well-timed prevention of malfunctions (failures) of functional elements from which

the system is constructed (see, for example, [21]). Following this reliability-theory guide-line, a special series of experiments was performed by our group, in which it has been shown that antioxidants do provide the preventive protection from free radicals *In vivo*. At this, the particular protection mechanisms are different for antioxidants of different types.

For BHT, this antioxidant prevents generation of the $O_2^{\cdot-}$ radicals as by-products of the mitochondrial electron transport. In the studies of low-temperature ESR (electron spin resonance) signals of rat tissues, it was found that BHT increases the myocardium oxygenation [22]. Meanwhile, hypoxia results in structural damage of mitochondrial membranes resulting in considerable decrease in the reliability of electron transport, so that the mitochondria become generators of intense fluxes of the $O_2^{\cdot-}$ radicals [23]. It stands to reason that BHT prevents the development of hypoxia by increasing the degree of myocardium oxygenation, thereby preventing the transformation of the electron-transport nanoreactors of the myocardial mitochondria into the intensive generators of $O_2^{\cdot-}$. Furthermore, it has been proved that BHT produces the dramatic hormonal changes in the animal's blood, i.e., the increase of corticotropin and corticosteroids along with the decrease of thyrotropin and triiodothyronine in blood plasma of rats after the BHT administration [24,25]. It means that BHT induces the substantial shift in the activity of adenohypophysis gland which is the source of corticotropin and thyrotropin hormones, and this is accompanied with the relevant shifts in the activity of peripheral endocrine glands, the adrenal cortex (the source of corticosteroids) and the thyroid gland (the source of triiodothyronine). It is common knowledge that the release of corticotropin into blood, followed by increase in synthesis of corticosteroids and decrease in synthesis of thyroid hormones, is a significant phase of the systems adaptation to stress. It seems that, with regular introduction into animals' food, BHT as a mild stress (hormetic) factor trains the neuro-hormonal system and, thus, increases the systems reliability, i.e. -adaptive capabilities of the organism [25]. Hence, BHT is actually able to decrease the level of active oxygen species in myocardial cells and, probably, other cells too. However, the beneficial effect of this antioxidant is manifested not through direct elimination (scavenge) of free radicals but in the preventive manner, i.e., upon decrease in the probability of generation of the radicals.

Another example of synthetic antioxidant is 2-ethyl-3-hydroxy-6-methylpyridine hydrochloride (Emoxipine). This water soluble antioxidant was first introduced in biomedicine as the anti-radiation protector [26]. The mechanism of the antioxidant prophylaxis in this case differs from that for BHT. The antioxidants based on hydroxypyridines are analogs of pyridoxine and pyridoxal phosphate which are the group B_6 vitamins. Meanwhile, pyridoxal phosphate performs the cofactor functions for glutamate aspartate aminotransferase, RNA polymerase and some other enzymes of biosynthesis of the nitrogen-containing compounds [15]. This implies that hydroxypyridine antioxidants are the anti-metabolites of vitamin B_6 and, as such, they inhibit the key enzymes of synthesis of amino acids and nucleotides. In particular, this allows understanding of the efficiency of 2-ethyl-3-hydroxy-

6-methylpyridine as the anti-radiation protector. Inhibition of biosynthesis retards cell division and, thus, provides the cells with additional time for restoring the genetic structures damaged by ionizing radiation [26].

The so-called mitochondria-targeted antioxidants, Mito Vit-E (vitamin E derivative [18]) and Sk Q (ubiquinone derivative [19]), can also act in a preventive manner. As the phenolic compounds, Mito Vit-E and Sk Q have weakly acidic properties and, as such, they can serve as protonophore uncouplers of oxidative phosphorylation, i.e., they can uncouple electron transport and ATP synthesis in mitochondria like, for example, 2,4-dinitrophenol. Besides, as the hydrophobic cations, they can transfer counterions through the mitochondrial lipid membrane and, thereby, they can decrease the transmembrane potential. Meanwhile, it is generally known that the electron transport in mitochondria experiences a "back pressure" of the transmembrane potential [15]. Therefore, as the transmembrane transfer agents of protons and anions, Mito Vit-E and Sk Q decrease the transmembrane potential thereby decreasing the generation of $O_2^{\cdot-}$ and its active products in mitochondria. This provides grounds for believing that mitochondria-targeted antioxidants not so much scavenge directly the $O_2^{\cdot-}$ (or its protonated form HO_2^{\cdot}) but prevent the formation of these radicals in mitochondria [11,12].

Mechanisms of antioxidant activity of natural antioxidants are being revised too. For example, glutathione (GSH), the physiological antioxidant and second messenger in cells, is considered now as the post-translational modifier that control over epigenetic mechanisms at different levels, i.e., substrate availability and enzymatic activity for DNA methylation, changes in the expression of micro RNAs, and participation in the histone code [27]. Furthermore, there are a wide class of natural antioxidants, called flavonoids, among them - quercetin, flavones and resveratrol. The last one is especially abundant in grapes and red wine [13]. These antioxidants can provide the preventive protection against oxygen radicals by induction of specific antioxidant enzymes. Indeed, the induction of synthesis of SOD and catalase was detected in blood erythrocytes of humans who received the food additive *Protandim* (extracts from five medical plants) [28]. The authors concluded that modest induction of the antioxidant enzymes, SOD and catalase, may be a much more effective approach to the problem of defense from free radicals than supplementation with antioxidants "that can, at best, stoichiometrically scavenge a very small fraction of total oxidant production" [28]. Noteworthy, that resveratrol activates expression of the sirtuin proteins thereby providing, in part, the increase in expression of mitochondrial SOD *In vivo* (see refs. in [29]).

Inasmuch as expression of SOD and other antioxidant enzymes in humans and animals is under the hormonal control, flavonoids also seem to make their preventive maintenance defense through hormonal regulation mechanisms. Indeed, in the experiments with *Macaca mulatta* monkeys it was found that the diurnal changes (circadian rhythms) in the SOD activity in erythrocytes tightly and positively correlate with the diurnal changes in the levels of cortisol and dehydroepiandrosterone sulfate (DHEAS) in blood plasma

[30]. For young animals the values of correlation coefficient were $0.92 + 0.09$ (cortisol versus SOD) and $0.99 + 0.02$ (DHEAS versus SOD). With aging, the circadian rhythms of SOD, cortisol and DHEAS are smoothed out although the correlation between the diurnal changes in cortisol and in SOD still maintains even for old animals. These results, like the above-mentioned experiments with BHT, testify that corticosteroid hormones do play the essential role in the regulation of SOD activity.

Quite a lot of data have now been accumulated demonstrating that even vitamin E (α -tocopherol), a "key antioxidant", can hardly serve as a free radical inhibitor *In vivo* and, hence, this issue in handbooks should be revised too [31,32]. There are four tocopherol isomers: α -, β -, γ -, and δ -tocopherol. All four isomers react *in vitro* with the RO_2^{\cdot} radical with approximately the same rate constants, of about $106 \text{ L mol}^{-1} \text{ s}^{-1}$. In the living nature, however, mainly α -tocopherol is encountered. As shown in experiments on cell cultures and isolated enzymes, the vitamin E inhibits a key regulator enzyme of biosynthesis, protein kinase C. Besides, it inhibits 5-lipoxygenase and phospholipase A_2 and activates protein phosphatase 2A and diacylglycerol kinase. It was proved that α -tocopherol modulates the expression of genes encoding synthesis of a number of protective proteins including α -TTP, α -tropomyosin, and collagenase. Moreover, α -tocophenyl phosphate, rather than the antioxidant phenolic form of vitamin E, serves as the bioregulator. It has been suggested that α -tocopherol acts as a ligand for yet unidentified specific proteins, membrane receptors or transcription factors, capable of regulating signal transduction and gene expression [31,32].

Furthermore, there are more and more data indicating that therapeutic effects of many pharmaceutical drugs are due to their beneficial action not only on the cells and tissues of the host organism but also on gastric and intestinal microbiota. Most of flavonoids, which are traditionally regarded as natural antioxidants, refer to the extensive class of physiologically active compounds long known as phytoalexins. Moreover, the phytoalexins are synthesized in plant tissues just for fighting, like antibiotics, against bacterial and fungal infections and for acting as inhibitors of transcription and translation of particular proteins in the cells of the infecting organisms [33]. Meanwhile, the number of microbiota cells in the gastrointestinal tract, on the skin and in some other organs and tissues nearly exceeds the number of cells of the host organism. Of even greater importance is that the microbial cells produce physiologically active substances that markedly affect all organs and tissues including immune system. More and more increasing number of experimental data are coming that the microbial metabolites promote metabolic benefits in the brain cells via gut-brain neural circuits (see refs. in [11,12,34,35]). As a matter of fact, a new synthetic biomedical concept has been emerged that the human microbiota is a source of therapeutic drug targets.

In view of the advances in systems biology, one can suggest that the so-called antioxidants, both natural and synthetic ones, attack the organism's microbial population. In high doses, these substances are toxic, as implied, because of their deleterious effects

on the microbiota. In low doses, however, the same compounds produce favorable effects on the organism's microbiota, in a hormetic-like fashion, and, thereby, increase the system reliability and lifespan of the organism. One can further assume that the so-called mitochondria-targeted compounds like Mito Vit-E and Sk Q actually affect the microbiotic cells. Thus, in this century, which is the century of systems biology, the theory which was put forward in the early 20th century by Metchnikoff about the considerable effects of the microbial population on the body health and aging [36] is actually revived. One can say with reasonable confidence that "Metchnikoff arises".

It has rather long been questioned whether the synthetic and natural antioxidants work *in vivo* in the same way, as *In vitro*, i.e. - as simple chemical scavengers of OH[•] and other active radicals [10-12,22,24-26]. Indeed, SOD was discovered by J M Mc Cord and I Fridovich long ago, in 1968 (see refs. in [15,17]). Shortly thereafter, in the chemical kinetics experiments it was found that neither natural nor synthetic antioxidants are able to compete for the reactive oxygen species with the specialized anti-oxidative enzyme systems of cells and tissues. Over the years, more and more experimental results indicate that the true mechanisms of the antioxidant effects are to be studied on the ways of systems biology instead of free-radical chemistry. Last years, such terms as "polyphenols", instead of antioxidants, and "redox regulation/redox signaling pathways", instead of oxidative stress, came into use, at last [37]. Moreover, the Society for Free Radical Biology and Medicine has been recently renamed in the Society for Redox Biology and Medicine. Yet, the paradigm that the antioxidants directly intercept free radicals *in vivo* in the same manner as *in vitro* had a very long-lived existence. As said in [38], "it is harder to overcome old ideas, rather than create the new ones". In other words, "never attribute to malice that which is adequately explained by stupidity" (Hanlon's Razor).

Conclusion

Natural and artificial antioxidants, or polyphenols, effectively protect living cells and tissues from chemically reactive oxygen species. However, the efficiency of the antioxidants as scavengers of free radicals in living systems is low, compared to the natural antioxidant enzymes, since the rate constants and concentrations of the antioxidants in cells and tissues are negligibly small to compete with the antioxidant enzymes for the oxygen radical anions (O₂⁻) and other reactive oxygen species. *In vivo*, the antioxidants do decrease concentrations of the reactive oxygen species but they do it not as inhibitors of the free radical reactions but in the preventive manner, i.e., as physiologically active compounds that prevent the formation of the oxygen radicals. Actually, the antioxidants provide prophylactic maintenance against the reactive oxygen species. At this, the protection mechanisms are different for antioxidants of different types. For example, the synthetic antioxidant BHT prevents the formation of the oxygen radical anions as the by-products of mitochondrial electron transport while natural flavonoids exert the preventive antioxidant action through the induction of synthesis of the antioxidant enzymes, superoxide dismutase and catalase. The prophylactic antioxidant maintenance realizes via the organism's

neuro-hormonal (endocrine) system. The antioxidant injection, as a mild stress factor, trains the neuro-hormonal system in a hormetic-like fashion and, thus, increases adaptive capabilities, i.e. - the systems reliability of the organism. Besides, there are more and more data indicating that the therapeutic effects of many pharmaceutical drugs, including antioxidants, are due to their beneficial action not only on the cells and tissues of the host organism but also on the gastric and intestinal microbiota. Thus, true mechanisms of the antioxidant prophylaxis are to be studied on the ways of systems biology instead of free-radical chemistry. It might be said now that antioxidant biomedicine has passed a long way from "false knowledge to true ignorance".

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