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Review

Vitamin Free Radicals and their Anticancer Action

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Abstract. *The antimumor effect of antioxidant vitamins (C, E and β-carotene) as well as of the vitamins B1 up to B6 and B11 under the action of oxidizing (OH, $O_2^{\bullet-}$) and reducing free radicals (e_{aq}^-, H) is discussed. In addition, the synergistic action of vitamins on cytostatic agents under the influence of free radicals and the involved reaction mechanisms are briefly discussed. The very fast kinetics of electron transfer taking place within a biological molecule (vitamin B11) is shown for demonstration of the complicated free radical processes in the organism.*

The human organism is an extremely complicated biological system. Biological processes are interlaced harmonically into one another and are very sensitive. Their course can be strongly influenced by various factors, such as environmental milieu, nutrition and microorganisms. The consequences of such influences can lead to a disorder in the course of biological functions and initiate diseases. Nevertheless oxidizing and reducing free radicals are permanently generated as well as consumed in the human organism, which consists of about 70% of water. These free radicals, however, can also be produced by treatment of biological systems with ionizing radiation in aqueous media, making it possible to investigate their properties individually or in combination with desired compounds, outside the organism, thus offering a pathway to study and get a deeper insight into complicated biological processes *e.g.* aging, initiation of cancer *etc.* (1).

Generation of Free Radicals

Oxidizing free radicals (OH, $HO_2^{\bullet}/O_2^{\bullet-}$, peroxides, oxygen species) are mostly generated in the liver, whereas reducing ones (e_{aq}^-, H, $R^{\bullet}$ *etc.*), mainly the "solvated electron (e_{aq}^-, representing the basic form of the H-atom) are emitted by enzymes, hormones and redox-systems (as a sequence of energy transfer processes) in the organism.

The oxidizing transients are also involved in the degradation of consumed nutrients ("wet oxidation"). However, their surplus attacks the cell-membrane and its components (2), causing an accelerated aging process (1) among other effects. Both, OH and e_{aq}^-, as well as radical ions are involved in a number of reactions with purine bases, nucleosides and nucleotides. An excellent review on this topic has been presented by Steenken (3). The involvement of free radicals in biological processes, *e.g.* DNA damage and repair has been previously discussed from different points of view (4-8).

The solvated electrons (e_{aq}^-) are also involved in the nervous system, brain functions and in various electron-transfer processes and communication between cells and biological systems, causing synergistic effects, *e.g.* cytostatic agents (2, 10-13). Some of these processes will be discussed with respect to carcinogenesis. The existence of e_{aq}^- was first experimentally proved by γ-ray transformation of CO_2 into simple organic compounds (14). It was later shown, that ea_q^- can be also formed by photo-induced oxidation of ions and of certain organic compounds in aqueous solution (15-17). Some examples are shown in Scheme 1 for illustration.

By systematic investigations of the fate of excited states of organic molecules in aqueous solution it was stated that, in addition to fluorescence, those molecules having substituents like, *e.g.* –OH, $-O^-$-OCH_3, -OPO_3H_2, -OPO3H–, -COO^-, -NH_2, -$NHCH_3$ can eject electrons. The highlights of the published results are discussed in a review paper (18). The photo-induced electron emission process is schematically presented for phenol in Figure 1.

The electron ejection from electronically excited organic substances takes place essentially below the ionization potential of the corresponding substrate. This is possible due to the very strong dipole action of the surrounding water molecules, as illustrated by Figure 1.

The ability of compounds, such as vitamins (1), flavines (19), indol and derivates (20), and hormones (21) to emit electrons is of special biological interest.

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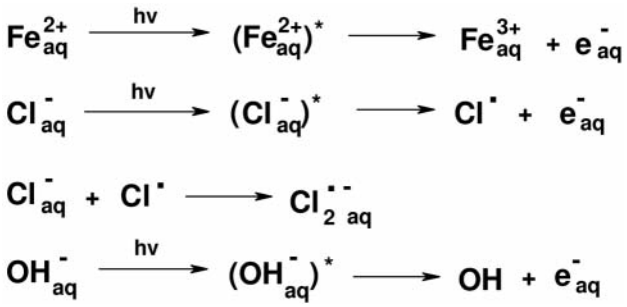


Scheme 1. *Examples for formation of e_{aq}^- from excited cations and anions in aqueous solution.*

Water Radiolysis

For the sake of completeness it should be mentioned that the formation of free radicals generated in the human organism (*e.g.* ea_q^-, H, OH, $O_2^{\bullet-}$) can also be produced by radiolysis of water. This fact makes it possible to study precisely the action of individual free radicals in respect to specific biological processes taking place in certain systems. The water radiolysis as well as the transformation reactions of reducing species (ea_q^-, H) into oxidizing ones in the presence of air and reverse, are presented in Table I.

Electron-transfer from Antioxidant Vitamins to Cytostatic agents

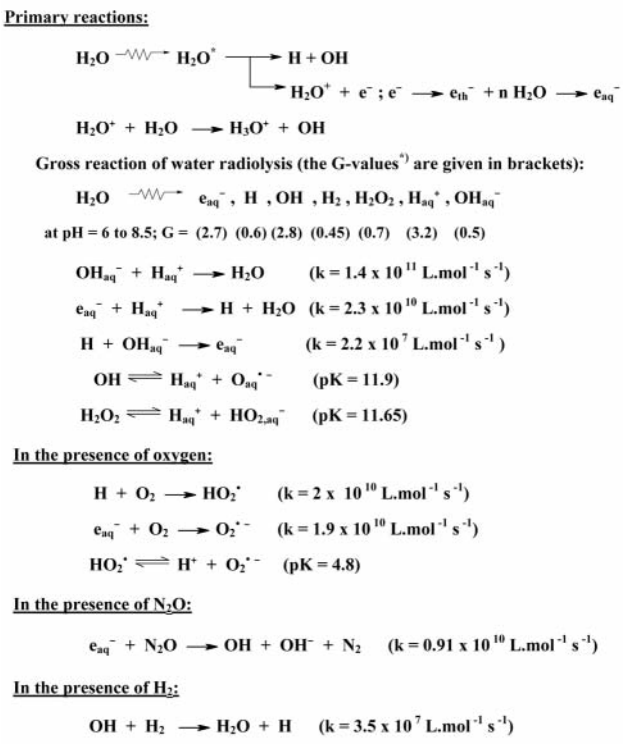
Electron-transfer, as already mentioned, plays a fundamental role in the human organism (10-12, 22, 23). This kind of processes were found to essentially govern the enhancement of cytostatic agents efficiency, especially under the implementation of vitamins (2, 24, 25). Vitamins C, E and β-carotene are very efficient antioxidants (electron donors) (26), vitamin C (ascorbate) having the greatest activity. As a sequence of the electron emission, vitamin C converts into the ascorbate radical, whereas the other two vitamins are converted into the corresponding radical cations (2). The ascorbate radical ($AH^{\bullet}$) disappears by disproportion forming dihydroascorbate (DHA): $AH^{\bullet} + AH^{\bullet} \rightarrow AH_2 + DHA$, which can act as a sensitizer for cytostatic agents. On the other hand, it is known that mitomycin C (MMC) can be activated by the acceptance of one electron (formation of semiquinone, $MMC^{\bullet-}$ radical anion (27-30). The resulting $MMC^{\bullet-}$ species are involved in the formation of a covalent cross-link adduct with DNA of cancer cells preventing their further partition.

The effect of the antioxidant vitamins (vit.C, vit.E and β-car) as electron donors on the MMC-activation, was studied in airfree as well as in aerated media, using *Escherichia coli* bacteria (AB1157) as a model (2, 24, 25). The course of the resulting survival curves (N/N_0-ratio) was registered as a function of the absorbed radiation dose of gamma-ray (Gy) and is presented in Figure 2. In order to get information about the biological effect of the resulting products higher radiation doses have been applied.

The D_{37}-value of each curve represents the N/N_0-ratio at a given dose (Gy); the corresponding ΔD_{37}-value (Gy) is calculated by substracting the buffer D_{37}-value (not given in Figure 2) from the individual D_{37}-data, *e.g.* D_{37}(sample) - D_{37}(buffer)=ΔD_{37}(sample in Gy) at N/N_0=0,37. The positive ΔD_{37}-values indicate the radiation protection property of the system, whereas the negative ones show its cytostatic efficiency. The calculated ΔD_{37}-value for each survival curve is given as an insert in Figure 2.

Obviously, the expected MMC-enhancement in airfree media in the presence of antioxidant vitamins is not realized. On the contrary, the MMC-activity is reduced by 32%, ΔD_{37}=–110 of MMC drops to ΔD_{37}=–0.75 in the presence of vitamins. This fact can be explained by the strong radiation protection ability of the vitamins in airfree media as radical scavengers, having rate constants for reactions with e_{aq}^-/H and OH in the range of 10^8 upto 10^{10} $L.mol^{-1}$ s^{-1}.

Table I. *Radiolysis of water and some primary reactions.*



*) G-value = number of changed molecules per 100 eV (1.60×10^{17} J) absorbed energy. For conversion into SI-units: multiply the G-value by 0.10364 to obtain G(x) in $\mu mol.J^{-1}$.
1 Gy (Gray)=100 rad=6.24×10^{15} $eV.g^{-1}$
1 Gy generate 6.344×10^{-7} $mol.L^{-1}$ (e_{aq}^- + H + OH)

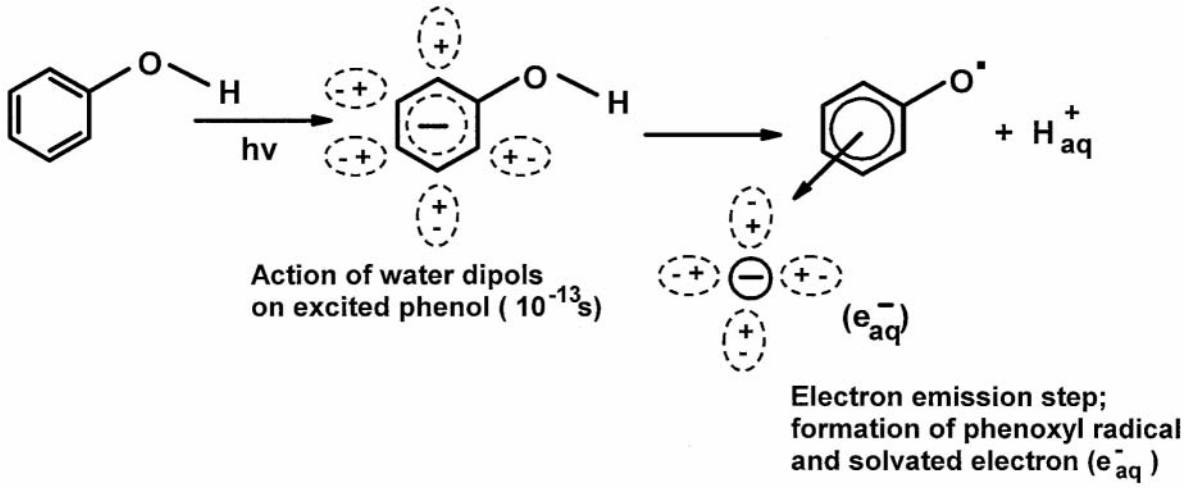


Figure 1. *Schematic presentation of electron ejection process (formation of e_{aq}^-, phenoxyl radical and H_{aq}^+).*

In the presence of air, however, the reducing species (e_{aq}^- and H) are converted into peroxyl radicals ($O_2^{\bullet-}$, $HO_2^{\bullet}$) as indicated in Table I. Hence, in aerated neutral media only oxidizing radicals (46% OH and 54% $O_2^{\bullet-}$) are operating, which obviously leads to a higher efficiency of MMC (Figure 2). This effect was elucidated by pulse radiolysis studies of MMC in aerated aqueous solution. It was established, that MMC react with OH radicals, producing MMC-adducts (MMC.OH), which can add oxygen forming the corresponding peroxyl radicals (MMC.OH)$O_2^{\bullet}$ (31). In addition to this it has been established that in an oxidizing environment MMC can emit electrons converting into an MMC-radical cation, $MMC^{\bullet+}$ (32). All these oxidizing forms of MMC are able to form covalent cross-link adducts with DNA in cancer cells, preventing their propagation (Figure 2, curves C and D and insert).

For the sake of completeness the formation and decay kinetics as well as spectroscopic results of all MMC transients are summarized in Table II. The presented MMC-data demonstrate the complexity of the processes under participation of the free radicals generated in human organism in the presence of air.

Carefully performed kinetic measurement concerning the role of oxygen in respect to the above discussed processes, showed, that MMC-semiquinone ($MMC^{\bullet-}$) can transfer one electron to O_2, forming $O_2^{\bullet-}$ oxidizing species (25). This process emphasizes once more the role and importance of competition reactions in organisms initiated by oxygen (Figure 3).

In order to complete the picture concerning the role of the antioxidant vitamins (C, E, β-car) some of the more important kinetic and spectroscopic characteristics of their transients are summarized in Table III. Both vit. E and β-car possess bifunctional properties, they can donate electron forming radical cations (vit $E^{\bullet+}$ and β-$car^{\bullet+}$) as well as accepting one electron resulting into a radical anion (vit $E^{\bullet-}$ and β-$car^{\bullet-}$). The complete data for the formation of vit $E^{\bullet-}$ transients are not yet published (see 1).

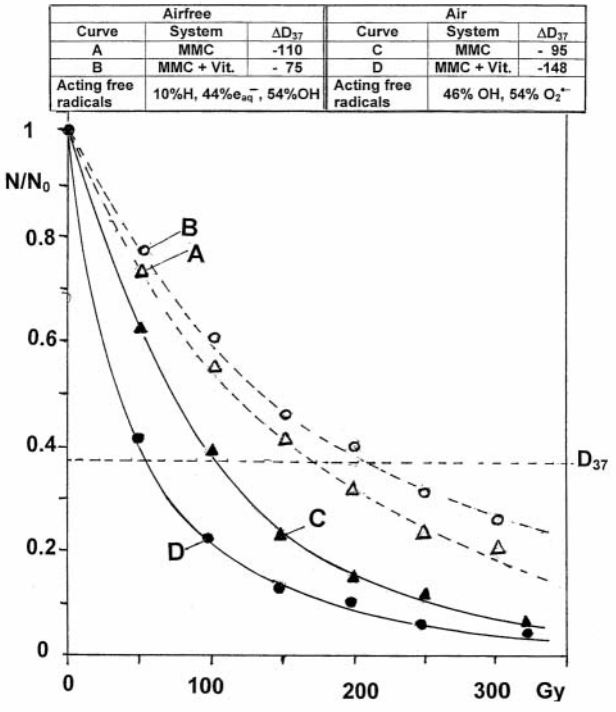


Figure 2. *Survival curves (N/N_0) of Escherichia coli (AB1157), as a function of absorbed radiation dose (Gy) showing the effect of oxygen as well as of antioxidant vitamins (C, E and β-carotene) on MMC activity in aqueous media (pH=7.4). Insert: ΔD_{37}-values for the experimental series (see text). [MMC]=10^{-5} mol/L; [vit.C]=[vit.E]=[β-car.]=10^{-4} mol/L; dose rate=120 Gy/min at 25°C. N_0=starting number and N=number of colonies after irradiation treatment.*

Table II. *Formation and decay rate constants as well as molar extinction coefficients (ε) of various MMC-transients. Formation: k in L.mol^{-1}.s^{-1} decay: k1 in s^{-1} and 2k in L.mol^{-1}.s^{-1}.*

MMC transients	Kinetics*		εmax (L.mol^{-1}.s^{-1})	Ref.
	Formation	Decay		
MMC$^{\bullet-}$ (Semiquinone)	MMC + eaq– → MMC$^{\bullet-}$ k=2.2×10^{10}	at 290-300 nm and 470-500 nm: k1=2.1×10^{3} at 420-460 nm: k1=1.2×10^{3} at 530 nm (maximum): 2k=4.6×10^{8}	ε_{510}=1×10^{4} ε_{475}=4.76×10^{3} ε_{310}=4.05×10^{3}	2
MMC.OH$^{\bullet}$ (OH-adduct)	MMC + OH → MMC.OH$^{\bullet}$ k=5.8×10^{9}	at 280-320 and 500 nm: k=0.85×10^{3} thereupon: 2k=1.3×10^{8}	ε_{295}=9.95×10^{3} ε_{410}=1.45×10^{3} ε_{505}=5.24×10^{3}	31
(MMC.OH).$O_2^{\bullet}$ (Peroxyl radicals)	MMC.OH$^{\bullet}$ + O_2 → (MMC.OH).$O_2^{\bullet}$ 290-420 nm: =5×10^{7} 480 nm: k=1.6×10^{8} >510 nm: k=3×10^{8}	290 – 420 nm: k1=1.5×10^{3} >510 nm: k1=7.0×10^{3}	ε_{295}=9.95×10^{3} ε_{410}=1.45×10^{3} ε_{505}=5.24×10^{3}	31
MMC$^{\bullet+}$ (Radical cation)	MMC + SO_4^{2-} → MMC$^{\bullet+}$ + SO_4^{2-} k=1.2×10^{10}	2k=4.6×10^{8}	ε_{295}=5.63×10^{3} ε_{400}=2.9×10^{3} ε_{500}=2.32×10^{3}	32

*The k- and 2k-values are given in: L.mol-1.s^{-1}, whereas k1 in s^{-1}, ε-values in L.mol^{-1}.cm^{-1}.

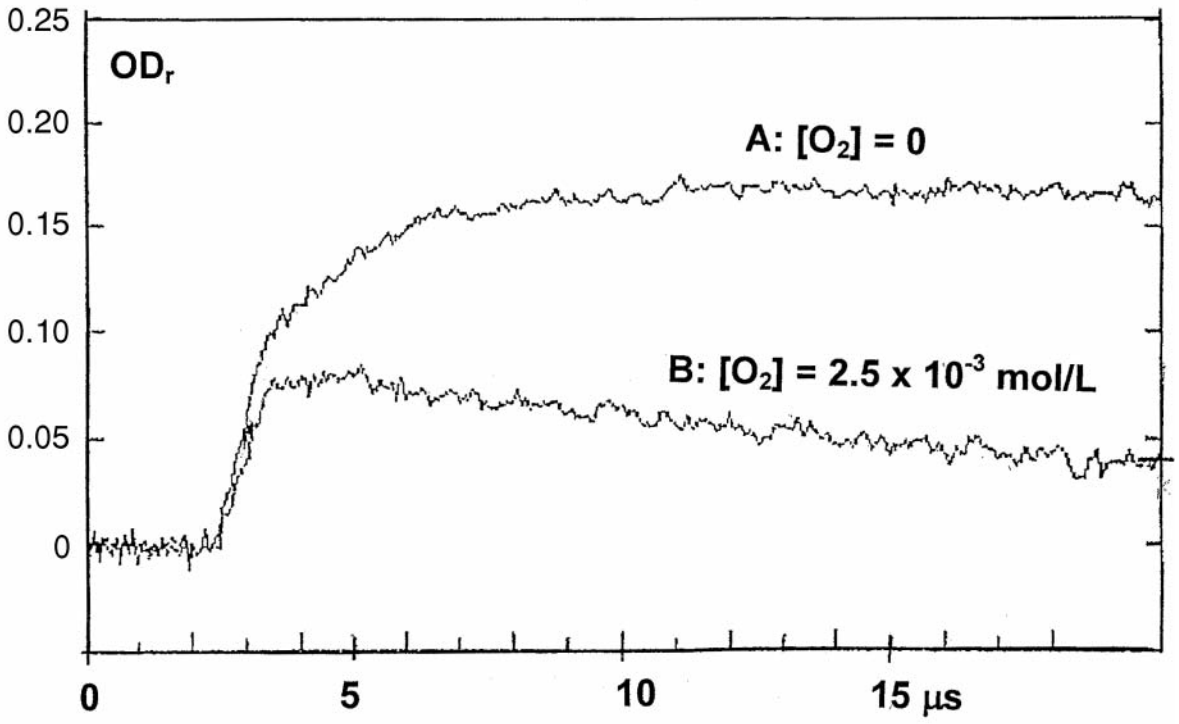
Table III. *Reactions as well as spectroscopic and kinetic data of transients from Vit.C, Vit.E-ac and β-Car.*

Substrate	Reaction	Kinetics (a)		ε_{max}(L.mol^{-1}.s^{-1})	Ref.
		Formation	Decay		
Vit.C (AH–) (b)	$AH^- + N_3^{\bullet} \rightarrow AH\bullet + N_3^-$ 2 AH → AH_2 + DHA	3.2×10^{9}	2k=1.7×10^{9}	ε_{360}=3.4×10^{3}	2
Vit.E-ac (c)	Vit.E-ac + DMSO$^{\bullet+}$ → Vit.E-ac$^{\bullet+}$ + DMSO	8×10^{8}	k_1=6×10^{3}	ε_{300}=6.2×10^{3} ε_{850}=3×10^{3}	2
β-Car (d)	β-Car + es^- → β-Car$^{\bullet-}$	5.1×10^{9}	k_1=3.8×10^{3}	ε_{850}=1.3×10^{4}	2
β-Car (e)	β-Car + DMSO•+ → β-Car$^{\bullet+}$ + DMSO	4.8×10^{8}	k_1=2.1×10^{3}	ε_{850}=1.6×10^{4}	33

(a) Formation: k in L.mol^{-1}.s^{-1}; decay: k_1 in s^{-1} and 2k in L.mol^{-1}.s^{-1}
(b) Aqueous solution, pH 8.4
(c) DMSO solution
(d) 16.8 mol/L ethanol in water
(e) DMSO solution

As mentioned before the ascorbate radical (AH$^{\bullet}$), resulting by the electron donation of vit. C undergoes disproportion forming dehydroascorbate (DHA) and ascorbate (vit. C). In order to learn more about the biological behaviour of DHA in respect to MMC action, some studies in this respect have been performed (33). Experiments *in vitro* using *Escherichia coli* bacteria (AB1157) as a model in the presence of various combinations of DHA and MMC with the vitamins C, E and β-car in aerated media (pH=7.4) were performed. The survival curves, N/N_0-ratio as a function of absorbed radiation dose, which is proportional to the concentration of the acting radicals (46% OH, 54% $O_2^{\bullet-}$) are presented in Figure 4.

Under these experimental conditions DHA shows a slight antitumor effect, ΔD_{37}=–8 and MMC, ΔD_{37}=–93 (see insert in Figure 4). The various combinations of vit. C, D, E and β-car with DHA affect essentially the MMC efficiency from ΔD_{37}=–96 up to ΔD_{37}=–141 (Figure 4, insert). As a matter of fact the exact reaction mechanism is not yet elucidated. It should be noted that in addition to MMC, the effect of antioxidant vitamins has also been studied on other antitumor compounds, *e.g.* sanazole (34).



Conversion reactions:

$HCOO^- + OH \rightarrow {}^\bullet COO^- + H_2O$, $(k = 2.7 \times 10^9\ L.mol^{-1}.s^{-1})$

$HCOO^- + H \rightarrow {}^\bullet COO^- + H_2$, $(k = 2.5 \times 10^8\ L.mol^{-1}.s^{-1})$

$MMC + {}^\bullet COO^- \rightarrow MMC^{\bullet +} + CO_2$

$MMC + e_{aq}^- \rightarrow MMC^{\bullet -}$, $(k = 2.2 \times 10^{10}\ L.mol^{-1}.s^{-1})$

Electron transfer from $MMC^{\bullet -}$ to O_2:

$MMC^{\bullet -} + O_2 \rightarrow MMC + O_2^{\bullet -}$, $(k = 2 \times 10^9\ L.mol^{-1}.s^{-1})$

Figure 3. *Kinetics of electron transfer from $MMC^{\bullet -}$ (radical anion) to O_2 in aqueous solution (pH=8.5) containing 3×10^{-4} mol/L MMC and 0.1 mol/L $HCOO^-$ in: (A) airfree and (B) containing 2.5×10^{-3} mol/L O_2, studied by pulse radiolysis technique (0.1 μs pulse, 5.5 Gy/pulse)(ODr=relative optical density; time: μs). Conversion reactions: $HCOO^- + OH \rightarrow {}^\bullet COO^- + H_2O$, $(k=2.7\times10^9\ L.mol^{-1}.s^{-1})$; $HCOO^- + H \rightarrow {}^\bullet COO^- + H_2$, $(k=2.5\times10^8\ L.mol^{-1}.s^{-1})$; $MMC + {}^\bullet COO^- \rightarrow MMC^{\bullet +} + CO_2$; $MMC + e_{aq}^- \rightarrow MMC^{\bullet -}$, $(k=2.2\times10^{10}\ L.mol^{-1}.s^{-1})$; Electron transfer from $MMC^{\bullet -}$ to O_2: $MMC^{\bullet -} + O_2 \rightarrow MMC + O_2^{\bullet -}$, $(k=2\times10^9\ L.mol^{-1}.s^{-1})$.*

Antitumor Properties of B-series Vitamins

The B-series vitamins (B1 up to B6 and B11) have been intensively investigated with regard to their anticancer behaviour and their strongly expressed synergistic effect on cytostatic agents. Two kinds of approach to the subject matter have been considered: (i) experiments *in vitro* implementing the individual vitamins as well as in mixtures with cytostatic agents under specific experimental conditions, where oxidizing or reducing primary free radicals as well as both radical types are acting, were performed. As a model for living systems *Escherichia coli* (AB1157) or cancer cell line MCF-7 were used. (ii) Chemical analysis of the products resulting by the attack of the corresponding free radicals on the given system. This point is of special interest, since very frequently the products can efficiently influence the biological effect of the substrate and cause undesired side-effects.

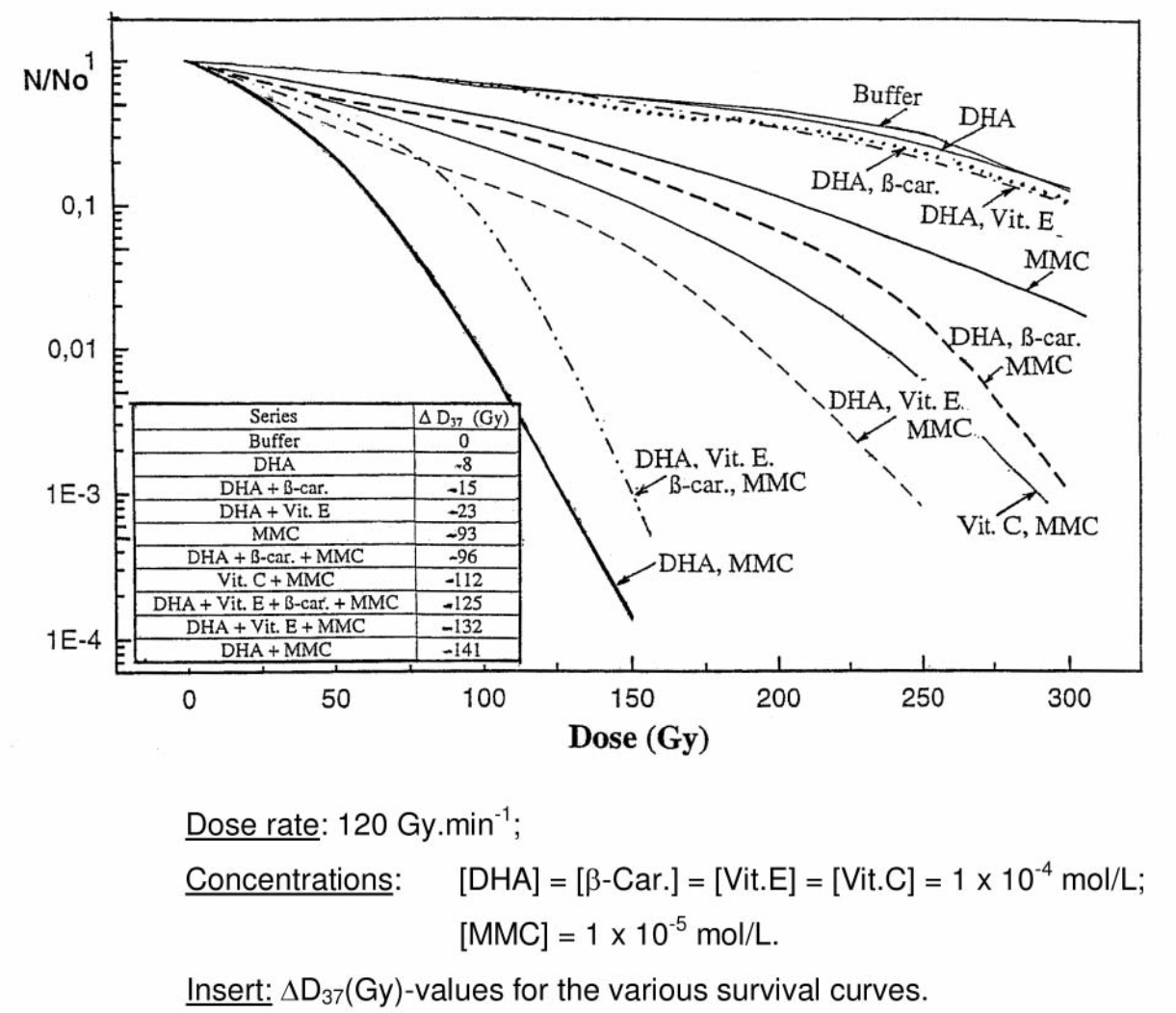


Figure 4. *Synergistic effect of DHA and various mixtures of DHA with β-Car., Vit.E and Vit.C on MMC-efficiency in the presence of air at pH=7.4. The presented survival curves: N/N0-ratio as a function of absorbed radiation dose (Gy) were obtained using Escherichia coli (AB 1157). Dose rate: 120 Gy.min^{-1}; Concentrations: [DHA]=[β-Car.]=[Vit.E]=[Vit.C]=1×10^{-4} mol/L; [MMC]=1×10^{-5} mol/L. Insert: ΔD37(Gy)-values for the various survival curves.*

Vitamin B1 (thiamine). Depending on the environment and on the kind of cytostatic agent, vitamin B1 (vit.B1; 3-(4'-amino-2'-methyl-pyrimidin-5'-ylmethyl)-5-(hydroxyethyl)-4-methylthiazolium) can act in different ways: as protecting agent against the attack of OH and $O_2^{\bullet-}$ radicals or/and it acts synergistically on agents, such as sanazole (Sz) (34, 35) and mitomycin C (MMC) (36). This has been shown by experiments *in vitro*, using *Escherichia coli* (AB 1157; pH=7.4). Some experimental data (ΔD_{37}-values) are given in Table IV.

The involved reaction mechanisms are not yet well understood, however, very likely the observed effects of vit. B1 are based on its radical scavenging ability and on electron transfer to the reaction partner.

Vitamin B2 (riboflavin). Vitamin B2 (vit.B12; riboflavin; 7,8-dimethyl-10(1'-D-ribityl)-isoalloxazine) acts as a reversible redox-system in enzymes. This is based on its ability to eject electrons (e_{aq}^--formation) from the singlet excited states (19). On the other hand riboflavin (RF) is a very potent scavenger of primary free radicals: k(e_{aq}^-+RF)=2.3×10^{10} L.mol^{-1}s^{-1} (pH=5.9), k(OH+RF)=1.2×10^{10} L.mol^{-1}s^{-1} (pH=1-11) and k(H+RF)=3.1×10^9 L.mol^{-1}s^{-1} (37). Based on these facts vitB2 functions as a strong radiation protection agent. In addition to this it has also been found to act synergistically to mitomycin C (MMC) in airfree media (38). For illustration of these biological properties some ΔD_{37}-values, obtained by experiments *in vitro* (model: *Escherichia coli*, AB 1157), are presented in Table V.

Table IV. *Vitamin B1 (Thiamine): ΔD_{37}-values*) derived from survival curves (N/N_0-ratio) as function of radiation dose in (Gy) using Escherichia coli (AB 1157) in the presence of Vit.B1 as well as in mixtures with sanazole or mitomycin C in aqueous media (pH=7.4).*

Vitamin B1 and additives	ΔD_{37}(Gy)			Ref.
	Airfree (44% e_{aq}^-, 10% H, 46% OH)	Air (46% OH, 54% $O_2^{\bullet -}$)	N_2O (90% OH, 10% H)	
Vitamin B1 (Vit.B1)(10^{-4} mol/L)	–151	+14	+110	34
Sanazole (Sz)(5×10^{-5} mol/L)	–123	–46	–120	
Vit.B1 + Sz	–151	–82	–142	
Sanazole (Sz)(5×10^{-5} mol/L)*)	–55	–75	–95	35
Sz + Vit.B1 + C	–110	–120	–170	
Sz + Vit.B1 + C + E + β-Car. [Vit.]=10^{-4} mol/L for each one	–140	–50	+110	
Mitomycin C (MMC) (10^{-6} mol/L)	–72	–34	–168	36
MMC + Vit.B1	–148	–132	–80	
MMC Vit.B1 + C	–34	–16	–67	
MMC Vit.B1 + C + E + β-Car. [Vit.]=10^{-4} mol/L for each one	–127	–87	–217	

*The absorbed radiation dose ranged up to 300 Gy in order to get the ΔD37(Gy)-values. They are relative, hence each series of experiments is treated individually.

Table V. *Some ΔD_{37}-values showing: (A) synergistic action of vitamin B2 to MMC in airfree media and (B) radiation protection ability in oxygenated aqueous solution, respectively (38).*

System	ΔD_{37}-values (Gy)	
	Airfree 44% e_{aq}^-, 10% H, 46% OH	Air 44% OH, 54% $O_2^{\bullet -}$
Vit.B2	---	+40
Vit.C	---	+60
MMC	–60	–20
Vit.B2+MMC	–95	+15
Vit.B2+MMC+Vit.C	---	+80

Aqueous solutions: [Vit. B2]=1×10^{-5} mol/L; [Vit.C]=1×10^{-4} mol/L; [MMC]=3×10^{-7} mol/L ; pH~7.4

Table VI. *ΔD_{37}-values (Gy) resulting from experiments in vitro in aqueous media (pH=7.4) with vitamin B3 (NA) in the presence of air.*

System*)	ΔD_{37}-values
NA	–30
NA+β-car	–50
NA+Vit.E	–65
NA+Vit.C	–105
NA+(C,E, β-car)	–120

*)[NA]=[β-car]=[Vit.E]=[Vit.C]=1×10^{-4} mol/L

The increase of the MMC-efficiency in airfree media from ΔD_{37}=–60 to –95 in the presence of vit. B2 is based on electron transfer from the vitamin to the cytostatic agent. The very strong antioxidant ability of vit. B2 predominates even in air containing solution. The reaction mechanisms are not yet completely elucidated.

Vitamin B3 (nicotinamide). Vitamin B3 (vit.B3; nicotinamide; niacinamide; pyridine-3-carboxamide) has versatile biological functions. It is an efficient scavenger for reducing as well as for oxidizing free radicals: $k(e_{aq}^-+NA)=2.4\times10^{10}$ L.mol^{-1}s^{-1} and $k(OH+NA)=1.5\times10^{9}$ L.mol^{-1}s^{-1} (37). Pulse radiolysis studies of nicotinic acid (39) as well as of carboxy, carbamido and other derivatives of pyridine (40) contributed to the understanding of their reactions with the primary radicals.

Vit. B3 is also involved in the redox processes of coenzyme couple NAD^+ (nicotinamide-adenine dinucleotide)-$NADP^+$ (nicotinamide-adenine-dinucleotide phosphate). These actions are associated with the reversible acceptance of one electron by reducing of the pyrene ring and is the basis for its radiosensitizing ability (41, 42). Thereby vit.B3 has a rather low toxicity in comparison to other sensitizers.

In addition to the above properties it has been shown that the antitumor action of vitamin B3 in aqueous solution is strongly increased in the presence of the antioxidant vitamins: C, E and β-carotene (43). Some data, derived from experiments *in vitro* (*Escherichia coli,* AB 1157) in aerated aqueous media (pH=7.4) containing nicotinamide (NA) and the antioxidant vitamins under the influence of γ-ray, are presented in Table VI. The irradiation is used in order to generate the primary free radicals.

The anticancer action of vit. B3 is efficiently enhanced in the presence of each antioxidant vitamin, which under the influence of radiation transfer an electron to nicotinamide.

Table VII. *ΔD(5 Gy)-values show the N/N_0 ratio of MCF-7 cells observed for vitamin B4 in different media (pH=7.4) after absorption of radiation dose of 5 Gy (for detail see text). The negative ΔD(5 Gy)-values express the antitumor effect of the system and the positive ones, the radiation protection, respectively.*

System*)	ΔD(5 Gy)			
	Air 44% OH, 54% $O_2^{\bullet-}$	N_2O 90% OH, 10% H	Airfree 44% e_{aq}^-, 10% H, 46% OH	Airfree (1 mmol formate) 100% e_{aq}^-
2.5 μmol A	---	---	---	+25
50 μmol A	---	---	---	+36
2.5 μmol MMC	–20	–36	–46	–70
2.5 μmol MMC 2 μmol A	- 26	–40	–41	–43
2.5 μmol MMC 50 μmol A	- 35	–44	–54	–53

*)MM =mitomycin C; A=adenine; 1 Gy=100 rad = 6.24×10^{15} eV/ml absorbed energy.

By joint use of vit. C, E and β-car a 4-fold increase of the ΔD_{37}-value is observed (Table VI). The involved reaction mechanisms are complicated. In addition to electron transfer processes from the antioxidant vitamins to NA, the generated OH and $O_2^{\bullet-}$ oxidizing radicals are participating in the process. The involvement of the produced NA-transients in the subsequent mechanisms cannot be excluded.

Vitamin B4 (adenine). Vitamin B4 (vit.B4; adenine; 3,6-dihydro-aminopurine) forms, along with β-D-ribose, the nucleoside adenosine. In combination with phosphoric acid it represents the low molecular nucleotide, the adenosine-5-phosphate, which appears as a component of DNA and of various co-enzymes.

It has been reported, that the application of adenosine-5'-monophosphate (AMP) or of adenosine-5'-triphosphate (ATP) inhibits the growth of tumors (44, 45). Recently, it has been established by experiments *in vitro* (model: breast cancer cells, MCF-7 line) in aqueous media (pH=7.4), that depending on vit.B4 concentration and the type of attacking free radicals it can act as an efficient radiation protecting agent and inhibit cytostatic properties as well as increase the cytostatic efficiency of mitomycin C (MMC) (46). The radiation protecting ability of adenine (A) can be explained by the very high rate constants for its reaction with the primary free radicals: $k(e_{aq}^-+A)=9\times10^9$ $L.mol^{-1}s^{-1}$, $k(OH+A)=6.1\times10^9$ $L.mol^{-1}s^{-1}$ and $k(H+A)=1\times10^8$ $L.mol^{-1}s^{-1}$ (37).

Some recent experimental data (Table VII), obtained from experiments *in vitro* (46) in the absence and presence of MMC in different media (pH=7.4) under irradiation with γ-ray, characterize the many-sided biological properties of adenine. As a mark for comparison, ΔD(5 Gy)-value was chosen: $\Delta D(5\ Gy)=N/N_0$ (5Gy, sample)-N/N_0(5 Gy, buffer), where: N_0=number of cells before irradiation, N=cell number after absorption of 5 Gy radiation dose.

It should be mentioned that in the presence of formate the OH and H radicals are converted into $^{\bullet}COO^-$-radical, which can transfer one electron to MMC as well as to adenine (A) (see conversion reactions in Figure 3).

Comparing the reactivity of MMC with e_{aq}^- ($k=2.2\times10^{10}$ $L.mol^{-1}s^{-1}$) and that of A ($k=9\times10^9$ $L.mol^{-1}s^{-1}$) (2) it is obvious that there is a competition for e_{aq}^- between MMC and A. The positive ΔD(5Gy)-values (Table VII, last column) are based on the adenine ability to act as a potent radical scavenger. This is also reflected by the reduced MMC-efficiency under these conditions.

The bifunctional biological property of adenine depends on its concentration and on the media composition. The fact that adenine on the one hand acts as scavenger of e_{aq}^-, but also can transfer electrons, makes it an electron mediator. This property may play a role in the electron transfer along DNA. The versatile mechanisms of the vit. B4 involved in various processes is rather complicated and presently not well understood.

Vitamin B5 (pantothenic acid). The vitamin B5 (vit.B5; pantothenic acid; 2,4-dihydroxy-3,3-dimethyl-1-oxobutyl-β-alanine) is an essential part of coenzyme A (CoA) and the acyl carrier protein (ACP). Vit.B5 occurs in all animal and plant tissues, however, the richest source is the jelly of the queen bee and liver. Vit.B5 and its derivatives have versatile biological properties. Among its antioxidant action (47, 48), it exhibits protective ability against ionizing radiation (49, 50) and UV-light (51), which is mainly referred to its reactivity to: OH-radicals, $k=4.5\times10^9$ $L.mol^{-1}.s^{-1}$, and e_{aq}^-, $k=1.2\times10^8$ $L.mol^{-1}.s^{-1}$ (52).

Very recently it has been reported, that in the frame of experiments *in vitro*, vitamin B5 shows an essential antitumor effect (53). In addition to this it exhibits a rather strong synergistic effect on mitomycin C (MMC). These biologically

Table VIII. *ΔD_{37}-values derived from survival curves of experiments in vitro (aqueous media, pH=7.4), showing the action of oxidizing and reducing free radicals on vitamin B5, MMC and mixtures of both (model: Escherichia coli AB 1157) (53).*

System	Concentr. (mol/L)	ΔD_{37} (Gy)			
		Air 44% OH, 54% $O_2^{\bullet-}$	N_2O 90% OH, 10% H	Concentr. (mol/L)	Airfree 44% e_{aq}^-, 10% H, 46% OH
Vit.B5	1×10^{-4}	–60	–45	5×10^{-5}	–70
MMC	5×10^{-6}	–110	–115	3×10^{-7}	–30
Vit.B5+MMC	mixture of both	–150	–165	mixture of both	–130

important properties of vitamin B5 are clearly expressed by comparing the ΔD_{37}- values presented in Table VIII.

The presented ΔD_{37}- values observed in the presence of vit.B5 demonstrate its rather high antitumor effect in all media studied. In addition to this vit.B5 acts with a very strong synergistic effect to MMC, particularly in airfree media.

It should be also mentioned, that the products resulting from the attack of free radicals (OH, $O_2^{\bullet-}$, ea_q^-) on vit.B5 (54) are very likely involved in the observed anticancer effect.

Vitamin B6 (pyridoxine). Vitamin B6 (vit.B6; pyridoxine; 5-hydroxy-6-methyl-3,4-pyridine-dimethanol) shows many biological properties and is present in various foodstuffs (55). It is important as a transaminase coenzyme factor. Vit.B6 in the organism is partly transformed into pyridoxal and pyridoxamine, whereby all three of them are converted irreversibly to their 5'-phosphates (56). It is also a very efficient scavenger for OH-radicals, $k(OH + vit.B6)=6.3\times10^9$ $L.mol^{-1}.s^{-1}$ at pH=7.2 as well as for e_{aq}^-, $k(e_{aq}^-+vit.B6)= 2.2\times10^{10}$ $L.mol^{-1}.s^{-1}$ (37). On the other hand it has been found that vit.B6 exhibits a strongly pronounced antitumor effect under the attack of oxidizing as well as of reducing free radicals (57). It can also act synergistically to cytostatic agents by electron transfer process. A rather high increase of MMC-efficiency was observed in the presence of vit.B6, as shown in Table IX.

The highest synergistic effect of vit.B6 on MMC was registered particularly in airfree media, where the action of reducing radicals (e_{aq}^-, H) dominate (Table IX, airfree media). However, the addition of vit.C to the vit.B/MMC-system caused a strong reduction of the MMC-efficiency. This can be explained by the radical scavenging effect of vit.C, which compete with that of vit.B6. Vit.B6 acts as a relative good antitumor agent, which also is able to increase the MMC-efficiency by electron transfer.

Vitamin B11 (folic acid). Vitamin B11 (vit.B11; folic acid; FA; folacin; N-[[4-[(2-amino-1,4-dihydroxy-4-oxo-6-pteridinyl) methyl]amino]benzoyl]-L-glutamic acid) is classified as a hematopoietic vitamin, although it does not meet all criteria for vitamins (58). It is a compound composed of 6-methylpterin, 4-aminobenzoic acid and glutamic acid.

Table IX. *ΔD_{37}-data derived from survival curves of experiments in vitro (Escherichia coli, AB1157; pH=7.4) in various media, showing the action of oxidizing and reducing free radicals on vitamin B6, MMC and mixture of both (57). The systems were treated with γ-ray to generate the desired free radicals.*

System*)	ΔD_{37}-values (Gy)		
	Air 44% OH, 54% $O_2^{\bullet-}$	N_2O (90% OH, 10% H)	Airfree 44% e_{aq}^-, 10% H, 46% OH
Vit.B6	–25	–90	–95
MMC	–45	–125	–160
Vit.B6 + MMC	–90	–110	–200
Vit.B6 + MMC + Vit.C	–140	+90	–55

*)[MMC]=1×10^{-6} mol/L; [vit.B5]=10^{-5} mol/L; [vit.C]=10^{-5} mol/L.

Vit.B11 is present free or combined with one or more additional molecules of L-glutamic acid in kidney, liver, mushrooms, spinach, yeast, green leaves, grasses *etc*. *In vivo* vit.B11 is converted by several enzymatic processes into a coenzyme form (5,6,7,8-tetrahydrofolic acid; THFA). This catalyzes reactions participating in the protein and nucleic acid metabolism (59). A deficiency of THFA causes a failure of the purines and pyrimidine synthesis. As a result, anemia, and disturbed formation of thrombocytes and granulocytes occurs. The folates also act as acceptors and donors of 1-carbon units (methanol, formaldehyde, formate) in these processes. Folic acid is also a very good scavenger of e_{aq}^- and OH-radicals: $k(e_{aq}^-+FA)=2.2 \times 10^{10}$ $L.mol^{-1}.s^{-1}$, $k(OH + FA)=5.6\times10^9$ $L.mol^{-1}.s^{-1}$ at pH=5.1 (37). These values are in very good agreement with more recently determined values in neutral media, namely: $k(e_{aq}^-+FA)=1.9\times10^{10}$ $L.mol^{-1}.s^{-1}$ and $k(OH + vit.B6)= 1.1\times10^{10}$ $L.mol^{-1}.s^{-1}$ (60).

It might also be mentioned, that vit.B11 is applied for the recovery of patients after methotrexate (MTX) therapy (61, 62), since both compounds, vit.B11 and MTX, have a very similar structure.

In the frame of investigations concerning the antitumor effect of vitamins, it was shown that vit.B11 may exhibits a cytostatic ability. Experiments *in vitro* (model: *Escherichia coli* in various aqueous media, pH=7.4) demonstrated this effect. It depends on the vitamin concentration, pH of the media, presence of air and the kind of the free radicals involved in the process (63). Some ΔD_{37}-data are presented in Table X. For the production of the desired free radicals under given conditions γ-ray were used.

The ΔD_{37}-values in Table X show that the oxidizing (OH, $O_2^{\bullet -}$) as well as the reducing free radicals (e_{aq}^-, H *etc*.) attacking vit.B11 lead to formation of intermediates with anticancer properties. In airfree media, where the reducing free radicals predominate, the antitumor effect is the highest. On the other hand, under the same conditions vit.B11 acts strongly synergistically to MMC increasing its efficiency by 2.5-fold (63).

Reaction mechanisms. The elucidation of mechanisms concerning the action of vitamins in various processes for prevention and in therapy is of basic importance. The better the understanding of the complicated, interfering processes, the better and more efficient therapy can be applied.

In order to meet these demands more broad comprehensive investigations are necessary. An overall good picture about the subject matter can be achieved on the basis of data, obtained by various research methods. The following combination of procedures appear to be rather promising: Experiments "*in vitro*" or/and "*in vivo*", which deliver very valuable information about the biological efficiency of anticancer drugs; Chemical analysis of products originating from the implemented medicaments. In most cases the products initiate undesired side-effects, or sometimes they can also act synergistically. The products resulting by attack of free radicals generated in the human organism can be simulated by using radiation (γ-ray, high energy electrons *etc*.) under specific conditions. This pathway has been implemented by the studies of products of vitamins, *e.g*. of vit.B4 (64), vit.B5 (54), vit.B11 (63).

Pulse radiolysis. This technique makes possible the determination of the spectroscopic and kinetic characteristics of free radicals (65-68). It can also essentially contribute to get a deeper insight on very fast biological processes. An example is the intramolecular electron transfer processes observed for the first time as a result of the OH-attack on vit. B11 (folic acid) molecule (60). The OH-radical can principally react by addition on double bonds (formation of OH-adducts) or abstract a H-atom from *e.g*. $–NH_2$ and –NH-groups. Because the OH radicals are strongly electrophilic, they can exclusively react with the negative charged sites of the molecule abstracting one electron. The electronic stability of the first formed transient is based on the preceding negatively charge force of the corresponding molecule site. Hence, vit.B11 transients with different electronic stability (life times) are produced: some of them with lower stability (very short lived) and others with more stable electronic structure (longer lived). Therefore, the short-lived radicals are tending to convert into such with more stable electronic structure by intramolecular electron transfer, which is registered by kinetic measurements (Figure 5). These processes are in competition with *e.g*. concerning reactions with adjacent molecules, hydrolysis, disproportion, leading to the formation of various products. Similar reaction mechanisms initiated by OH-attack on procarbacine were observed (68). In this case the pulse radiolysis data and computer simulation studies were used to resolve the superimposed absorption spectra of produced transients.

Table X. *ΔD_{37}-values of vitamin B11 calculated from the course of survival curves (N/N0-ratio as a function of absorbed radiation dose, Gy) using Escherichia coli (AB1157) in different media (63).*

System (mol/L)	ΔD_{37}(Gy)-values		
	Air (46% OH, 54% $O_2^{\bullet -}$)	N_2O (90% OH, 10% H)	Airfree (44% e_{aq}^-, 10% H, 46% OH)
10^{-4} Vit.B11	–40	–60	–90
10^{-3} Vit.B11	–60	–80	–160
7.5×10^{-7} MMC	----	----	–80
7.5×10^{-7} MMC + 5×10^{-5} Vit.B11	----	----	–200

Conclusion

The purpose of this brief review is to turn attention of researchers to the effect of oxidizing and reducing free radicals generated in the organism, on their reactivity with vitamins as well as on the antitumor action of the resulting vitamin transients. These rather fast processes proceed permanently; they grasp into each other initiating desired or/and undesired biological processes.

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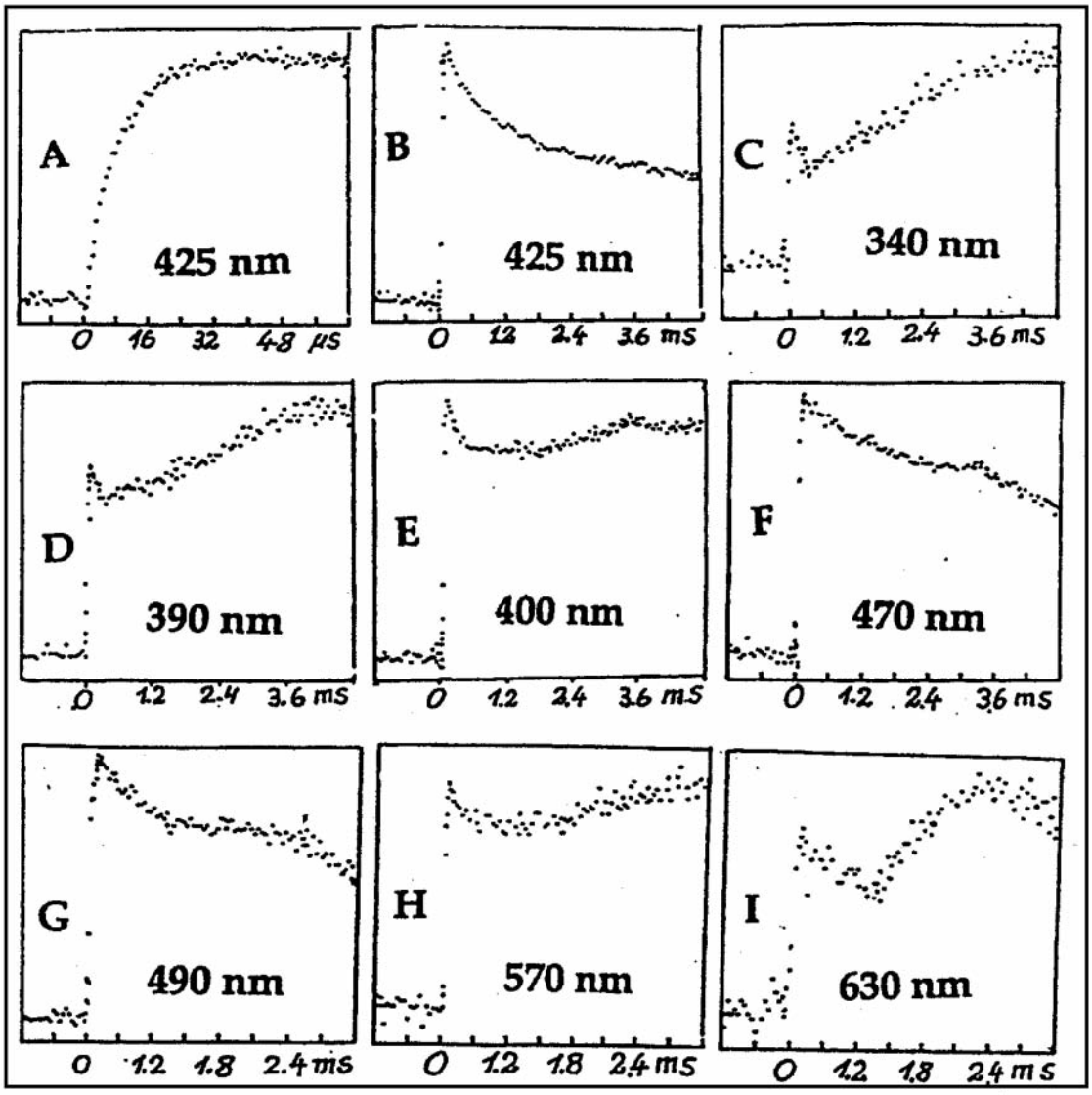

Figure 5. *Kinetic profiles registered by OH attack on 1×10^{-5} mol/L FA in aqueous solutions saturated with N_2O (pH 7.8). (A) Formation and (B) decay of FA radicals at 425 nm. (C-I) Kinetic traces showing the transformation of FA by intramolecular electron transfer processes observed at various wavelengths. (Reproduced from "Transient absorption spectra and kinetics of folic acid, vitamin B11 and some kinetic data of folic acid and methotrexate", Oncology Res 15: 295-300, 2005; with permission of the Journal) (13).*

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