

Anti-aging and aging factors in life. The role of free radicals[☆]

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Abstract

The present review deals with some factors determining the anti-aging as well as the aging process. In order to get a deeper insight in the subject matter, firstly some less known spectroscopic and kinetic data of antioxidant vitamins (C, E, β -carotene) acting as anti-aging factors by electron transfer are briefly discussed. The generation of oxygen transients (OH, ROO[•], ¹O₂, ozone radicals, etc.) by sunlight, ultrasonic and microwave radiation are causing “oxygen stress” and contribute to early ageing is also reviewed. Particular attention is paid to external environmental aging factors. Their action is based on the incorporation of various pollutants contained in water and air in the human organism. In this respect the polycyclic aromatic hydrocarbons (PAHs) play an essential role by initiating DNA-mutation, leading to an accelerate aging, carcinogenesis and diseases.

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1. Introduction

The aging is a very complex biological process. In addition to individual genetic factors also external influences, such as nutrition, smoking, alcohol, environmental conditions, etc. can strongly contribute to its anticipated appearance. A particular attention in this respect has been paid to the biological action of free radicals, especially to oxygen species (OH, peroxy, ozone and other oxidizing species), which are causing “oxidative stress” (Stocker and Frei, 1991). It appears that these transients in addition to the nitrogen oxides are one of the major factors for a forced aging, DNA-damage, carcinogenesis and for initiation of a number of diseases.

It might be mentioned that the oxidizing and reducing free radicals generated in the human organism can also be produced by ionizing radiation in aqueous media. In such case it is possible to obtain each desired kind of transients, such as OH (hydroxyl), RO[•] (alkoxyl), ROO[•] (peroxy), NO[•] (nitric oxide), ONOO[−] (peroxynitrite) as well as

radical cations, radical anions, ozonides, etc. in wanted concentration in a given media. Consequently, using radiation, more precise studies can be performed on model systems for a better understanding of involved mechanisms and for development of preventive anti-aging measures.

Based on these premises a brief overview is presented on less known formation and characterization of the most important free radicals resulting from the antioxidant vitamins (β -carotene, E and C), which are involved in the anti-aging process. The antioxidant properties of flavonoides, polyphenols, etc. are also mentioned. The “oxidative stress” initiated by uv-light: formation and scavenging of singlet oxygen (¹O₂) and other reactive oxygen species, which are leading to an accelerated skin aging and carcinogenesis, plays nowadays a particular role for the population, is also briefly discussed (Kaiser et al., 1990; Lang et al., 2006). The involved reaction mechanisms and some protection measures are reviewed.

2. Water radiolysis and primary products

The living systems consists of more than 70% of water. By treatment of aqueous media with ionizing radiation the water is radiolyzed to reducing (e_{aq}[−], H) and oxidizing (OH) free radicals, as well as molecular products (H₂, H₂O₂).

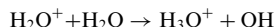
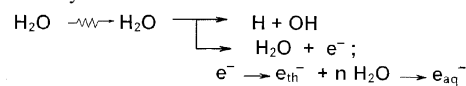
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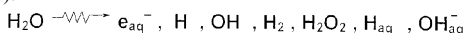
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Table 1
Radiolysis of water and some primary reactions

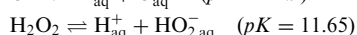
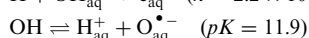
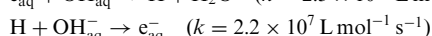
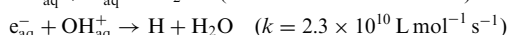
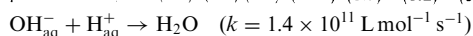
Primary reactions:



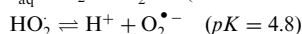
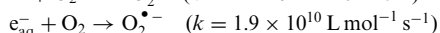
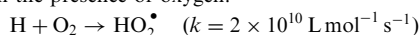
Gross reaction of water radiolysis (the G -values^a at pH 7 are given in brackets):



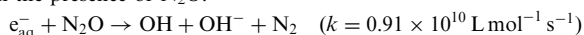
at pH = 6 to 8.5: $G = (2.7) (0.6) (2.8) (0.45) (0.7) (3.2) (0.5)$



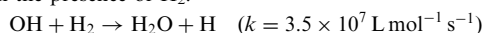
In the presence of oxygen:



In the presence of N_2O :



In the presence of H_2 :



1 Gy (Gray) = 100 rad = $6.24 \times 10^{15} \text{ eV g}^{-1}$.

1 Gy generates $6.344 \times 10^{-7} \text{ mol L}^{-1}$ ($\text{e}_{\text{aq}}^- + \text{H} + \text{OH}$).

^a G -value = number of changed molecules per 100 eV ($1.60 \times 10^{17} \text{ J}$) absorbed energy. For conversion into SI-units: multiply the G -value by 0.10364 to obtain $G(x)$ in $\mu\text{mol J}^{-1}$.

For better understanding of the subject matter, the formation of primary products of water radiolysis, their yields (G -values) and some common transformation reactions are compiled in Table 1. Thereby the “solvated electrons” (e_{aq}^-) represent the second form of the reducing species and are of special biological interest.

3. Antioxidant vitamins

The vitamin C (ascorbate AH^-), E (α -tocopherol) and β -carotene were taken as representatives of this group and some of their somewhat less known behaviors are briefly discussed. The antioxidant effect is based on the vitamin's ability to act as electron donor in various biological processes. After the electron ejection, β -carotene results in an oxidizing radical cation ($\beta\text{-car}^{\bullet+}$). The same is valid for vitamin E, yielding ($\text{vit.E}^{\bullet+}$), whereas ascorbate converts into a neutral free radical (AH^{\bullet}). These processes are very fast and are investigated by time resolved methods, pulse radiolysis and laser flash photolysis (see e.g. Benssasoun et al., 1983; Getoff et al., 1999; Getoff, 2001).

The radiation induced formation of ascorbate (vitamin C) transients has been a subject of several studies having various objectives (e.g. Bielski et al., 1971; Schöneshofer, 1972; Bielski, 1982). Our interest in this respect was the characterization of the vitamin C transients, formed as a sequence just after the electron donation process in neutral,

aqueous media. For this purpose the electron transfer from ascorbate to azide radical (N_3^{\bullet}), acting as a specific electron acceptor, was studied. The rate constant for the formation of the ascorbate free radical (AH^{\bullet} ; $k = 3.2 \times 10^9 \text{ L mol}^{-1} \text{ s}^{-1}$), as well as of its decay by disproportionation ($k = 1.7 \times 10^7 \text{ L mol}^{-1} \text{ s}^{-1}$) were determined (Getoff, 2001). The absorption spectrum of AH^{\bullet} radical, kinetic traces of its formation and decay are given in Fig. 1. The biological fate of the resulting dehydroascorbate (DHA) is discussed in respect to its synergistic effect to mitomycin C (Kammerer and Getoff, 2004). Vitamin C can scavenge efficiently OH, peroxy radicals, hypochlorite and $^1\text{O}_2$ (singlet oxygen), especially in human plasma lipids inhibiting lipid peroxidation. Consequently, based on all these properties it appears that vitamin C is acting as an excellent anti-aging compound.

The transient absorption of α -tocopherol (vitamin E) radical cation ($\text{vit.E}^{\bullet+}$) is observed in oxygenated DMSO solution, using pulses of 3 and 400 ns of 1.6 and 3 MeV electrons, respectively (Getoff et al., 1999). The formation

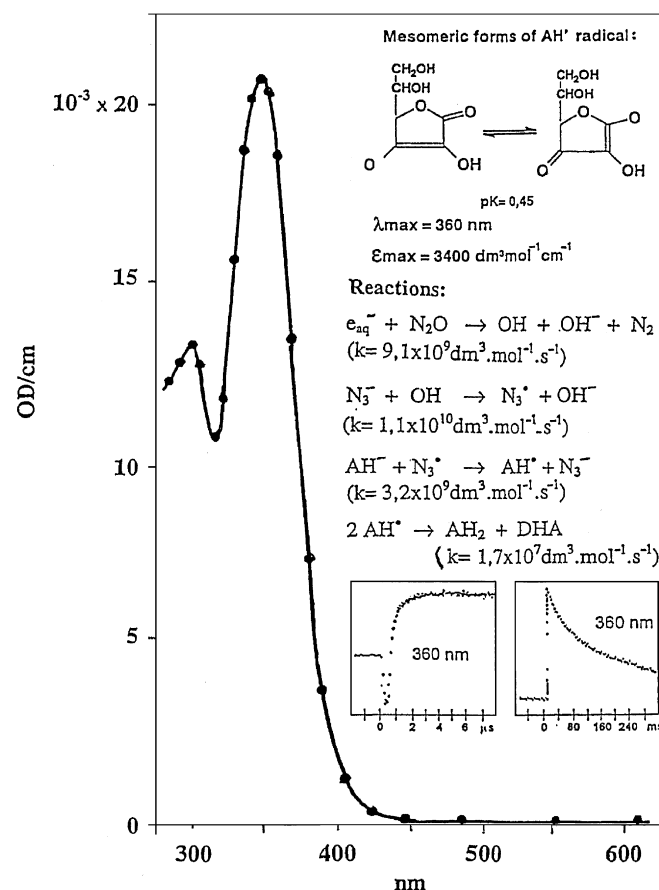


Fig. 1. Transient absorption spectrum resulting by N_3 attack on aqueous ascorbate saturated with N_2O at pH = 7.4, OD/cm values are normalized to 10 Gy. Inset: mesomeric forms of AH^{\bullet} species, spectroscopic data as well as formation and decay kinetic traces at 360 nm; $\epsilon_{360} = 3.670 \text{ L mol}^{-1} \text{ cm}^{-1}$. (Reprinted from Radiat. Phys. Chem. 60, 351–358, 2001, by N. Getoff: Cytostatica efficiency enhancement by vitamins C, E, and β -carotene under irradiation. State of the art, with permission from Elsevier).

of $\text{vit.E}^{\bullet+}$ by electron transfer from vit.E to $\text{DMSO}^{\bullet+}$ proceeds with a rate constant, $k = 1 \times 10^9 \text{ L mol}^{-1} \text{ s}^{-1}$ and the resulting $\text{vit.E}^{\bullet+}$ decays by a second order reaction with $k = 4.7 \times 10^8 \text{ L mol}^{-1} \text{ s}^{-1}$. The transient absorption spectrum as well as the molar extinction coefficients (ϵ -values) of the corresponding maxima are presented in Fig. 2. The radical cation of vitamin E-acetate has been also determined by pulse radiolysis (Getoff, 2001). Preliminary experiments showed that vitamin E under certain conditions can also react with e_{aq}^- generating $\text{vit.E}^{\bullet-}$ (radical anion). Studies by various researchers (see e.g. Machlin, 1991; Kammerer et al., 2001) have shown that vitamin E, likewise as vitamin C is a very potent scavenger of oxidizing species: OH, peroxy and ozone radicals, etc. Hence, it protects the cell components against destruction, operating as anti-aging agent.

The formation of β -carotene radical cation ($\beta\text{-car}^{\bullet+}$) was determined in oxygenated DMSO solution and is presented in Fig. 3 at various times (μs) after the end of a $0.1 \mu\text{s}$ pulse of 10 MeV electrons. The spectrum shows a strong absorption maximum at 942 nm ($\epsilon = 1.6 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$), whereas the absorption band of β -car triplet state at $520\text{--}680 \text{ nm}$ is strongly quenched by oxygen at the same time (Getoff, 2000).

Based on the kinetics (Fig. 3, insert) it was established that the species are formed with a rate constant $k = 4.8 \times 10^8 \text{ L mol}^{-1} \text{ s}^{-1}$. They disappear in a first order reaction, $k_1 = 2.1 \times 10^3 \text{ s}^{-1}$, resulting in a dimer radical cation $(\beta\text{-car})_2^{\bullet+}$. For further details about $\beta\text{-car}^{\bullet+}$ species see the original paper (Getoff, 2000). It might be mentioned that $\beta\text{-car}^{\bullet+}$ is a oxidizing specie. In addition to the formation of dimer radical cation, it can also undergo

hydrolysis, where OH radicals are formed or it is regenerated to $\beta\text{-car}$ by electron acceptance from, surrounding molecules (see later).

It was further established that β -carotene can also react with e_{aq}^- , yielding the corresponding radical anion ($\beta\text{-car}^{\bullet-}$). The absorption spectrum of β -carotene radical anion ($\beta\text{-car}^{\bullet-}$) is shown in Fig. 4. It was registered by pulse radiolysis in an airfree solvent mixture of 86% ethanol and 14% three times distilled water (Getoff, 1999). The transient absorption spectrum possesses a strong absorption band at 850 nm ($\epsilon = 1.3 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$) as well as a small one at about 270 nm . The $\beta\text{-car}^{\bullet-}$ radical anion is formed with $k = 4.3 \times 10^9 \text{ L mol}^{-1} \text{ s}^{-1}$ and disappears by a first order reaction with $k_1 = 3.8 \times 10^3 \text{ s}^{-1}$, forming a dimer radical anion, $(\beta\text{-car})_2^{\bullet-}$. The ability of vitamin E to form various types of transients can explain, at least to certain extent, its versatile biological properties. Naturally, the formation of either $\beta\text{-car}^{\bullet+}$ or $\beta\text{-car}^{\bullet-}$ radical ions in the organism should strongly depend on the redox-processes of the involved systems. Based on its multiple reactivity $\beta\text{-car}$ plays an important role in the aging process as a mediator in redox processes (see next chapter).

The antioxidant properties of the vitamin C, E and β -carotene have been also studied from various points of view (e.g. Benssason et al., 1983; Sies and Stahl, 1995).

4. Protection of cell membrane damage by antioxidant vitamins

In general the biological cell membrane consists of lipoproteins, where also enzymes and 3–5% antioxidant vitamins (β -carotene, vitamin E and C) are also contained. Based on the above briefly mentioned spectroscopic and kinetic data of vitamins, an attempt was made to investigate their effect on the membrane protection. The studies concerned the electron transfer between them in airfree DMSO solutions. It was established a *cascade electron transfer process* with the following pathway: β -carotene ($\beta\text{-car}$) contained at distinct areas at the outer layer of the cell membrane is getting first in contact with oxidizing agents (e.g. OH radicals). The ejected electron from $\beta\text{-car}$ forms the passive OH^- ions and a strong oxidizing $\beta\text{-car}^{\bullet+}$ radical cation. The last one as an oxidizing specie could destroy the membrane layer, however, it is regenerated to $\beta\text{-car}$ by electron transfer from vitamin E (formation of $\text{vit.E}^{\bullet+}$). In turn each $\text{vit.E}^{\bullet+}$ specie is regenerated to vit.E by an electron provided by vitamin C (AH^-). The resulting AH^\bullet radicals undergo disproportionation, forming DHA. This cascade electron transfer can be illustrated by the reactions and the scheme shown in Fig. 5. It appears, therefore, vitamin C to be the most important electron source for the protecting process (Getoff, 2001).

It should be mentioned that the electron transfer reaction in the cell membrane, very likely the lipoproteins as well as enzymes are involved. These processes are connected with the mechanisms concerning the rather

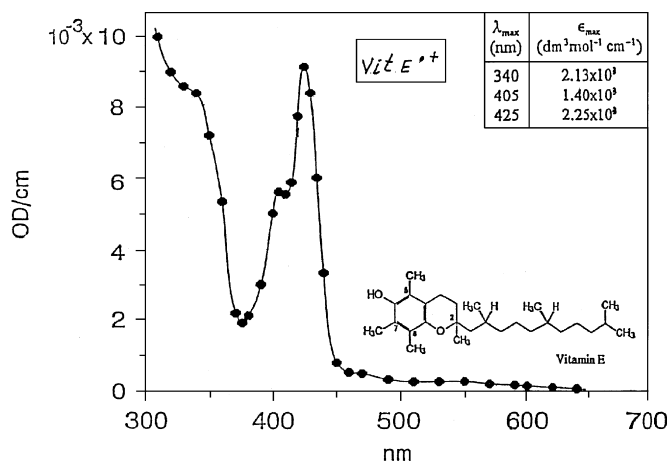


Fig. 2. Transient absorption spectrum ($5 \mu\text{s}$ after $0.4 \mu\text{s}$ electron pulse) resulting from $9.3 \times 10^{-4} \text{ mol L}^{-1}$ α -tocopherol in aerated DMSO (OD/cm-values normalized to 10 Gy). Insert: spectroscopic data of $\text{Vit.E}^{\bullet+}$ transients.

Formation and decay reactions: $\text{Vit.E} + \text{DMSO}^{\bullet+} \rightarrow \text{Vit.E}^{\bullet+} + \text{DMSO}$ ($k = 1 \times 10^9 \text{ L mol}^{-1} \text{ s}^{-1}$), $\text{Vit.E}^{\bullet+} + \text{Vit.E}^{\bullet+} \rightarrow \text{products}$ ($k = 4.7 \times 10^8 \text{ L mol}^{-1} \text{ s}^{-1}$). (Reprinted from Radiat. Phys. Chem. 55, 699–704, 1999 by N. Getoff, I. Platzer and C. Winkelbauer: Transients and cooperative action of β -carotene, vitamin E and C in biological systems in vitro under irradiation, with permission from Elsevier).

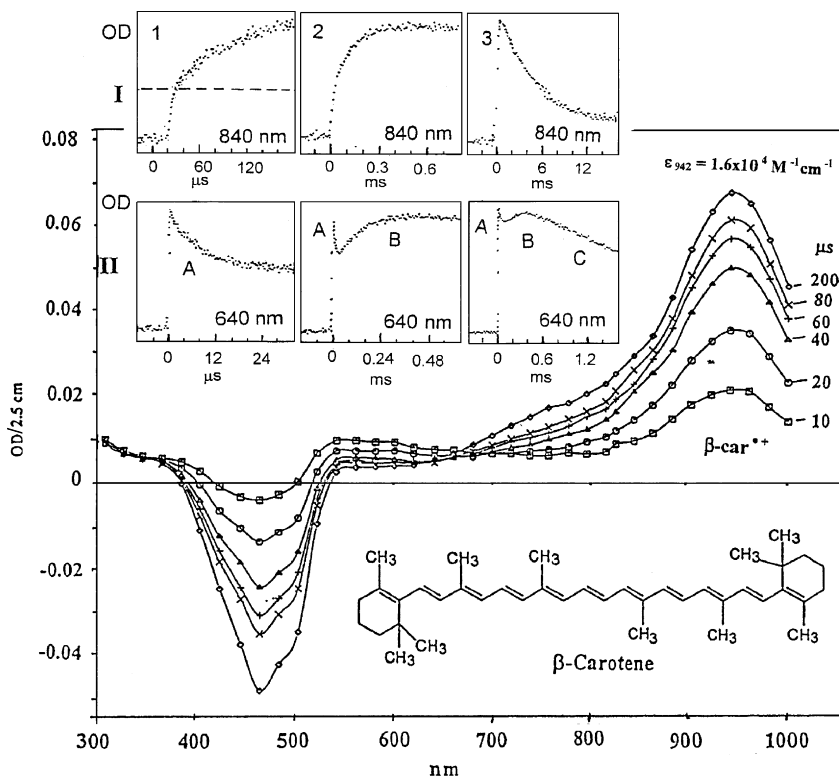


Fig. 3. Absorption spectra and kinetic traces of $\beta\text{-car}^{\bullet+}$ resulting by $0.1 \mu\text{s}$ pulse of 10 MeV electrons of $1.5 \times 10^{-5} \text{ mol L}^{-1}$ $\beta\text{-carotene}$ in aerated DMSO solution.

Formation: $\beta\text{-car} + \text{DMSO}^{\bullet+} \rightarrow \beta\text{-car}^{\bullet+} + \text{DMSO}$ ($k = 4.8 \times 10^8 \text{ L mol}^{-1} \text{ s}^{-1}$). Decay: $\beta\text{-car}^{\bullet+} + \beta\text{-car} \rightarrow (\beta\text{-car})_2^{\bullet+}$ (dimer radical cation) ($k_1 = 2.1 \times 10^3 \text{ s}^{-1}$). (Reprinted from Radiat. Res. 154, 692–696, 2000, by N. Getoff: Pulse radiolysis studies of $\beta\text{-carotene}$ in oxygenated DMSO solution. Formation of $\beta\text{-carotene}$ radical cation, with permission of Radiation Research Journal).

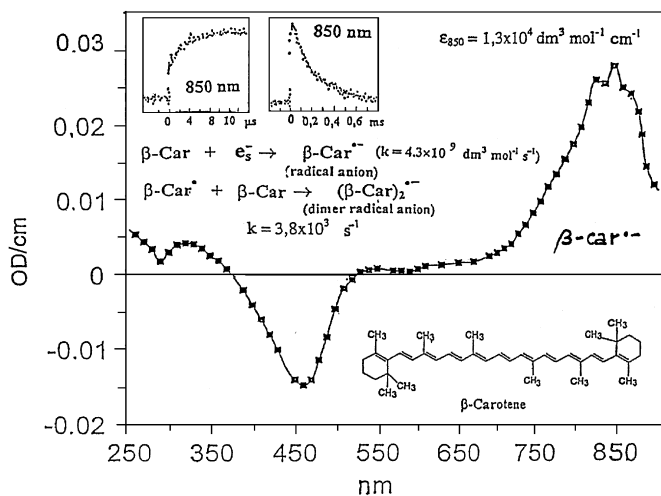


Fig. 4. Absorption spectrum of $\beta\text{-car}^{\bullet-}$ radical anion resulting from airfree $5 \times 10^{-3} \text{ mol L}^{-1}$ $\beta\text{-carotene}$ in a mixture of 86% ethanol and 14% four-times distilled water. OD/cm-values are normalized to 10 Gy. Inset: formation and decay kinetics and reactions. (Reprinted from Radiat. Phys. Chem. 55, 395–398, 1999, by N. Getoff: Radical anion of $\beta\text{-carotene}$ studied by pulse radiolysis in ethanol-water and tert-butanol-water mixtures, with permission from Elsevier).

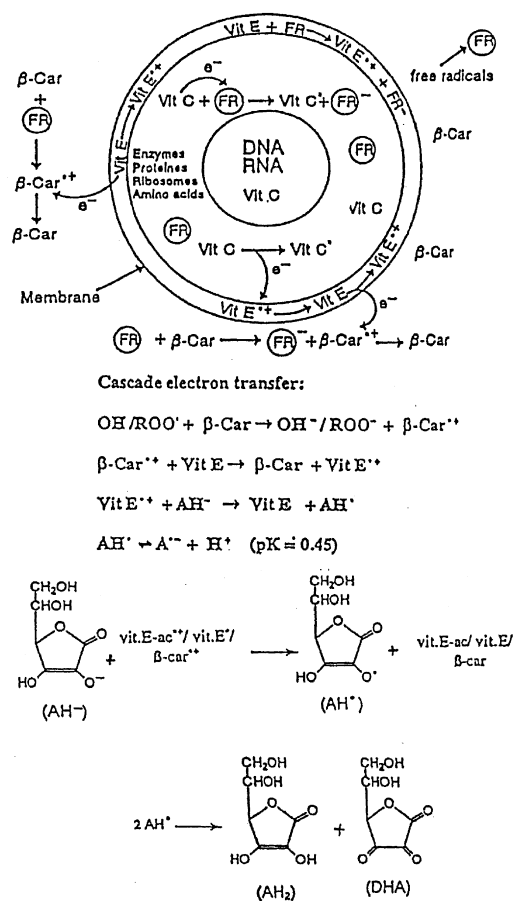
complex problem of the communication between cells, which is here not further discussed.

It may be noted that DHA in aqueous media is able to eject electron from its electronically excited state (like the

antioxidant vitamins). Hence, in aqueous media DHA can strongly enhance the efficiency of mitomycin C (MMC) by formation of $\text{MMC}^{\bullet-}$ (semiquinone), the active form of the cytostaticum (Kammerer and Getoff, 2004). Naturally, DHA plays a positive role in the anti-aging process.

5. The nature of antioxidants. General considerations

Early investigations showed that the structure of a molecule, having substituents like $-\text{OH}$, $-\text{O}^-$, $-\text{OPO}_3\text{H}^-$, $-\text{COO}^-$, $-\text{NH}_2$, $-\text{NH}_3\text{CH}_3$, $-\text{N}(\text{CH}_3)_2$, etc. can act as electron donor, that means they have antioxidant properties. This fact has been demonstrated by studies on formate (Zechner and Getoff, 1974), phenol and related compounds (Zechner et al., 1976; Grabner et al., 1977), aromatic amines (Köhler et al., 1977), flavines (Getoff et al., 1978), etc. In the last decades it was stated that the above quoted functional groups are contained in natural compounds in numerous plants, fruits, etc., e.g. as polyphenols (see resveratrol: e.g. Bader and Getoff, 2006), flavonoides, proteins, natural dye stuffs, etc. In aqueous media these substances can eject electron (formation of e_{aq}^-) from their excited singlet states, whereas this effect is in strong relation to the fluorescence ability of the compound. The yield of e_{aq}^- obtained from a given substance depends on several factors: excitation energy, structure of the molecule, kind of substituent, temperature, and pH of the aqueous



Reactions of the cascade electron transfer	k ($\text{L}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}$)
$\text{DMSO}^{**} + \beta\text{-Car} \rightarrow \text{DMSO} + \beta\text{-Car}^{**}$	2.3×10^9
$\beta\text{-Car}^{**} + \text{Vit.E} \rightarrow \beta\text{-Car} + \text{Vit.E}^{**}$	2.7×10^9
$\text{Vit.E}^{**} + \text{AH}^{-} \rightarrow \text{Vit.E} + \text{AH}^{\cdot}$	3.1×10^9

Fig. 5. Cascade electron transfer from vitamin C→E→ β -carotene and rate constants of the involved reactions. (Reprinted from Radiat. Phys. Chem. 60, 351–358, 2001, by N. Getoff: Cytostatica efficiency enhancement by vitamins C, E, and β -carotene under irradiation. State of the art, with permission from Elsevier).

media (Getoff, 1989). For example, with temperature increase of the solution, the e_{aq}^{-} yield is enhanced, but the fluorescence quantum yield is correspondingly decreased.

The precise knowledge about the reaction mechanism of the antioxidant action in human organism is not yet clear enough. Very likely a gradually electron transfer takes place within the involved systems, which is activated by enzymatic redox processes.

6. Formation of peroxy radicals and action of antioxidant vitamins

The most reactive oxidizing species are the OH radicals. They can split off an H-atom or electron of an organic molecule. However, their preferred reaction sites are the double bonds. In the case of aromatic compounds,

the OH-species react on various ring positions, where the ortho- and para-sites are the preferred ones. All resulting transients can subsequently add O_2 , resulting into peroxy radicals. For illustration of the reaction mechanisms the absorption spectra and kinetics of 4-chlorobenzaldehyde (4-ClBzA) is taken as a model for pulse radiolysis studies (Fig. 6) (Getoff, 2002). Obviously, the transient absorption spectrum (A), formed in a media, where 90% OH and 10% H are the acting species, represents practically the OH-adduct on various positions (ortho, meta, para and ipso) of the aromatic ring. In the media containing a N_2O /air-mixture the reduced spectrum (B) is observed, since a part of e_{aq}^{-} is converted into the much less reactive $\text{O}_2^{\cdot-}$ radical anions. Subsequently (155 μs after 0.4 μs electron pulse), the addition of O_2 -molecule to the OH-adduct takes place, resulting into the peroxy radical formation (spectrum C, Fig. 6). The corresponding rate constants (k) are calculated from the kinetic profiles, given as insert in Fig. 6. The peroxy radicals are generally long-lived species and can introduce a number of harmful biological sequences and accelerate the aging process.

The antioxidant vitamins and related compounds are rather efficient scavengers for oxidizing species and act as protectors against the oxidative stress, initiated by OH, ROO^{\cdot} (peroxy radicals) and $^1\text{O}_2$ (singlet excited oxygen), etc. (see e.g. Stocker and Frei, 1991). For illustration of this vitamin ability some rate constants (k) of reactions with free radicals are given in Table 2.

The data in Table 2 clearly demonstrate the broad reactivity spectrum of the antioxidant vitamins. It has been also shown, that ascorbate is more efficient in inhibiting lipid peroxidation caused by peroxy radicals (ROO^{\cdot}) than vitamin E, protein thiols, bilirubin, etc. Ascorbate protects also DNA against endogenous oxidative damage in human sperm (Frei et al., 1989; Fraga et al., 1991). It has been also found that the three vitamins (C, E, β -carotene) are potent radiation protectors (Kammerer et al., 2001).

7. Effect of sunlight: generation of oxygen transients and ozone

The sunlight reaching the earth surface ($\lambda \geq 315 \text{ nm}$) has a manifold effect on all life systems. Since oxygen in the atmosphere is in excited triplet state ($^3\text{O}_2$), it can be activated to the very reactive singlet state ($^1\text{O}_2$), where organic compounds, like chlorophyll, flavines, various phyto-dye-stuffs, etc. as well as inorganic materials, e.g. dust particles can act as sensitizer. Such substances can mediate energy or/and electron transfer, which can initiate cell damage.

Some basic sunlight induced processes are presented in Scheme 1, where β -carotene is taken as a model for photosensitizer and generation of very reactive oxygen species.

The resulting very active oxygen transients can strongly accelerate the skin aging, as well as cause various harmful biological effects by inhalation of ozone.

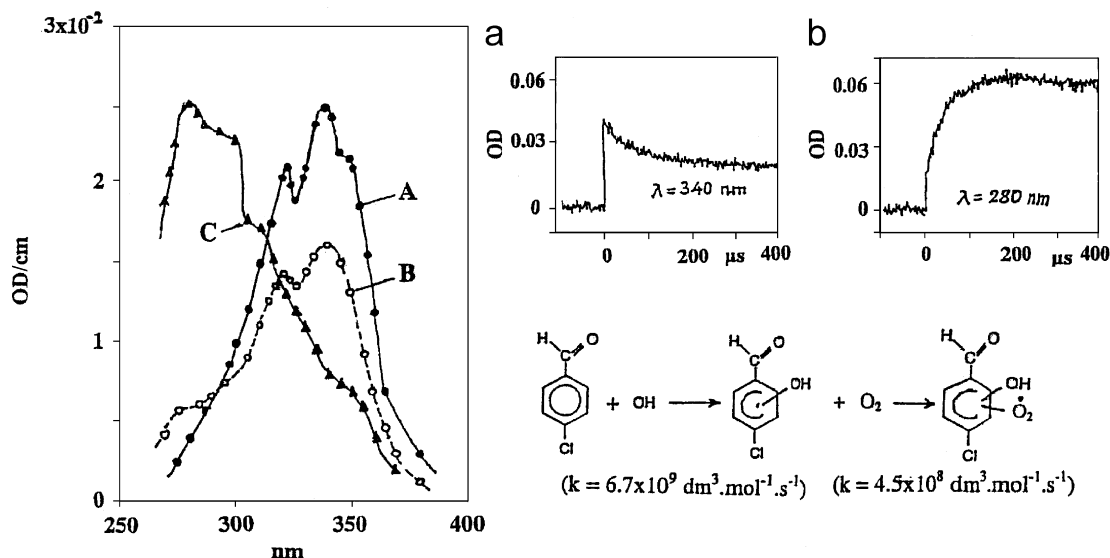


Fig. 6. (A) Total absorption at pulse end (OH-adduct) resulting from $2 \times 10^{-4} \text{ mol dm}^{-3}$ 4-chlorobenzaldehyde saturated with N_2O . (B) Saturated with $1.4 \times 10^{-2} \text{ mol dm}^{-3} \text{ N}_2\text{O}$ and $0.62 \times 10^{-3} \text{ mol dm}^{-3} \text{ O}_2$ at pulse end. (C) The same medium, spectrum measured at 155 μs after pulse end; pH = 10.4, absorbed dose: 5 Gy/0.4 μs electron pulse. Kinetic profiles: (a) decay of OH-adducts, (b) formation of peroxy radicals in solution (B). (Reprinted from Radiat. Phys. Chem. 65, 437–446, 2002, by N Getoff: Factors influencing the efficiency of radiation-induced degradation of water pollutants, with permission from Elsevier).

Table 2

Rate constants ($k, \text{L mol}^{-1} \text{s}^{-1}$) for reactions of some free radicals and singlet oxygen ($^1\text{O}_2$) with antioxidant vitamins

Vitamin	Rate constant ($k, \text{L mol}^{-1} \text{s}^{-1}$) of			
	OH	e_{aq}^-	ROO^\bullet	$^1\text{O}_2$
Ascorbate	3×10^{9a}	3.5×10^{8a}	2×10^{8c}	1×10^{7c}
Ascorbic acid	1×10^{10a}	–	–	–
Vitamin E	4.6×10^{9b}	4.6×10^{9a}	5×10^{8c}	8×10^{7c}
β -Carotene	–	2×10^{8a}	1.5×10^{9c}	5×10^{9c}

^aBuxton et al. (1988).

^bGetoff (1999).

^cSies and Stahl (1995).

A particular role in the aging process is attributed to *ozone*. It is a very strong oxidant and in addition to this by reaction with the free radicals generated in the human organism, a number of various oxidizing species are formed. In order to give an impression in this respect, some reactions of ozone degradation and corresponding rate constants (k -values) are compiled in Table 3 (Getoff, 1997 and references therein).

As mentioned above, ozone like OH radicals, attack preferentially the double bonds in organic compounds. For illustration of such reactions, the gradual course of the ozone attack on tyrosine is presented in Scheme 2.

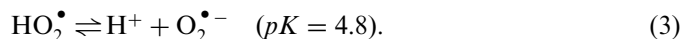
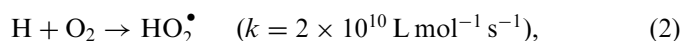
Naturally, each of the shown intermediate products can be involved in a number of other processes, e.g. formation of peroxy radicals, etc., leading to harmful consequences to living systems and contributing to the aging process of men.

Interesting features in this respect appears the ozone action on halogenated organic compounds, which are air

pollutants. In Scheme 3 the stepwise ozonation course of aqueous trichloroethylene (TCIE) is given as an example. As final products of the process very toxic phosgene, chloroaldehyde and hydrogen peroxide are formed. The first two products undergo hydrolysis. The products of such processes can seriously contribute to accelerated aging, cell damage and carcinogenesis.

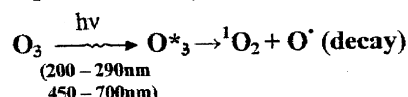
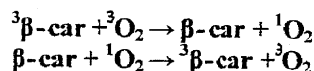
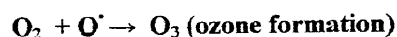
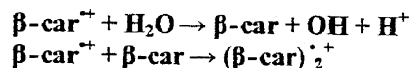
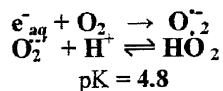
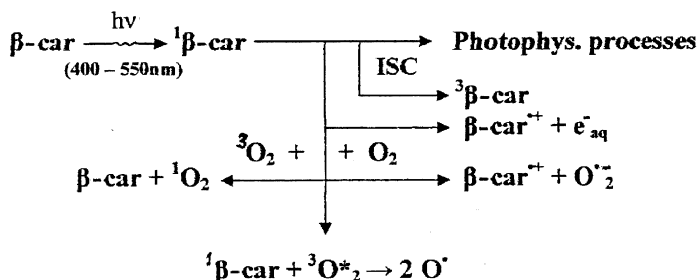
8. Ultrasonic radiation

Electromagnetic radiation with a frequency in the range of 2×10^5 to about 10^8 Hz is indicated as *ultrasonic radiation*. It can lead to *sonolysis of water*, meaning water splitting to H and OH radicals, whereby in the presence of air peroxy radicals are formed (El'Piner, 1964; Getoff, 1995):



It might be noted that under certain conditions ultrasonic treatment of nutrients can evoke chemical changes, which consequently may contribute to the aging process.

Summing up, as a sequence of the sonolysis of aerated aqueous solutions and water containing food, oxidizing radicals: OH, $\text{HO}_2^\bullet / \text{O}_2^{\bullet -}$, ROO^\bullet , etc. are produced. They can initiate numerous of chemical and biological processes as already discussed above. Naturally, the implemented frequency and duration of the ultrasonic treatment are determining factors for the biological damage. Hence,

Example: β -carotene (β -car); simplified mechanisms:

The resulting oxygen transients: ${}^1\text{O}_2$, O^{\cdot} , $\text{O}_2^{\cdot-}$, O_3 are leading to cell death and tissue damage.

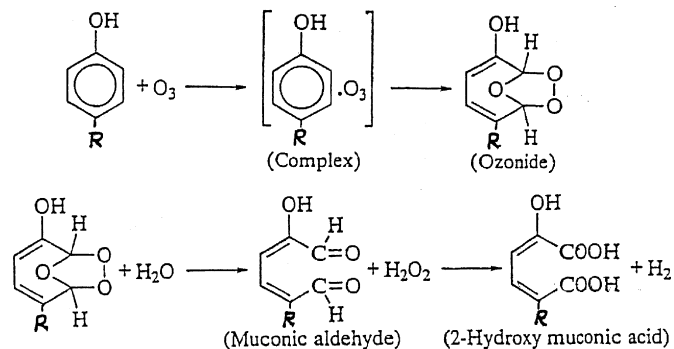
Scheme 1. Photo-generation of oxygen transients from biological sensitizers, e.g. β -carotene, flavines, pigments, chlorophyll, etc.

Table 3
Formation of ozone- and peroxy transients in aqueous media (Getoff, 1997)

Reaction	k ($\text{dm}^3 \text{mol}^{-1} \text{s}^{-1}$)
$\text{H} + \text{O}_3 \rightarrow \text{HO}_3^{\cdot}$	3.6×10^{10}
$e_{\text{aq}}^- + \text{O}_3 \rightarrow \text{O}_3^{\cdot-}$	3.7×10^{10}
$\text{HO}_3^{\cdot} \xrightleftharpoons[\text{b}]{\text{a}} \text{H}^+ + \text{O}_3^-$ ($\text{pK}_a = 8.2$)	(a) 3.3×10^{2a}
$\text{O}_3^{\cdot-} \xrightleftharpoons[\text{b}]{\text{a}} \text{O}^{\cdot-} + \text{O}_2$	(b) 5.2×10^{10}
$\text{O}_3^{\cdot-} + \text{H}^+ \rightarrow \text{OH} + \text{O}_2$	(a) 3.3×10^{3a}
$\text{O}_3^{\cdot-} + \text{H}^+ \rightarrow \text{HO}_3$	(b) 3.0×10^9
$\text{OH} + \text{O}_3 \rightarrow \text{HO}_2^{\cdot} + \text{O}_2$	9.0×10^{10}
$\text{OH} + \text{O}_3 \rightarrow \text{HO}_4^{\cdot}$	1.1×10^8
$\text{HO}_4^{\cdot} \rightarrow \text{HO}_2^{\cdot} + \text{O}_2$	2.0×10^9
$\text{HO}_4^{\cdot} + \text{HO}_4^{\cdot} \rightarrow 2\text{O}_3 + \text{H}_2\text{O}_2$	2.8×10^{4a}
$\text{HO}_4^{\cdot} + \text{HO}_3^{\cdot} \rightarrow 2\text{O}_3 + \text{H}_2\text{O}_2 + \text{O}_2$	5.0×10^9
$\text{HO}_4^{\cdot} + \text{HO}_2^{\cdot} \rightarrow \text{O}_3 + \text{H}_2\text{O}_2 + \text{O}_2$	5.0×10^9
$\text{OH} + \text{O}_3^{\cdot-} + \text{OH}^- \rightarrow 2\text{O}_2^{\cdot-} + \text{H}_2\text{O}$	8.5×10^8
$\text{HO}_2^{\cdot} + \text{O}_3 \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$	$< 10^4$
$\text{HO}_2^{\cdot} + \text{O}_3 \rightarrow \text{OH} + 2\text{O}_2$	$< 10^4$
$\text{O}_2^{\cdot-} + \text{O}_3 \rightarrow \text{O}_3^{\cdot-} + \text{O}_2$	1.6×10^9
$\text{OH} + \text{HO}_2^{\cdot} \rightarrow \text{O}_2 + \text{H}_2\text{O}$	7.9×10^9
$\text{HO}_2^{\cdot} + \text{HO}_2^{\cdot} \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$	7.5×10^5
$\text{HO}_2^{\cdot} + \text{HO}_2^{\cdot} \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$	3.7×10^6
$\text{HO}_2^{\cdot} + \text{O}_2^{\cdot-} \rightarrow \text{HO}_2^- + \text{O}_2$	1×10^8
$\text{O}_2^{\cdot-} + \text{O}_2^{\cdot-} \rightarrow \text{O}_2 + \text{O}_2^{2-}$	< 10
$\text{O}^{\cdot-} + \text{HO}_2^- \rightarrow \text{O}_2^{\cdot-} + \text{OH}^-$	4×10^8

^aUnits in s^{-1} .

special protection measures are necessary by using ultrasound in medical treatments as well as by frequent observation of unborn babies in pregnant women.



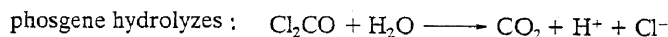
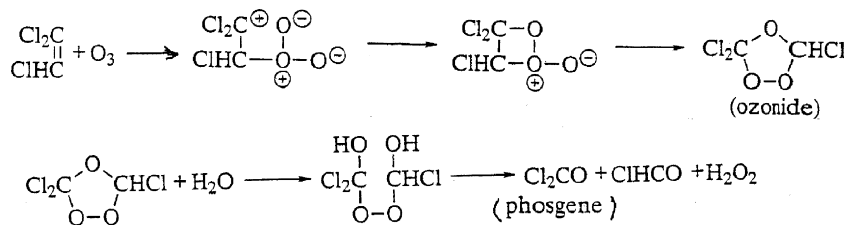
Scheme 2. Ozone reactions with aromatic compounds, e.g. tyrosine, where $\text{R} = \text{CH}_2\text{CH}(\text{NH}_3^+)\text{COO}^-$.

9. Microwave radiation

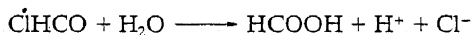
It covers the electromagnetic radiation range from about 3×10^8 up to 10^{12} Hz and is partly overlapping with the far infrared. It has broad practical application, e.g. as microwave oven for quick cooking (2.45×10^9 Hz).

The heat results by excitation of dipoles of molecules with polar structure, e.g. water, amino acids, proteins and ions. In the last case the absorbed microwave radiation causes very intense motions and frictions, leading also to heat.

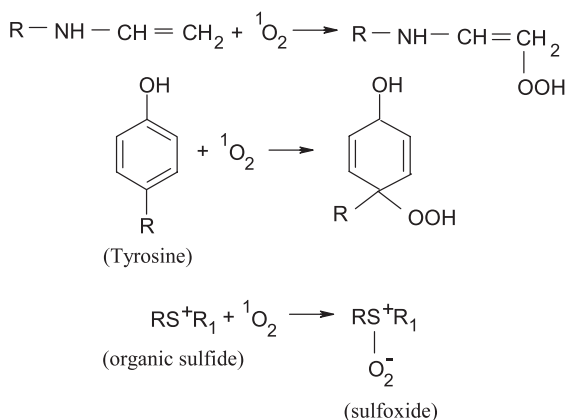
Microwave radiation can produce singlet oxygen (${}^1\text{O}_2$), which can react in the gas phase as well as in solution. Principally, ${}^1\text{O}_2$ is attacking double bonds of organic compounds, forming unstable peroxide. Similarly, ${}^1\text{O}_2$ can react with organic sulfides resulting by several steps into the corresponding sulfoxides: Eq. (4)–(6) (Foote, 1976; Getoff, 1995):



The halogenated aldehyde can likewise undergo hydrolysis:



Scheme 3. Ozonation of three chloroethylene (TCIE) in aqueous media.



Based on these facts it is recommendable to avoid implementation of microwave cooking for the second time of the same food. Otherwise the produced peroxides can seriously contribute to an accelerated aging process!

10. Environmental influences

The strong development of various industries and the rapid growth of the world population in the last decades led to a rather heavy pollutant-overloading of the atmosphere and water resources. The combustion of fossil fuels (coal, oil, gas) in the industry and in caloric plants, as well as in private households are associated not only with the emission of NO_x , SO_2/SO_3 (acid rain precursors) in addition to CO_2 in the atmosphere (green house effect and damaging of the world climate), but also with a number of polycyclic aromatic hydrocarbons (PAHs). The last ones are content in the air and even in trace amounts they are highly carcinogenic. By rain and snow they can be brought also to ground water.

PAHs can be incorporated in the human organism (e.g. in DNA), leading to strong biological changes, accelerating the aging process, inducing carcinogenesis, etc. Taking diphenylether (DPE), one of the simplest PAHs as a model, a possible incorporation reaction mechanism in DNA is presented in Scheme 4. As a sequence of several oxidation/

hydratization reaction steps in the liver, the resulting carcinogenic oxacyclopropane of DPE can react with the NH_2 -group, e.g. of the guanine base, leading to a DNA modification and initiation of tumour. This process can also occur on adenine or cytosine groups of the DNA, likewise.

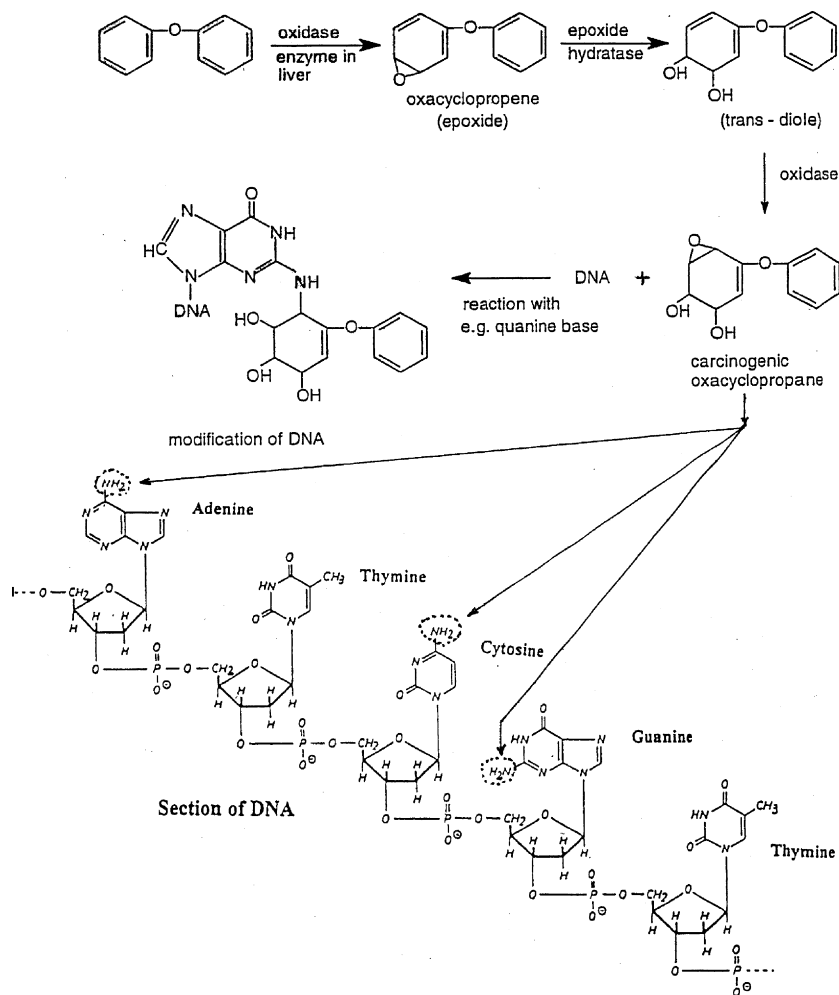
It might also be mentioned that pyrene, belonging to the PAHs group, is forming simultaneously radical cations and radical anions (Getoff et al., 2003). It is expected, each of these species to have different biological effects.

On the other hand biological resistant compounds originating from chemical, pharmaceutical and other industries, dyestuffs from dyehouses, etc. admitted to rivers, lakes and oceans are strongly influencing the sensitive marine life. These substances are subsequently incorporated in the plankton and accumulated in fish, which is finally consumed by the population. As a sequence of the above mentioned influences a premature aging as well as various diseases can appear.

11. Conclusion

In the present review some anti-aging as well as aging factors are very briefly discussed. Basic data characterizing vitamins (C, E, β -carotene), acting as anti-aging compounds and their protecting action of the cell membrane are reviewed. The production of reactive oxygen transients (OH , ROO^* , ${}^1\text{O}_2$, ozone species, etc.) by sunlight, as well as by ionizing, ultrasonic and microwave radiation are leading to "oxygen stress". All these factors contribute to a forced aging. Finally, the PAH substances produced by combustion of fossil fuels and discharged in the air, play nowadays a particular role in this respect.

Summing up, it can be stressed, that in addition to the genetic features, such as free radicals, enzymes, hormones, etc. in the human organism, also external influences are determining factors in aging. To them belong wrong nutrients and preparation of the food, enjoyment of alcohol, smoking and the kind of profession are strongly implemented in the aging process.



Scheme 4. Possible enzymatic oxidation and hydratization of diphenylether (DPE) leading to DNA-mutation of a cell.

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