Int J of Cancer Prevention (2007)
Volume 3, Issue 3, pp.
ISSN: 1554-1134

Metal Ions Mediated Pro-Oxidative Reactions with Vitamin C: Possible Implications for Treatment of Different Malignancies

John G. Ionescu[*1,2] and Borut Poljsak[3]
[1] Spezialklinik Neukirchen, Neukirchen Germany
[2] Dept. of Medical Nutrition, Donau University Krems, Austria
[3] Laboratory for ageing process research, Chair of Environmental Health, Faculty of Health Studies, University of Ljubljana, Slovenia

Abstract

Vitamin C is an acidic molecule with strong reducing activity. It is an essential micronutrient in man, due to the absence of L-gulonolactone oxidase. Vitamin C has several important roles and there are many enzymes utilizing ascorbate as a co-factor. Besides, vitamin C protects human health by scavenging toxic free radicals and other reactive oxygen species (ROS) formed in cell metabolism. On the other side, it is well established by *in vitro* experiments that vitamin C is reactive with free iron and other transition metals and produces free radicals, while causing oxidative damage to biomolecules. The interaction of ascorbic acid with transition metal ions could promote their reduction, accompanied by increased H_2O_2 production and consequently $OH^{\bullet}$ formation. There is still debate on whether supplements of vitamin C could act as antioxidant or pro-oxidant *in vivo*. Recent research suggests that 3 factors are responsible for the pro- or antioxidant behaviour of vitamin C in biological systems, e.g. cellular environment:1.) the redox potential of the cellular environment (oxidosis/redosis), 2.) the presence or absence of transition metals and 3.) the local concentration of ascorbate. This may also explain the observed quite specific pro-oxidant activity of high dose intravenous vitamin C against metal rich malignant tumours. In this paper anti- and pro- oxidant effects of vitamin C will be presented and their potential impact on cancer prevention and treatment will be discussed.

1. Introduction

Natural antioxidants are generally considered to be beneficial fruit and vegetable components. Vitamin C is present in almost all foods of plant origin. It is an essential micronutrient in man, due to the absence of L-gulonolactone oxidase. Vitamin C has several important roles and there are many enzymes utilising ascorbate as a co-factor. The term vitamin C refers to both ascorbic acid (AA) and dehydroascorbic acid

[*] E-mail: John.Ionescu@gmx.com

(DHA), since both exhibit anti-scorbutic activity. Ascorbic acid, the functional and primary *in vivo* form of the vitamin, is the enolic form of an α-ketolactone (2,3-didehydro-L-threo-hexano-1,4-lactone). The two enolic hydrogen atoms give the compound its acidic character and provide electrons for its function as a reductant and antioxidant. Its one-electron oxidation product, the ascorbyl radical, readily dismutates to ascorbate and DHA, the two-electron oxidation products. Both the ascorbyl radical and DHA are readily reduced back to ascorbic acid *in vivo*. Because of its ability to donate electrons, ascorbic acid is an effective antioxidant. The vitamin readily scavenges reactive oxygen species (ROS) and reactive nitrogen species (RNS) (e.g., hydroxyl, superoxide, singlet oxygen, and peroxynitrite, nitroxide radicals, respectively) as well as peroxyl and hypochlorite (Frei et al., 1989; Halliwell and Whiteman 1997). The one- and two-electron oxidation products of ascorbate are relatively nontoxic and easily regenerated by the ubiquitous reductants glutathione and NADH or NADPH. Both the one- and the two-electron oxidation products of the vitamin are readily regenerated *in vivo* —chemically and enzymatically— by reduced glutathione, nicotinamide adenine dinucleotide (NADH), and nicotinamide adenine dinucleotide phosphate (NADPH) dependent reductases (May et al. 1998; Park and Levine 1996). In addition to scavenging reactive oxygen species and reactive nitrogen species, vitamin C can regenerate other small molecule antioxidants, such as α-tocopherol, glutathione (GSH), urate, and β-carotene, from their respective radical species (Halliwell 1996). Many cells possess enzymes that can convert dehydroascorbate or ascorbate radical back to ascorbate at the expense of GSH or NADH. Glutathione dependent dehydroascorbate reductase enzymes have been identified in plants and in several mammalian tissues. Evidence that GSH and ascorbate interact *in vivo* is provided by studies on animals treated with the inhibitors of GSH synthesis (Halliwell and Gutteridge 1999). Severe glutathione depletion in newborn rats is lethal, but death can be prevented by high doses of ascorbate (but not DHA).

1.1. ROS Formation in Biological Systems and the Role of Metal Ions at Their Formation

With the exception of unusual circumstances such as the influence of ionizing radiation, free radicals are generally produced in cells by electron transfer reactions (Scheme 1). These can be mediated by the action of enzymes, or non-enzymatically, often through redox chemistry of metal ions (Halliwell and Gutteridge 1999). Free radical production in cells can be either accidental or deliberate (Halliwell and Gutteridge 1999).

All transition metals, with the exception of copper contain one electron in their outermost shell and can be considered free radicals. Copper has a full outer shell, but loses and gains electrons very easily making itself a free radical (Halliwell and Gutteridge 1985). In addition iron has the ability to gain and lose electrons (i.e. $Fe^{2+} \leftrightarrow Fe^{3+}$) very easily.

This property makes iron and copper two common catalysts of oxidation reactions. Iron is major component of red blood cells. Many metal ions are essential for normal cellular metabolism. However, if they are free in the cell and higher concentrations this can increase oxidative stress. The transformation of less reactive intermediates into highly reactive forms needs the participation of free metal ions. Oxidative state and bioavailability of redox active metals are the key determinants of their possibility to form ROS. The reduced forms of metal ions are involved in Fenton reaction where $OH^{\cdot}$ radicals are produced. The oxidative forms participate in Haber-Weiss reaction where reduced forms of metal ions are generated which can again re-enter Fenton reaction (Reactions 6 and 7).

It is clear that any increase in the levels of superoxide anion, hydrogen peroxide or the redox active metals (e.g. Fe, Cu, Cr) are likely to lead to the formation of high levels of hydroxyl radical by chemical mechanisms listed below. Therefore, the valence state and bioavailability of redox active metal is a key determinant in its ability to participate in the generation of reactive oxygen species.

$O_2 + e^- \rightarrow O_2^{\bullet -}$ superoxide anion (1)

$O_2^{\bullet -} + e^- + 2H^+ \rightarrow H_2O_2$ hydrogen peroxide (2)

$H_2O_2 + e^- + H^+ \rightarrow H_2O + OH^{\bullet}$ hydroxyl radical (3)

$OH^{\bullet} + e^- + H^+ \rightarrow H_2O$ water (4)

Sum:

$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$ (5)

Scheme 1. The step-wise reduction of molecular oxygen via one electron transfers, producing and also consuming the ROS molecules.

Due to its strong reactivity with biomolecules, $OH^{\cdot}$ is probably capable of doing more damage to biological systems than any other ROS (Halliwell 1987). The radical is formed from H_2O_2 in a reaction catalyzed by metal ions (e.g. Cr(VI), Fe(III)), often found in complexes with different proteins or other molecules (Nordberg and Arner 2001). This is known as the Fenton reaction:

$$Metal^{(n)} + H_2O_2 \rightarrow Metal^{(n+1)} + HO^{\cdot} + HO^-$$

(Fenton reaction) (6)

$$H_2O_2 + {}^{\bullet}O_2^- \xrightarrow{Metal^{(n+1)}} O_2 + HO^{\bullet} + HO^-$$

(Haber-Weiss reaction) (7)

In the presence of ascorbic acid transition metals play an important role in the formation of superoxide radicals:

$$Metal^{(n+1)} + O_2/AA^- \rightarrow Metal^{(n)} + O_2^{-\bullet}/AA^{\bullet} \quad (8)$$

AA^- = ascorbic acid ion; $AA^{\bullet}$= ascorbic acid radical; see also Figure 1.

1.2. Metal Ions and Malignant Tumours

Increased levels of transition metals like iron, nickel, chromium, copper and lead are closely related to free radical generation, lipid peroxidation, formation of DNA-strand breaks, and tumour growth in cellular systems. Reports in the last two decades are closely relating the presence of transition metals, such as iron or copper to free radical generation via Fenton/Haber-Weiss-reactions, ascorbate autooxidation, lipid peroxidation processes, and the formation of DNA strand breaks (Aust et al. 1985; Mello and Meneghibi 1984; Minotti and Aust 1987; Scarpa et al. 1983). In turn, lipid peroxidation-induced malondialdehyde-DNA adducts can accumulate and reach high levels in the breast tissue of women with breast cancer leading to endogenous DNA modifications (Wang et al. 1996). Furthermore, ferric-EDDA- and –NTAcomplexes have been proven to induce free radicals and renal carcinomas in Wistar rats demonstrating the key role of transition metals in the abnormal proliferation process (Liu and Okada 1996; Okada 1996).

In a previous study (Ionescu *et al.* 2006) the accumulation of heavy metals in 8 healthy and 20 breast cancer biopsies by means of a standardized Atomic Absorption Spectrophotometry (AAS) methodology was investigated in order to determine the correlation to malignant growth in humans. A highly significant accumulation of iron ($p < 0.0001$), nickel ($p < 0.00005$), chromium ($p < 0.00005$), zinc ($p < 0.00001$), cadmium ($p < 0.005$), mercury ($p < 0.005$), and lead ($p < 0.05$) was recorded in the cancer samples when compared to the control group. Copper and silver showed no significant differences to the control group whereas tin, gold, and palladium were not detectable in any biopsies.

The higher heavy metal concentrations encountered in various tumor cells may be used for therapeutic interventions with ascorbic acid as already reported (Baader 1994, Ionescu 2005a, 2005b, Lode 1994). Reduction and mobilization of transition metals from their storage or transport proteins renders them extremely reactive in catalyzing free radical reactions according to the equations above (6-8), thus leading to apoptosis in tumor cells.

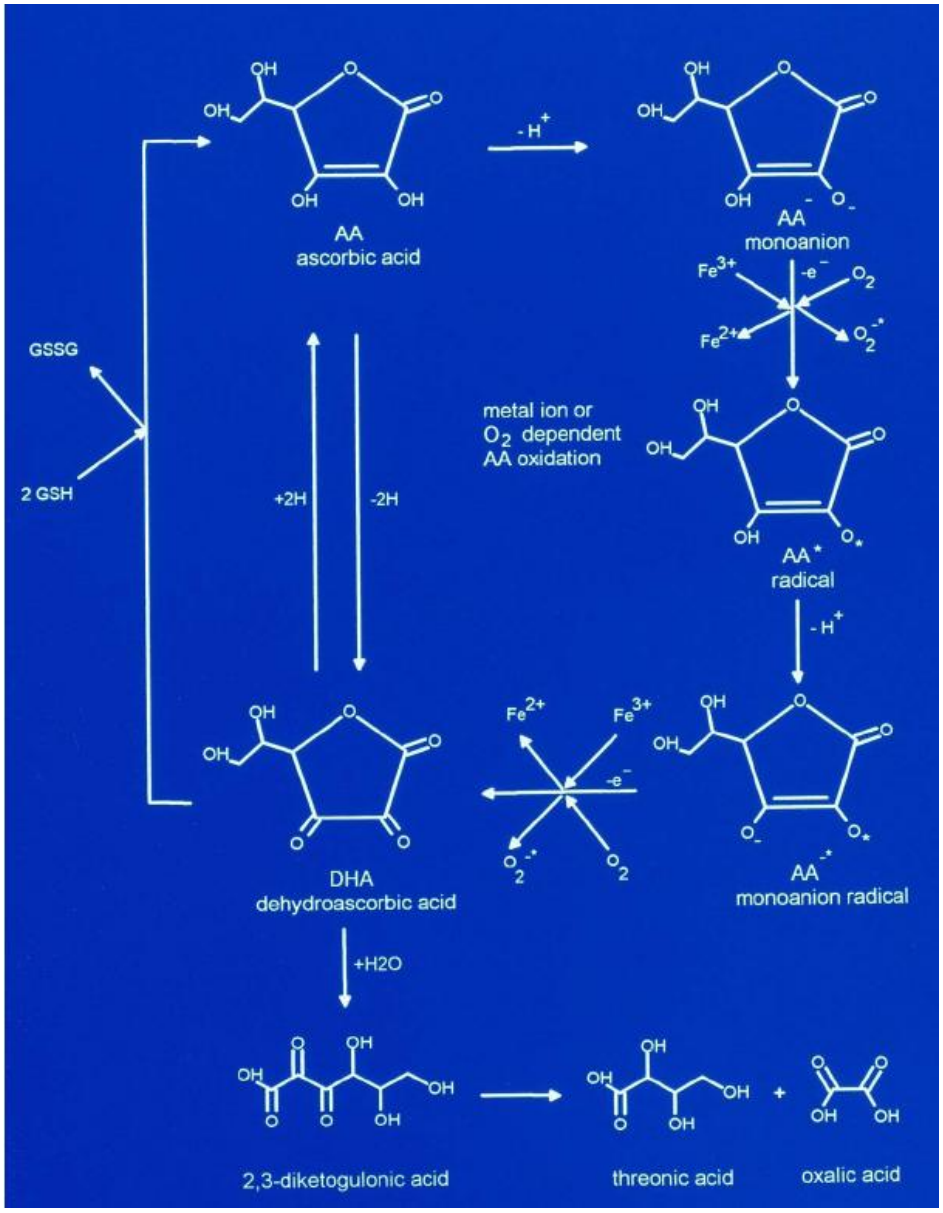

Figure 1. Ascorbic acid redox cycle and catabolic pathways.

2.1. The In Vitro Evidence for an Antioxidative Role of Vitamin C

At physiological concentrations, vitamin C is a potent free radical scavenger in plasma, protecting cells against oxidative damage caused by ROS (Carr and Frei 1999). The antioxidant property of ascorbic acid is attributed to its ability to reduce potentially damaging ROS, and forming a relatively stable ascorbyl free radical. Ascorbate has the ability to act as a reducing agent. One electron donated by ascorbate gives ascorbyl radical, also named monodehydroascorbate (MDHA) or semi dehydroascorbate (SDA). It can be further oxidized to give dehydroascorbate (DHA). DHA is unstable and breaks down rapidly, producing diketo-L-gulonic acid which breakes down to oxalic and L-threonic acid (see Figure 1, Ascorbic acid redox cycle). At physiological pH the acid form is largely ionized

(ascorbate) since the pKa_1 of ascorbic acid is 4.25 (Halliwell and Gutteridge 1999).

Basic intracellular reaction in which ascorbic acid and ROS are involved (McKersie 1996):

$$2^{\bullet}O_2^- + 2H^+ + \text{ascorbate} \rightarrow 2\,H_2O_2 + \text{+ dehydroascorbate}^{\cdot}$$

$$H_2O_2 + 2\ \text{ascorbate} \rightarrow 2H_2O + \text{+2 monodehydroascorbate}^{\cdot}$$

The indirect role of the ascorbate as an antioxidant is to regenerate membrane-bound antioxidants, such as alpha-tocopherol that scavenge peroxyl radicals and singlet oxygen, respectively:

Tocopheroxyl radical + ascorbate →
tocopherol + + monodehydroascorbate

The above reactions indicate that there are two different products of ascorbate oxidation: monodehydroascorbate and dehydroascorbate which represent one and two electron transfers, respectively. The monodehydroascorbate can either dismutate spontaneously, or is reduced to ascorbate by NAD(P)H monodehydroascorbate reductase:

2 monodehydroascorbate → ascorbate +
+dehydroascorbate

monodehydroascorbate + NAD(P)H → ascorbate + NAD(P)

The dehydroascorbate is unstable at pH greater than 6 and decomposes to tartrate and oxalate (McKersie 1996). To prevent this, dehydroascorbate is rapidly reduced to ascorbate (Figure 1) by dehydroascorbate reductase, using reducing equivalents from glutathione:

2 GSH + dehydroascorbate → GSSG + ascorbate

In vitro tests performed under physiological conditions show a better viability of ascorbic acid pretreated cells, which might be the consequence of ascorbic acid prevention of oxidant-induced apoptosis (Deutsch 1998). In the absence of added metal ions, however, vitamin C inhibits the formation of 8-oxodG in purified DNA exposed to peroxynitrite or UV light (Hu and Shih 1997¸ Fiala et al. 1996). Also the study of Panayiotidis et al. (1997) has shown reduced strand breakage, as determined by the comet assay in lymphocytes. Results of cytotoxicity tests in *S. cerevisiae* cells pretreated with ascorbic acid and subsequently treated with Cr(VI) indicate a preventive effect of ascorbic acid (Poljsak et al. 2005) regarding intracellular oxidation. These results are in agreement with those of Blankenship et al. (1997) on CHO cells.

When sufficient exogenous iron (as ferrous ammonium sulfate) is added to plasma to saturate transferrin and result in nonprotein-bound, bleomycin- detectable iron (BDI), endogenous and exogenous vitamin C inhibits rather than promotes lipid peroxidation (Berger et al. 1997).

Overall, *in vitro* studies have shown that vitamin C either has no effect (Dabbagh and Frei, 1995) or inhibits (Berger et al. 1997; Dasgupta and Zdunek 1992) metal ion dependent lipid oxidation in plasma and other biological fluids.

2.2. The In Vitro Evidence for a Pro-Oxidative Role of Vitamin C

Ascorbic acid quenches free radicals by providing hydrogen atoms that can pair up with unpaired electrons on free radicals. In this process ascorbic acid becomes an ascorbyl radical, which is relatively unreactive toward biomolecules (Buettner 1993; Halliwell and Gutteridge 1999). Relatively unreactive means that ascorbyl radical is not reactive enough to cause damage to biomolecules. However, ascorbic acid can also act as a pro-oxidant in the presence of transition metals, depending on the environment in which the molecule is present (Paolini et al. 1999 ; Halliwell 1999). The interaction of superoxide ($^{\bullet}O_2^-$) and ascorbic acid with transition metal ions could promote their reduction, accompanied by increased H_2O_2 production (Halliwell 1999; Clement 2001; Lay and Levina 1998), and consequently $OH^{\cdot}$ formation. The ascorbate acts as reducing agent to iron and other transition metals, easily permitted by their standard redox potentials (Fe^{3+}-ferritin/ ferritin+Fe^{2+}: SRP=-0.19V; $ascorbate^{\cdot -}$,H^+/$ascorbate^-$: SRP=+0.28V) (Halliwell and Gutteridge 1999).

$Fe(III) + ascorbate \rightarrow Fe(II) + ascorbate^{\cdot}$

$Fe(II) + H_2O_2 \rightarrow Fe(III) + OH^{\cdot} + OH^{-}$
(Fenton reaction)

Ascorbic acid reduces Fe(III) to Fe(II) which reduces oxygen to hydroxyl radical (Halliwell and Gutteridge 1999). Cells have a not well characterized pool of low molecular weight iron (Jacobs 1977; Voogd 1992). If these come into contact with ascorbate, pro-oxidant effects may occur. The ability of ascorbic acid to enhance the release of transition metals from protein complexes, and to reduce them to catalytic forms, has implicated this compound to be a prooxidant as well (Dreosti 1991). Iron and copper are essential for cellular life as enzyme cofactors. Thus they could participate in the autooxidation of ascorbic acid. Vitamin C in the presence of high body iron stores, reveals prooxidant properties (Food and Drug Administration 1993; Herbert 1993; Simopoulos et al. 1993.) Vitamin C is especially dangerous in the presence of high body iron stores, which make vitamin C violently prooxidant (Herbert 1993; Simopoulos et al. 1993). The reduction of transition metal ions by ascorbate could also have deleterious effects via the production of hydroxyl radicals or lipid alkoxyl radicals (LO•) by reaction of the reduced metal ions with hydrogen peroxide or lipid hydroperoxides (LOOH) (Halliwell 1996; Buettner and Jurkiewicz 1996). This effect can be prevented if enough anti-oxidants e.g. glutathione are available (Ionescu, 2002) [Figure 2, Figure 3].

However, due to the capacity of metal ions to undergo one-electron transfers, which enables them to become powerful catalysts of autooxidation reactions, cells sequestrate these metal ions into proteins since metal-bound ions are less effective than free-radical catalysts (Halliwell and Gutteridge 1999). Cells must establish fine-tuned mechanisms which allow cells to accumulate sufficient levels of Fe and Cu for normal biochemical reactions, yet prevent the accumulation of these metals to levels which unleash their toxic effects. Many autooxidation reactions within the cell produce superoxide by addition of an electron to molecular oxygen (Anderson and Phillips 1999). It was reported that $O_2^{-\bullet}$ is produced from ascorbate (AH^-) autooxidation by dioxygen (Scarpa 1983), yet is has been shown (Buettner 1993) that aerobic oxidation of ascorbate strictly requires a metal catalyst (Fig. 1). Antioxidants, which are reducing agents, capable of reacting with molecular oxygen (e.g. ascorbic acid) will generate superoxide radicals under aerobic conditions. This will dismutate to H_2O_2 that can enter cells and react with superoxide or reduced metal ions to form highly damaging hydroxyl radicals (Anderson and Phillips 1999). Even though H_2O_2 production from ascorbate and dioxygen is thermodynamically favored, a direct oxidation of ascorbate by dioxygen does not occur. Thus, the spin restriction of dioxygen is a kinetic barrier that prevents the oxidation of organic biomolecules regardless of thermodynamic considerations (Miller et al. 1990). In the absence of transitional metals, the rate constant for the reaction of dioxygen with ascorbate has been reported to be $6x10^{-7}$ s^{-1}, which results in an observed second rate order constant of approximately $2x10^{-3}$ M^{-1} s^{-1}. Mitochondrial respiration keeps $O_2 \approx$ 0-10 μM in the cell while intracellular concentration of GSH ≈ 1 mM (Buettner 1993).

In the study of Poljsak et al. (2005) it was demonstrated that hydroxyl radicals are generated following the interaction of Cr(VI) with ascorbic acid in vitro. This is believed to involve the reduction of the metal ion by ascorbic acid, followed by the reduction of oxygen to H_2O_2/$HO^{\bullet}$. Stearns et al. (1995) found out that the reduction of Cr(VI) by ascorbate under physiological conditions produced Cr(V) and carbon-based radicals as intermediates which reacted with DNA to produce Cr-DNA adducts and DNA single-strand breaks, respectively. Low concentrations of ascorbate enhance oxygen radical activity whilst high concentrations scavenge hydroxyl radicals, singlet oxygen and lipid peroxides.

A study by Anderson et al. (1997) examined DNA damage in lymphocytes with comet assay. Vitamin C supplementation significantly elevated plasma vitamin C concentration, but had no effect on oxidative DNA damage either with or without an ex vivo hydrogen peroxide challenge. However, a statistically significant increase in bleomycin-induced aberrations was found after vitamin C supplementation. In the study of Green et al. (1994) vitamin C acted as a pro-oxidant when added to isolated lymphocytes *in vitro.*

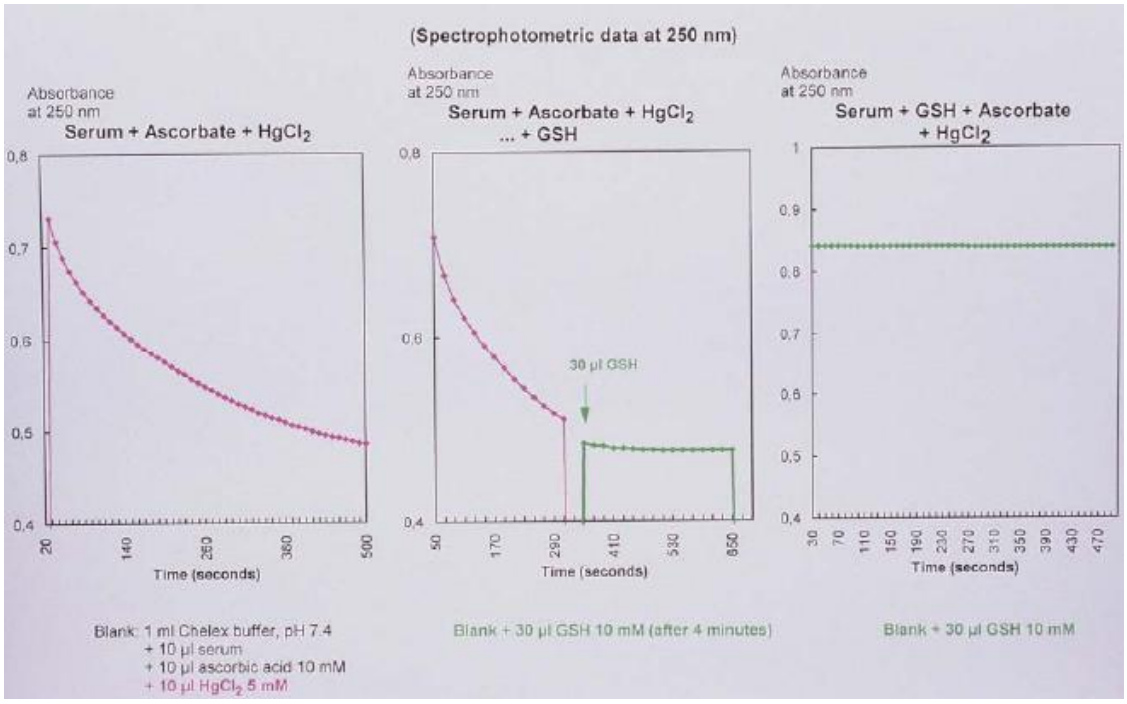

Figure 2. The prevention of mercury II induced ascorbate oxidation by reduced glutathione.

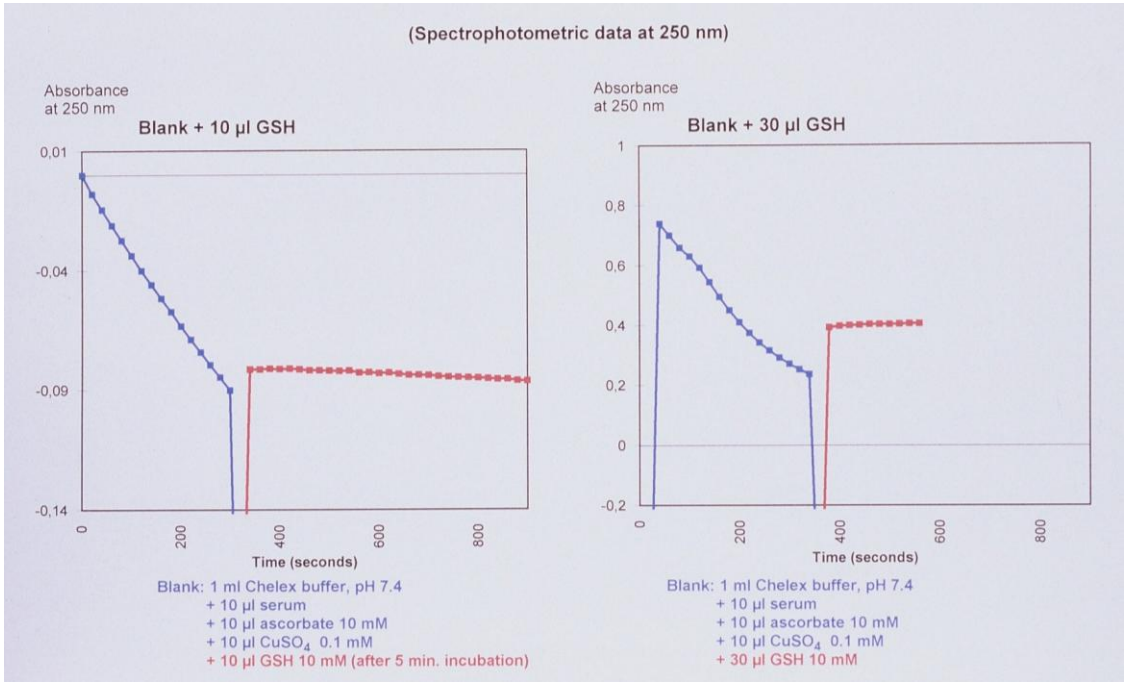

Figure 3. The prevention of copper II-induced ascorbate oxidation by reduced glutathione.

Addition of vitamin C to purified DNA or isolated nuclei in the presence of redox active metal ions in vitro results in single-strand breaks and base modifications such as 8-oxodG (Drouin et al. 1996; Fischer-Nielsen et al.1992, Hu and Shih 1997).The results of Sugiyama et al. (Sugiyama et al. 1991a; Sugiyama 1991b; Sugiyama 1991c) on Chinese hamster V-79 cells showed increased cytotoxicity of

Cr(VI) in ascorbic acid pretreated cells. Addition of Fe^{2+} (but not Fe^{3+}) and H_2O_2 to human serum results in rapid generation of hydroxyl radical (Fenton reaction, Figure. 4). On the other side, addition of ascorbate to Fe^{3+}-loaded serum leads to an immediate increase of superoxide generation, through ascorbate autooxidation (Figure 5) (Ionescu 2002).

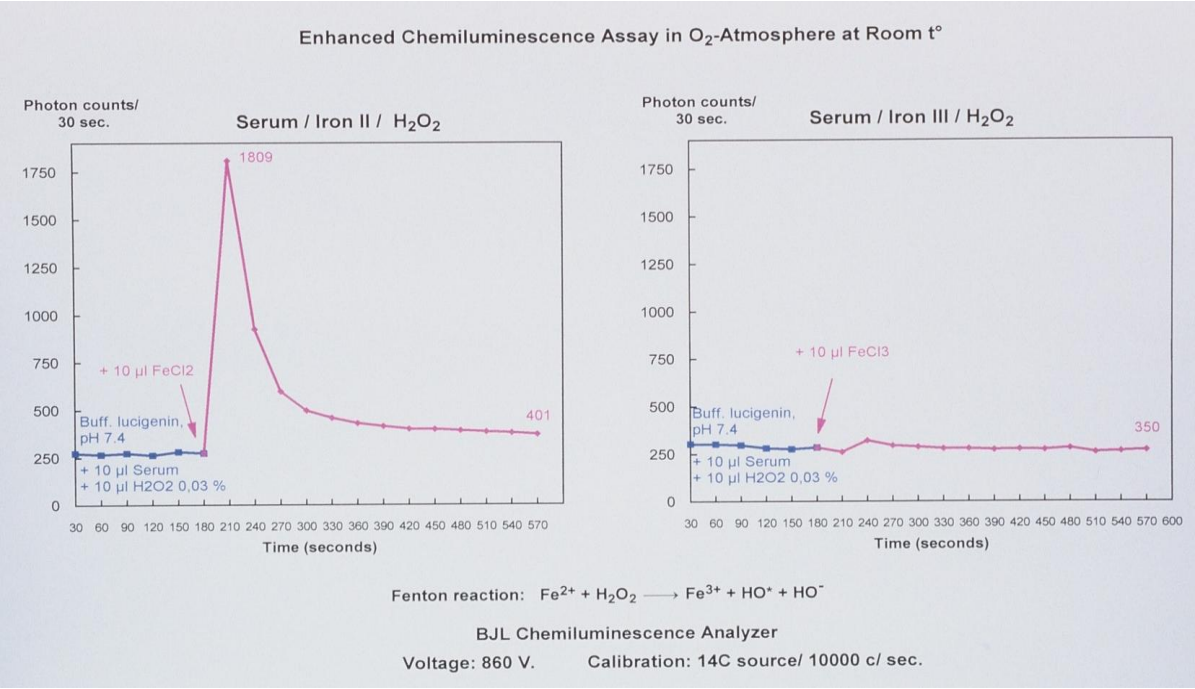

Figure 4. Iron II / Iron III / H_2O_2 dependent free radical generation.

3. The *In Vivo* Evidence for a Prooxidative or an Antioxidative Role of Vitamin C

The human oxidative biomarkers data on the role of vitamin C are controversial and appear inconsistent. Some studies examining different biomarkers after vitamin C treatment showed a vitamin C-dependent reduction in oxidative DNA damage, whereas some studies found either no change or an increase in the levels of selected DNA lesions. Carr and Frei (1999) examined nine human vitamin C supplementation studies, four of them showed a reduction in *ex vivo* or *in vivo* DNA oxidation (Cadenas et al. 1997; Fraga et al. 1991; Lee et al. 1998; Panayiotidis and Collins 1997), whereas two showed no change (Prieme et al. 1997; Anderson et al.1997); another three showed a decrease in some markers and an increase in others (Podmore et al. 1998; Cooke et al. 1998; Rehman et al. 1998). Porkkala-Sarataho et al. (2000) observed that neither vitamin E nor vitamin C, nor the combination influenced the urinary excretion rate of 7-hydro-8-oxo-2-deoxyguanosine. In the study of Podmore and colleagues (1998) the results of supplemented volunteers with 500 mg of vitamin C daily reported 8-oxogua levels were significantly reduced relative to baseline and placebo, whereas the levels of 8-oxoade were significantly elevated. Since 8-oxoade is at least 10 times less mutagenic than 8-oxogua, the authors conclude that the overall effect of ascorbate intake is "profound protective" (Podmore's reply to Levine et al, 1998). On the same line, Vojdani *et al.* (2000) reported in a placebo-controlled study that increasing concentrations of vitamin C administered to humans (500mg, 1000mg and 5000mg per day, respectively) showed no DNA oxidation products, but a decrease of apoptosis and an increase of NK-cell cytotoxic activity.

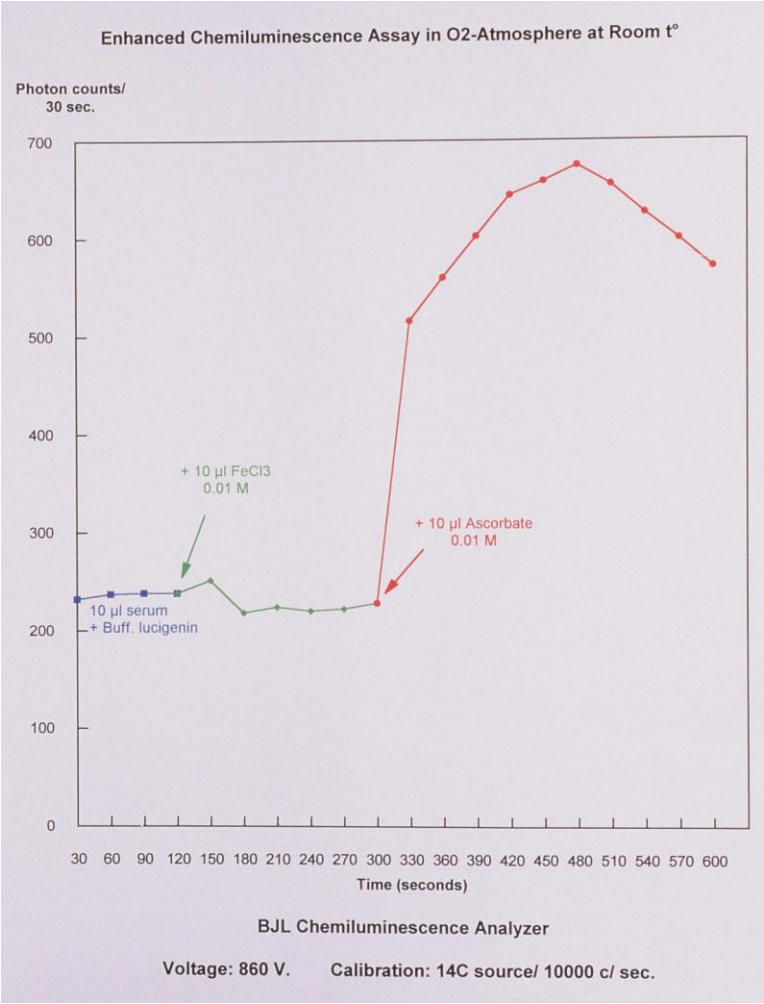

Figure 5. Iron (III) and ascorbate induced free radical activity in serum.

In the study from Rehman et al. (1998) volunteers were supplemented with iron II (ferrous sulphate) and vitamin C and levels of 13 different types of oxidized DNA bases in white blood was monitored. There were no control groups given either iron or vitamin C alone, nor was there a placebo group. Results revealed an inverse correlation between mean plasma vitamin C concentrations and total oxidative DNA damage. This study does not provide compelling evidence for a pro-oxidant effect of vitamin C and iron cosupplementation on DNA damage, but supports an antioxidant effect (Carr and Frei 1999). Inverse correlations of lymphocyte ascorbate and glutathione concentrations with oxidized DNA bases in another study of 105 apparently healthy adults suggest that these two intracellular antioxidants protect human lymphocytes against oxidative damage (Lenton et al. 1999).

Urinary excretion of DNA oxidant damage products, which is thought to represent the balance of total body DNA damage and repair has been investigated. This is a nonspecific measure used to assess changes due to micronutrient status. Except for the study by Cooke et al. (1998), no relationships between vitamin C intake and urinary markers of DNA damage were observed (DRI for vitamin C, Food and Nutrition Board, Institute of Medicine 2000).

The five measured DNA and chromosome damage *ex vivo* after supplementing the subjects with vitamin C were discussed by the Food and Nutrition Board, Institute of Medicine 2000 (DRI for vitamin C). Single large doses of vitamin C (1 g/day or more) provided protection against lymphocyte DNA strand break damage induced *ex vivo* by radiation or hydrogen peroxide (H_2O_2) as measured by the comet

assay (Green et al. 1994; Panayiotidis and Collins 1997). In contrast, Crott and Fenech (1999) reported that a single 2-g dose of vitamin C neither caused DNA damage nor protected cells against hydrogen peroxide-induced toxicity. Two other studies measured DNA chromosome damage after treatment of lymphocytes with bleomycin, a test for genetic instability. Following vitamin C supplementation for two weeks, Pohl and Reidy (1989) found decreased chromosome breaks and Anderson et al. (1997) reported no effects on DNA damage but increased chromosome aberrations. Since the findings of these studies were inconsistent, *ex vivo* damage cannot be used to estimate a vitamin C requirement (DRI for vitamin C, Food and Nutrition Board, Institute of Medicine 2000).

A study using rats challenged with paraquat showed a protective, antioxidant role for vitamin C when given *before* paraquat treatment, but a pro-oxidant role when given *after* the challenge, as determined by expiratory ethane (Kang et al. 1998). Similar effect was reported by Poljsak et al. (1995) on vitamin C pre-treated yeast cells which were later exposed to chromium.

Another animal study has reported an antioxidant role for vitamin C in guinea pigs co-supplemented with vitamin C and iron. In the study of Collins et al. (1997) autooxidation of liver microsomes obtained from iron-supplemented guinea pigs resulted in increased accumulation of MDA compared with control animals or animals co-supplemented with iron and vitamin C. An important point to note about studies in animals that can synthesize vitamin C, such as rats, is that the results may not reflect the situation in humans. According to Carr and Frei (1999) several vitamin C and iron co-supplementation studies, both in animals and humans, indicate that vitamin C inhibits rather than promotes iron-dependent oxidative damage. Similarly, a study carried out in humans to assess the effects of simultaneous iron(II) and vitamin C supplementation has yielded mixed results with respect to various types of oxidized DNA bases in leukocytes. Reanalysis of the data from this study (Rehman et al. 1998) suggest that vitamin C acts as an antioxidant, rather than a pro-oxidant, *in vivo* in the presence of minute amounts of iron(II) in healthy volunteers (Carr and Frei 1999).

Although vitamin C induced Fenton chemistry occurs readily *in vitro*, its relevance *in vivo* has been a matter of some controversy, the main point of contention being the availability of catalytic metal ions *in vivo* (Halliwell and Gutteridge 1986). It has yet to be proven that oxidative damage *in vivo* can be ameliorated by supplementation with large doses of ascorbic acid. The dose of ascorbate which is protective *in vitro*, may not be relevant *in vivo* (Griffiths 2001). According to Simopoulos (1993), for genetic reasons more than 10% of American whites and perhaps as many as 30% of Afro-Americans have high body iron. Vitamin C is known to increase the gastrointestinal absorption of nonheme iron by reducing it to a form that is more easily absorbed (Bendich and Cohen 1990). Individuals with iron overload generally have low plasma levels of vitamin C, possibly due to interaction with the elevated levels of 'catalytic' iron in these individuals, and therefore vitamin C administration has been proposed to be harmful in these people (Halliwell 1996; Herbert 1994). According to Herbert (1994) for consumer protection, every advertisement and label for vitamin C and/or iron supplements should warn: "Do not take this product until your blood iron status has been determined". Six percent of Americans are in negative iron balance, and this product may help them. Twelve percent of Americans are in positive iron balance and this product may hurt them.

Intravenous administration of large doses of ascorbate (20g) in metal-sensitive atopic eczema patients resulted in a worsening for 24-48 hours of their clinical symptoms, with increased erythema and itching. The simultaneous monitoring of the evolution of free radical generation in whole blood and serum showed a dramatic increase of superoxide and hydrogen peroxide in serum, and a moderate ROS increase in whole blood (Figure 6, Ionescu 2002).

Carr and Frei (1999) analyzed 44 *in vivo* studies done on vitamin C, 38 of them showed a reduction in markers of oxidative DNA, lipid, and protein damage, whereas only 6 showed an increase in oxidative markers. According to Carr and Frei (1999) the answer to the question: "Does vitamin C act as a pro-oxidant under physiological conditions?" appears to be 'no'. However, there is still debate on whether supplements of vitamin C could act as pro-oxidants *in vivo*.

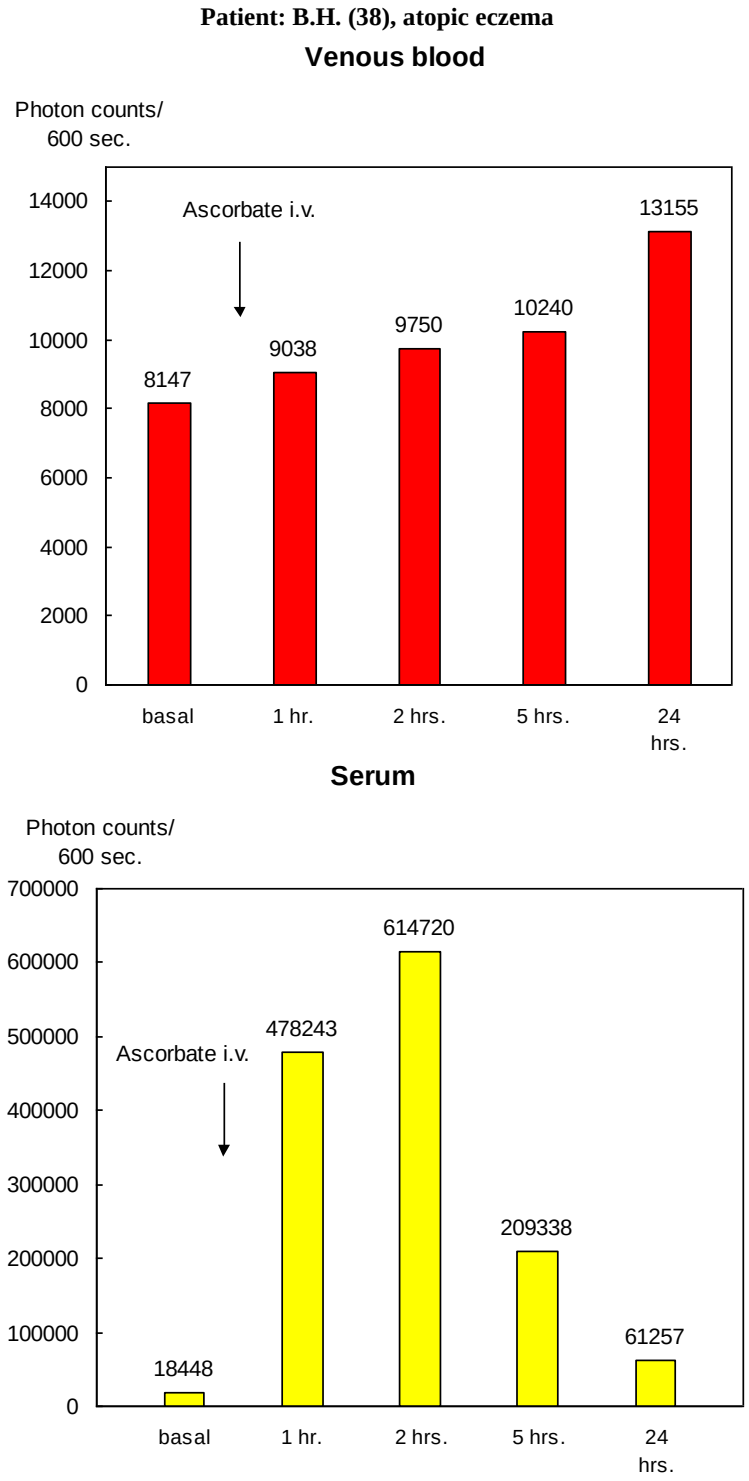

Figure 6. Free radical activity in (a) venous blood and (b) serum before and after 20 g ascorbate i.v.

Vitamin supplements taken by millions of people do not increase life expectancy and some of them, such as beta carotene, vitamin A, and vitamin E may raise the risk of a premature death, according to a recent review of 67 studies with more than 230,000 subjects (Bjelakovic et al. 2007). On the other hand, the same study concludes that "vitamin C and selenium had no significant effect on mortality".

3.1. The Possible Explanation of the Dual Role of Antioxidants vs. Pro-Oxidants

Numerous epidemiological studies have shown an inverse association between vitamin C intake, or plasma status, and the risk to develop different types of cancers (Block 1991; Jenner, et al. 1998). There are several possible explanations for the potential negative effect of antioxidant supplements. Reactive oxygen species in moderate concentrations are essential mediators of defense against unwanted cells. Thus, if administration of antioxidant supplements decreases free radicals, it may interfere with essential defensive mechanisms for ridding the organism of damaged cells, including those that are precancerous and cancerous (Salganik 2001). Thus, antioxidant supplements may actually cause some harm (Vivekananthan et al. 2003; Bjelakovic et al. 2004a; Bjelakovic et al. 2004b; Miller et al. 2005; Bjelakovic et al. 2007; Caraballoso et al. 2003). Our diets typically contain safe levels of vitamins, but high-level antioxidant supplements could potentially upset an important physiologic balance (Vivekananthan et al. 2003; Bjelakovic et al. 2004a; Bjelakovic et al. 2004b; Miller et al. 2005; Bjelakovic et al. 2007; Caraballoso et al. 2003). In the same line, a systematic Review and Meta-analysis done by Bjelakovic et al. (2007) conclude that long-term treatment with beta carotene, vitamin A, and vitamin E may increase mortality. There are still many gaps in our knowledge of the mechanisms of bioavailability, biotransformation, and action of antioxidant supplements.

Selman et al. (2006) suggest different possible explanations regarding the general inability of antioxidants, including vitamin C, in supplementation studies to consistently deliver the promise of increased lifespan in animal models, or reduced disease risk in humans, could have a varied causality (see McCall and Frei, 1999).

(I) Perhaps *in vivo* vitamin C may act more as a pro-oxidant than an antioxidant (Childs et al. 2001; Rehman et al. 1998), possibly necessitating increased activation of the defense system to maintain the status quo.

(II) Alternatively, vitamin C may successfully scavenge ROS (Carr and Frei 1999) but this may not be translated into damage reduction and lifespan enhancement. Vitamin C may negatively affect the endogenous scavenging and repair systems, either directly (Nemoto et al. 1997; Podmore et al. 1998), or indirectly via systems that sense reduced radical production.

The ability of vitamin C to decrease the activity of endogenous antioxidant systems was reported by Selman et al. in 2006. Mice exhibited a significantly reduced expression of several genes in the liver linked to free-radical scavenging, including Mn-superoxide dismutase and glutathione peroxidase in the vitamin C treated group. Authors suggest that high dietary doses of vitamin C are ineffective at prolonging lifespan in mice because any positive benefits derived as an antioxidant are offset by compensatory reductions in endogenous protection mechanisms, leading to no net reduction in accumulated oxidative damage. Carr and Frei (1999) suggested that if tissues are already saturated due to an adequate intake of vitamin C at baseline, subsequent supplementation cannot have an effect on tissue vitamin C levels and thus on oxidative stress biomarkers. Levine and co-workers (1996) investigated the pharmacokinetics of vitamin C and found that in healthy humans, tissue saturation (measured in peripheral blood leukocytes) occurred at vitamin C intakes of ~100 mg/day, which corresponds to a plasma concentration of ~50 mmol/l. On the other hand Zaidi et al. (2005) reported that a decrease of free radical scavenging enzymes such as superoxide dismutase (SOD), glutathione-S-transferase (GST) and catalase, as well as levels of total glutathione (GSH) besides an increase in level of malondialdehyde (MDA) may be effectively compensated by supplementation with vitamin C.

Carr and Frei (1999) pointed out that the ability of vitamin C to act as a pro- or anti-oxidant depends on the moment when the vitamin is added to the system (Kang et al. 1998; Otero et al. 1997). For example, vitamin C acts as an antioxidant if added before initiation of LDL oxidation by copper, but acts as a pro-oxidant if added to LDL that is already (mildly) oxidized (Otero et al. 1997). Since transition-metal ions are liberated from metalloproteins as a primary mechanism of injury by oxidative damage (Halliwell and Gutteridge 1999; Swain et al. 1994;

Kang et al. 1998), administration of a powerful antioxidant (i.e., powerful reducing agent) after oxidative damage has started could promote damage—i.e., be pro-oxidant—and the more powerful the antioxidant is as a reducing agent, the more problems it might cause (Halliwell 2000). As already observed *in vitro* (Ionescu 2002, Figure 2), the autooxidation of supplemented vitamin C in the presence of transition metals also depends on the concentrations of other antioxidants in the system, such as reduced glutathione, NADH, NADPH, vitamin E.

A reason why vitamin C and other antioxidant supplements would be expected to increase lung cancer and mortality in smokers ((The) alpha-tocopherol/beta-carotene cancer prevention (ATBC) study (1994)) is that vitamin C supplements drive nicotine out of the blood into the urine (Herbert et al. 1994), causing smokers to reach for that next cigarette (more carcinogens) much faster in order to sustain their nicotine 'high'.

More studies are warranted in which the effects of vitamin C supplementation on more than one biomarker of oxidative damage are determined. This is particularly important, according to Carr and Frei (1999) because several studies in which more than one oxidative biomarker was measured showed an antioxidant role of vitamin C in respect to lipid oxidation, but not DNA oxidation (Hu and Shih 1997) or protein oxidation (Frei et al. 1989; Frei, et al. 1988; Cross et al. 1993). These discrepancies may be due to the differential ability of the various macromolecules, i.e., DNA, lipids, and proteins, to bind metal ions and the redox activity of the bound metal ions (Halliwell and Gutteridge 1986).

3.2. Redox Balance and Cancer

The consensus opinion has been that five servings of fruits and vegetables containing the above nutrients would reduce the incidence of various cancers (Hwang et al. 1994; National Research Council 1992; Shklar and Schwartz 1994). The ingestion of these foods would provide a wide range of phytochemicals acting as chemopreventives. Hoffer et al. (2008) reported that scientific interest in the interaction between ascorbic acid and cancer has increased in recent years with evidence that in millimolar concentrations—which are attainable only after parenteral administration—it is selectively cytotoxic to many neoplastic cell lines (Bran et al. 1980; Sestili et al. 1996; Chen et al. 2005), potentiates cytotoxic agents (Song et al. 1995; Kurbacher et al. 1996; Kassouf et al. 2006; Grad et al. 2001; Abdel-Latif et al. 2005) and demonstrates anticancer activity alone and in combination with other agents in tumor-bearing rodents (Sarna end Bhola 1993; Verrax et al. 2006; Taper et al. 2004). Simultaneously, theoretical interest has arisen in the potential of redox-active molecules to modify cancer biology (Verrax et al. 2006) especially when administered together with cytotoxic drugs (Tetef et al. 1995; Diaz et al. 2005; Doroshow 2006).

DNA mutation is likely a major contributor to the age-related development of cancer (Deng et al. 1998; Halliwell 2000). Attenuation of oxidative stress induced mutations through vitamin C could provide a potential cancer prevention mechanism (Li and Schellhorn 2007). Paradoxically, ascorbic acid may also function as a prooxidant, promoting oxidative damage to DNA (Stich et al. 1976). This occurs in the presence of free transition metals, such as copper and iron, which are reduced by ascorbate and, in turn, react with hydrogen peroxide, leading to the formation of highly reactive and damaging hydroxyl radicals, *via* the Fenton reaction (Stich et al. 1976). The relevance of such abnormal physiological conditions *in vivo* has been questioned, as most transition metals exist in inactive, protein bound form *in vivo* (Halliwell and Gutteridge 1986). However, ascorbic acid may also display a pro-oxidant activity, which is more profound in cancer cells and causes cell death, when used at pharmacological concentrations (0.3–20 mmol/L, Chen et al. 2005). Increased generation of hydrogen peroxide (by ascorbic acid autooxidation) *in vivo* may be exploited as a means for inducing tumor-specific cytotoxicity (Gonzales et al. 2005). An explanation for this quite specific anticancer activity of vitamin C is provided by recent research reporting highly increased levels of transition metals in malignant tumors (Ionescu et al. 2006; Ionescu 2007a; Yaman et al 2005) (Figure 7-9), leading to *in situ* auto-oxidation of the vitamin and generation of H_2O_2/$HO^{\bullet}$ with apoptosis induction.

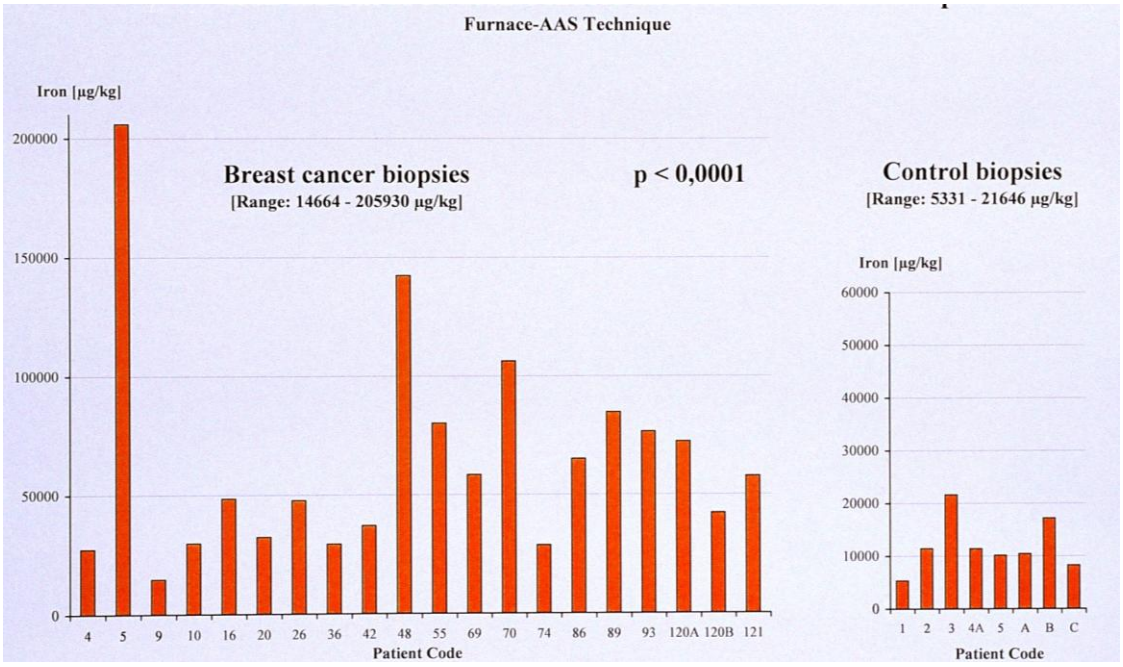

Figure 7. Iron content of 20 breast cancer and 8 control human biopsies.

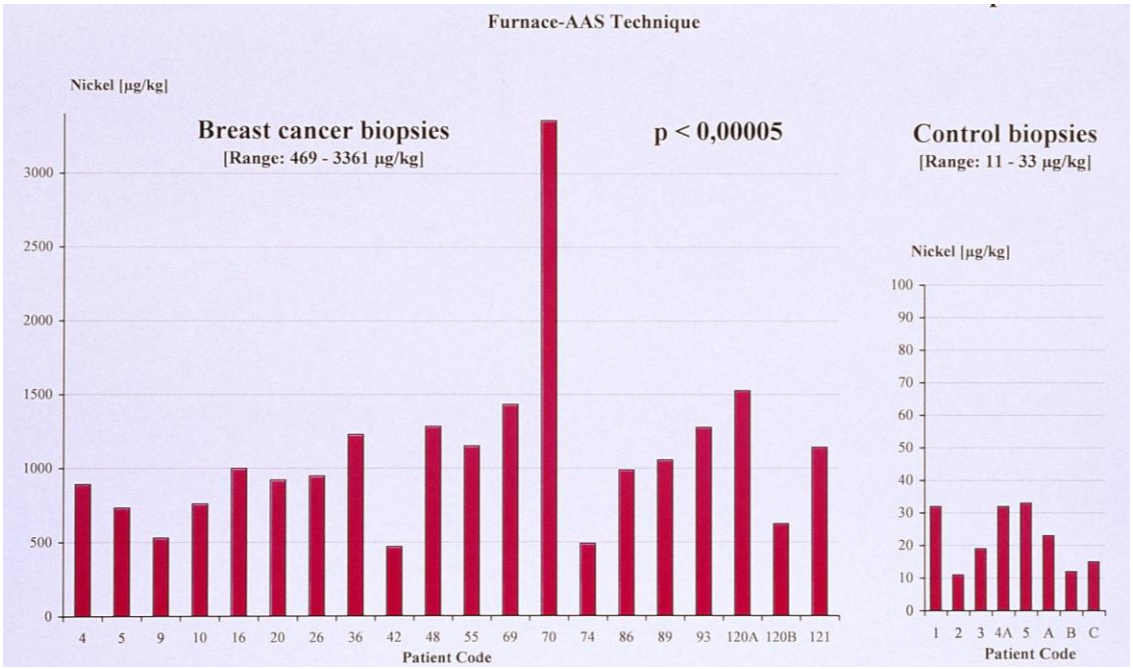

Figure 8. Nickel content of 20 breast cancer and 8 control human biopsies.

Antioxidants can modify cellular oxidative balance resulting in stimulation of proliferation (increased viability) or protect damaged cells from oxidative-stress induced suicide (apoptosis), and thereby accelerate cancer progression in higher eukaryotes. Antioxidants can sometimes suppress apoptosis, and sometimes facilitate it (Hampton and Orrenius 1998; Clement and Pervais 1999, Halliwell 2000). Apoptosis is accompanied by an intracellular shift towards increased oxidation, but too much

oxidation will stop apoptosis by oxidising and inactivating the caspase enzymes (Hampton and Orrenius 1998). On the other hand, low quantities of reactive oxygen species often stimulate cell proliferation (Burdon 1995).

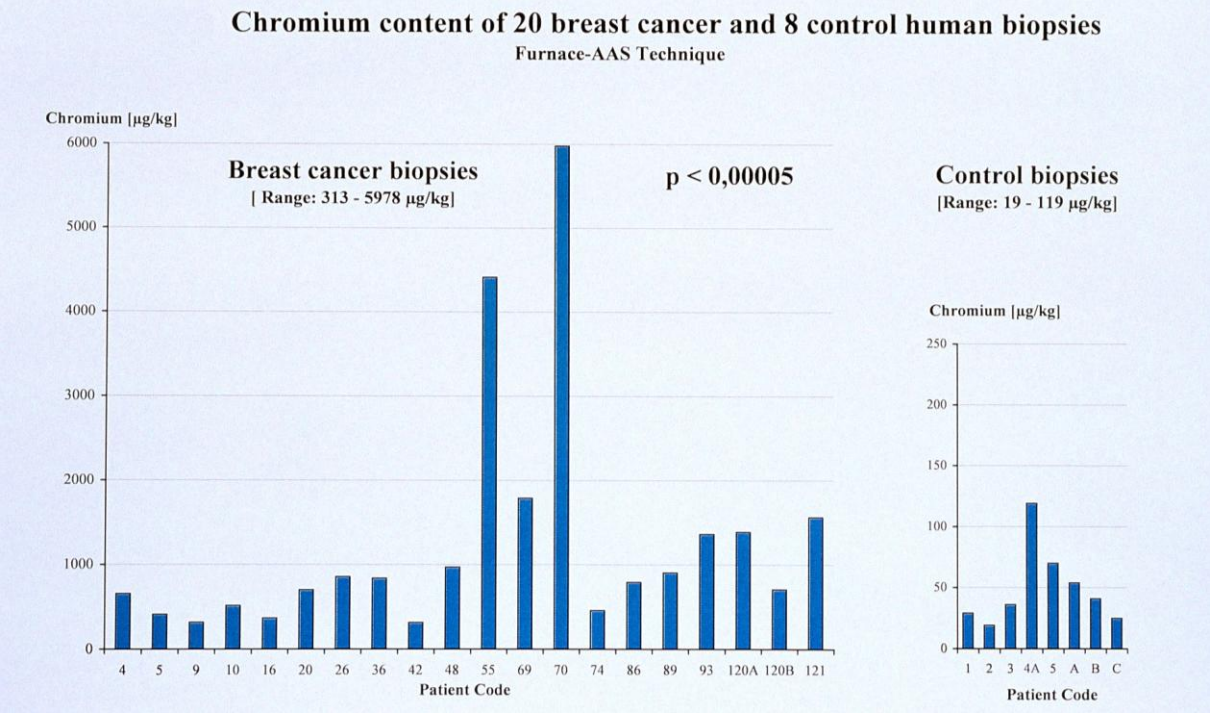

Figure 9. Chromium content of 20 breast cancer and 8 control human biopsies.

In addition, when an inappropriate pro-oxidant activity develops in normal cells, the reactive oxygen metabolites generated could damage the DNA and cellular membranes. The initiation of programmed cell death in tumor cells could result in the loss of malignant cellular integrity. In contrast, (reducing agents) antioxidants that quench free radicals or reactive oxygen products in transforming tumor cells may allow these selected cells to proliferate, enhance DNA repair and become therapeutically more resistant to treatment. Cancer cells are known to accumulate large amounts of antioxidants, such as glutathione, which, in turn, render these cells resistant to classic anticancer therapies (Luisini 2001, Yeh 2006). According to Schwartz (1996) when an antioxidant activity occurs in transformed cells an enhanced growth may result. The result of this modification of the tumor population would be the inadvertent enhanced survival and selection of tumor cell clones.

As a powerful antioxidant vitamin C may help to fight cancer by protecting healthy cells from free-radical damage and inhibiting the proliferation of metal-rich cancer cells. Vitamin C can affect cell growth by altering cell proliferation and/or inducing cell death in various cell systems (Brigelius-Flohe and Flohe 1996; Sakagami et al. (1997).

Increasing cellular viability with ascorbic acid pretreatment in Cr(VI)-induced toxicity was reported by Poljsak et al. (2002; 2005) in yeast cells. This might not always be beneficial. Chromium-induced growth-arrest and apoptosis are at the molecular decision point between chromium toxicity and chromium carcinogenesis (Singh et al 1998; Carlisle et al. 2000). When normal growing cells come in contact with carcinogenic forms of chromium, they may respond by undergoing growth arrest, apoptosis and necrosis. A population of genetically damaged cells may also emerge, which exhibits either intrinsic or induced resistance to apoptosis (Carlisle et al. 2000). Such cells may be predisposed to neoplasia as a result of their altered growth/death ratio, disrupted cell cycle control, or genomic instability. This, however, raises the question of whether ascorbic

acid-decreased Cr(VI) toxicity may actually increase the incidence of cancer (in higher eukaryotes) by allowing the inappropriate survival of genetically damaged cells. Besides, cells have their own endogenous antioxidants (superoxide dismutase, catalase, glutathion) and the addition of one single synthetic antioxidant could interfere with the complex antioxidant (redox) network and decrease the activity of endogenous defense. Because vitamin C is essential for collagen maturation and stabilization, it has been suggested that ascorbic acid may reduce tumor spreading by potentiating the stability of the extracellular matrix, especially since neoplastic invasion exhibits similar pathological manifestations as vitamin C deficiency (Gonzales et al. 2005). Unfortunately, the effects of vitamin C deficiency on metastasis caused by reduced collagen stabilization have not yet been examined *in vivo* due to the lack of appropriate animal models (Li and Schellhorn 2007). Though not fully understood, there are two opposing views on the role of the collagen-stabilizing function of vitamin C on tumor growth. First, by stabilizing collagen, ascorbic acid fortifies the extracellular matrix and stromal structures, leading to better confinement of neoplastic cells to their primary sites and preventing tumor growth and metastasis (Gonzales et al. 2005). Second, the same function may also facilitate the formation of new blood vessels, providing the prerequisite for malignant tumor growth (Telang et al. 2007). The interplay of these effects *in vivo*, especially under pharmacological levels of vitamin C, is far from clear (Li and Schellhorn 2007). In addition to angiogenesis, cancer cells can also modify their energy metabolic pathways to adapt to the low oxygen microenvironment in the interior of a solid tumor (Leo et al. 2004; Vaupel 2004). This is achieved by activation of hypoxia-responsive gene expression networks controlled by hypoxia-inducible factor-1a (HIF-1a) (Harris 2002; Schofield and Ratcliffe 2004). The negative impact of ascorbate on HIF-1a expression raises the question of whether intracellular vitamin C can inhibit the hypoxia-induced adaptation of solid tumor and thus restrict tumor growth and metastasis (Li and Schellhorn 2007). In high doses, ascorbic acid can trigger hemolysis in glucose-6-phosphate dehydrogenase deficient subjects, especially in the presence of infection and fever (Levine et al. 1999). Because oxalic acid is a major end metabolite of ascorbic acid oxidation, even limited oxidation of a large i.v. dose of ascorbic acid to oxalic acid could be dangerous. Acute tumor hemorrhage and necrosis have been reported within days after starting i.v. ascorbic acid in patients with advanced cancer (Cameron and Campbell 1974).

In previous studies, ascorbic acid inhibited the growth of various human melanoma cells (Bram et al. 1980); and induced apoptosis in human premyelocytic leukemia HL-60 cells (Satoh et al. 1998), and in fibroblasts (Denk and Knorr 1998; Peterkofsky and Prather 1977).

Uncontrolled studies reported clinical benefit from oral and intravenous vitamin C administered to patients with terminal cancer at dosage of 10 g daily (Cameron and Campbell 1974; Cameron and Pauling 1978). Similar results were also seen in a similarly designed Japanese study (Murata et al. 1982). Placebo controlled trials in patients with cancer reported no benefit from oral vitamin C at dosage 10 g daily (Creagan et al. 1979; Moertel et al. 1985). However, in vitro evidence showed that vitamin C killed cancer cells at extracellular concentrations higher than 1mM/L (Leung et al. 1993; Sakagami et al. 2000; Witte 1985), and thus its clinical use by some practitioners continues.

In 1997 the World Cancer Research Fund and the American Institute for Cancer Research issued an authoritative statement: "Food, nutrition and the prevention of cancer: a global perspective". They rated the anti-cancer effects of ascorbate as "probable" only for stomach cancer (its role is in its inhibitory effects on nitrosamine formation (Mirvish et al. 1998; Halliwell B. 2000) rather than antioxidant effects), "possible" for prostate, mouth, pharynx, oesophagus, lung, pancreas and cervical cancers and "insufficient data" for cancers of the colon, rectum, larynx, breast and bladder (Halliwell B. 2000). The controversy in beneficial vs. harmful vitamin C properties may also reflect a misinterpretation of epidemiology. Fruits, grains and vegetables contain multiple components that might exert protective effects against disease. It could be any, or any combination of those factors that is a true protective agent. High plasma ascorbate levels or high ascorbate intake could simply be a marker of a good diet rather than a true protective factor (Rietjens et al. 2001). However, the direct inverse

relationship between serum vitamin C levels and mortality cannot be neglected.

3.3. The Use of Vitamin C in Cancer Treatment

The use of vitamin C in different pathogenies is based on its role in collagen synthesis, protein hydroxylation, drug detoxification, phagocytosis, and bactericidal activities (Tsao 1997). However, in view of its redox cycling associated with antioxidant and prooxidant activities, (Halliwell and Whiteman 1997; Podmore et al. 1998; Ionescu et al. 2007a; Ionescu et al. 2007b) the appropriate decision pro or contra the high dose vitamin C drip remains a permanent challenge for the physician. Cancer treatment is one of the more controversial proposed uses of vitamin C although the antioxidant most widely used for treating cancer is perhaps vitamin C.

In biological systems, the concentration of redox-active transition metals capable of catalysing and/or generating free radicals such as superoxide, hydrogen peroxide, and the hydroxyl radical appears to be relatively low. However, under certain pathological conditions (e.g. different malignancies), transition metals and their transport proteins may accumulate in different target organs and induce cellular lipid peroxidation and DNA-attack.

In this respect, the ability of excess iron in mediating the formation of hydroxyl radicals, suppressing cellular immune functions, and promoting tumour growth is well established (Mello 1984; Liu and Okada 1996; Okada 1996; Weinberg 1996) and increased copper concentrations have also been found in human lung cancer biopsies (Adachi et al. 1991) and in other tumours (Ebadi and Swanson 1988).

The higher heavy metal concentration encountered in various tumours may be used for therapeutic intervention with ascorbic acid or substituted phenolic mixtures (Ionescu 2005a, 2007a).

The autoxidation of vitamin C and phenolic compounds in the presence of heavy metals strongly increase superoxide and H_2O_2 generation at the tumour site, resulting in a fast depletion of the malignant cell reducing equivalents with oxidosis shift and apoptosis induction. Reduction and mobilization of transition metals from their storage or transport proteins renders them extremely reactive in catalysing free radical reactions according to Fenton like and Haber-Weiss reactions. These reactions are strong generators of the hydroxyl radical, leading to lipid peroxidation, DNA strand breaks, and apoptosis ((Mello 1984; Okada 1996; Baader et al. 1994). The autoxidation of vitamin C with superoxide generation in the presence of transition metals like Fe^{3+}, Cu^{2+} or Hg^{2+} can be easily demonstrated with the chemiluminescence methodology in human serum [Figure 5].

In an acidic milieu (H^+ excess) the superoxide radical is further converted to H_2O_2. We therefore believe that the clinical improvement in cancer patients treated with high doses of ascorbate (Chen et al. 2005; Cameron and Pauling 1975) is based on the mechanism described above.

3.3.1. Vitamin C and Radioprotection

In recent years, more and more patients with cancer have been treated with radiation therapy. The destructive action of ionizing radiation is mainly due to ROS formation, including superoxide anion radical, hydroxyl radical and hydrogen peroxide (Ertekin and Sezen, 2007). Moreover, tissue irradiation results in a strong release of transition metals from the protein matrix. It is well known that radiotherapy not only increases the production of ROS but also reduces significantly natural antioxidants, such as vitamin A, C, E, selenium, and the activities of antioxidant enzymes in plasma and tissue (Ertekin et al. 2004; Borek 2004). Because radiotherapy leads to the generation of free radicals in excessive amounts, the endogenous antioxidant pool cannot offer optimal protection to protect body organs or healthy tissues. In this respect, to decrease radiotherapy-induced toxicity to the healthy cells, exogenous antioxidants may be supplemented after radiotherapy. On the other hand antioxidants could reduce the oxidizing free radicals created by radiation therapy, and thereby decrease the effectiveness of this treatment. An ideal radioprotectant is thus one that protects normal tissue while maintaining antitumor effectiveness, and is itself without moderate or severe toxicity (Ertekin and Sezen 2007). There are considerable in vitro and animal data showing that vitamin C can protect cells against radiation and chemotherapy (Lamson and Brignall 1999; Tewfik et al. 1982; Taper et al. 1996;

Okunieff 1991; Kurbacher et al. 1996; Chiang et al. 1994). Vitamin C is an antioxidant that can be used to reduce DNA damage and diminish lipid peroxidation and increase tissue radioresistance (Frei 1994; Sies and Stahl 1995). The administration of vitamin C through drinking water before and after x-irradiation decreased the survival of tumor cells in mice without causing a similar effect on normal cells (Tewfik et al. 1982). Vitamin C protected against radiation-induced chromosomal damage in mice even when administered after irradiation (Sarma and Kesavan 1993). On the other hand, some studies demonstrated that vitamin C at low doses may have protected cancer cells against free radical damage produced by chemotherapeutic agents or x-irradiation, and vitamin C, when given in a single low dose shortly before x-irradiation, reduced the effectiveness of irradiation on cancer cells in in vitro and in vivo models (Salganik 2001; Labriola and Livingston 1999; Witenberg et al. 1999). No in vivo evidence suggests that vitamin C decreases the effect of chemotherapy (Ertekin and Sezen 2007).

For further reading on radioprotective effects of other antioxidants see review done by Ertekin and Sezen (2007).

4. Conclusion

There will be continuous interest in the use of vitamin C for the treatment of human diseases, as well as in the vitamin C induced prevention of disease development. Herbert (1994) suggests that vitamin C (and other antioxidants) are mischaracterized by describing them solely as “antioxidants”. They in fact are redox agents, antioxidants in some circumstances (like the physiological quantities found in food), and pro-oxidants (producing billions of harmful free radicals) in other circumstances (often so in the pharmacologic quantities found in ill-designed supplements). However, epidemiological studies and clinical trials examining the ability of antioxidant vitamins (either individually or in combination) to affect disease outcome, rarely address possible underlying mechanisms. Thus, in these studies it is often assumed that antioxidant vitamins act by lowering oxidative damage, but evidence in support of this contention is not provided (DRI for vitamin C, Food and Nutrition Board, Institute of Medicine 2000). Whereas fruit and vegetable consumption decreases the amount of free-radical damage to DNA and the human body, supplements of vitamin C alone do not decrease the oxidative damage in some of the studies. Results from most intervention trials with single antioxidant in pharmacological doses do not support a protective effect. Recent studies suggest that well-known antioxidants (vitamin E, C, beta carotene) contribute a relatively small part of the total antioxidants. It should be noted that the protective effect of certain diet is not equivalent to the protective effect of antioxidants in the diet. Positive effects of the protective substances that originate from food are greater because of the synergistic activity between individual antioxidant substances (Rietjens et al., 2001), nutritional fibers and secondary vegetal substances. Dr. Bjelakovic's team (2007) evaluated 67 randomized clinical trials with 232,550 subjects. The evidence suggests it would be safer to obtain the compounds not as supplements, but by eating plenty of fruit and vegetables. Fruits and vegetable contain at least several hundred different types of antioxidants (i.e., electron or hydrogen donating reductants) which may directly react with free radicals. Another mechanism involves activation of genes encoding proteins involved in the antioxidant defense.

The outcome of latest epidemiologic studies is contradictory. Many studies show an inverse relationship between mortality and vitamin C intake. However, several studies show no relationship at all or no significant relationship after controlling for confounding variables. Several reports suggest a pro-oxidant or adverse effect from vitamin C in vitro and in vivo. It is well established by in vitro experiments that vitamin C is reactive with free iron and produces the ascorbate radical, while causing oxidative damage to biomolecules (DRI for vitamin C, Food and Nutrition Board, Institute of Medicine 2000). Scientists have claimed increases in DNA damage in healthy humans supplemented with vitamin C and iron salts, as well as ascorbyl radical formation in subjects with sepsis following ascorbate loading. However, other studies show vitamin C as protective antioxidant that can prevent oxidative stress. Whether vitamin C functions as an antioxidant or prooxidant is determined by at least 3 factors: 1) the redox potential of the cellular environment; 2) the presence/absence

of transition metals; and 3) the local concentrations of ascorbate (Ionescu 1998; Gonzales et al. 2005; Ionescu 2006).

Ascorbic acid has been described as "of all the paradoxical compounds, ascorbic acid probably tops the list. It is truly a two-headed Janus, a Dr. Jekyll-Mr. Hyde, an oxymoron of antioxidants" (Porter 1993). As already Paracelsus realized that "Sola dosis facit venenum", similar is the fact that intakes of vitamin C below the recommended daily allowance are associated with increased free radical damage to DNA (Rehman et al. 1998; Fraga et al. 1996) due to its ability to react with the "free" metal ions in the Fenton-like chemical reactions. However, these properties might have beneficial role in cancer treatment.

The thesis that pro-oxidant effect of vitamin C depends on its unbalance with other antioxidants, minerals and other nutrients, among them many still unknown, opens many questions regarding the best way to minimize the oxidative damage through food intake. Enough fruits and vegetables seem to be just the first step to this goal. Experiments on pigs show that supplementation of diet with different types of fruits and vegetables (apples, strawberries and tomatoes) have different effects on lowering the level of oxidative stress; so does their combinations (Pajk Žontar et al; 2006). Which combination of fruits and vegetable is best for humans? A controlled intervention should take into account the subject's blood redox potential and its total antioxidant activity, as already described (Ionescu 2007b), Figure 10.

According to well known biologists thesis, no animal species is optimally adapted to environment (Dawkins, 1999), especially in changeable environment. Despite the new finding on speeding up of genetic changes in humans (Hawkes, 2007), human genes didn't change much during last 10,000 years, but all produced food does due to normal selection that agriculture does (Watson, Berry 2007). Moreover todays fruits and vegetables are depleted of some essential micronutrients because of intensified type of production (Poljšak, 2006) or because of post harvest processes - transport, storage etc (Tijskens, 2004).

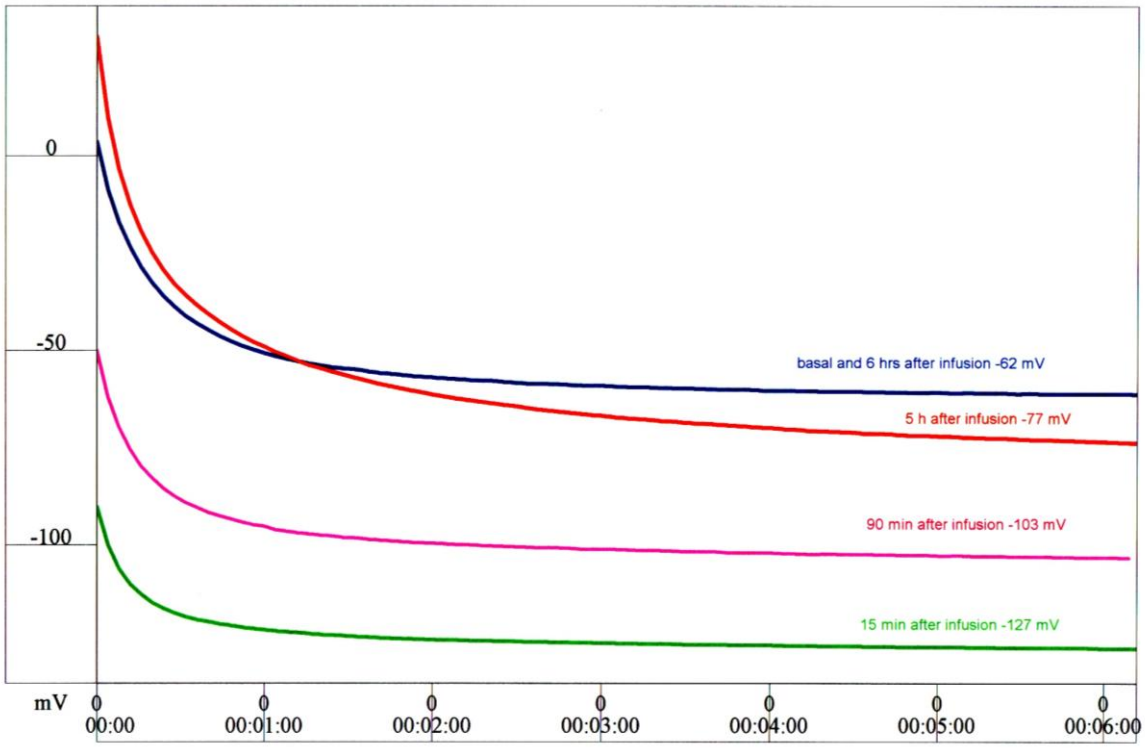

Figure 10. Effects of 8.5 g intravenous vitamin C administration on serum redox potential in a MCS patient (male, 42 yrs).

What is the content of nutrients of specific fruits and vegetables we eat? How to measure with non invasive methods the specific needs of vitamin C and other nutrients of each individual? The path of food supplements seems to be at the present time even more uncertain. However there is a general trend to increase of processed food and food supplements. In most countries of the world the consumption of fruit and vegetables is below the minimal level of 400 g per day advised by WHO and FAO (FAO/WHO 2004). Even in countries that had in the past high consumption of fruit and vegetables their consumption has been lowering (López-Torres, Barja, 2008). The addition of different food supplements to the diet seems to be, besides consumption of fruit and vegetables, for different reasons, and especially in different clinical conditions, a need as well. But more research is needed to find solutions that are closer to optimal human diet.

Acknowledgement

The authors would like to thank Dr. Alexandru Tudor Constantinescu for his valuable comments and suggestions when editing the manuscript.

References

Abdel-Latif, M.M.and Raouf ,A.A.and Sabra, K., et al. (2005). Vitamin C enhances chemosensitization of esophageal cancer cells in vitro. *J. Chemother*, 17, 539–549.

Anderson, D. and Phillips, B. J. and Yu, T. and Edwards, A. J. and Ayesh, R. and Butterworth, K. R. (1997). The effects of vitamin C supplementation on biomarkers of oxygen radical generated damage in human volunteers with 'low' or 'high' cholesterol levels. *Environ. Mol. Mutagen*. 30**,** 161–174.

Anderson, D. and Phillips, B.J. (1999). Comparative *in vitro* and *in vivo* effects of antioxidants. *Food Chem Toxicol, 37,* 1015-1025.

Alpha-tocopherol, beta-carotene cancer prevention study. (1994). *N. Engl. J. Med*. 330, 1029.

Baader S.L., and Bruchelt G., and Carmine T.C., and Lode H.N., and Rieth A.G., Niethammer D. (1994) Ascorbic-acid-mediated iron release from cellular ferritin and its relation to the formation of DNA strand breaks in neuroblastoma cells. *J. Cancer Res. Clin. Oncol*; 120 (7): 415–421.

Beatty, E.R. and England, T.G. and Geissler, C.A. and Aruoma, O.I. and Halliwell B. (1999). Effects of antioxidant vitamin supplementation on markers of DNA damage and plasma antioxidants. *Proc. Nutr. Soc, 58* (abstr), 44.

Bendich, A. and Cohen, M. (1990). Ascorbic acid safety: analysis of factors affecting iron absorption. *Toxicol. Lett., 51,* 189–201.

Berger, T. M. and Polidori, M. C. and Dabbagh, A. and Evans, P. J. and Halliwell, B. and Morrow, J. D. and Roberts, L. J., Frei, B. (1997). Antioxidant activity of vitamin C in iron-overloaded human plasma. *J. Biol. Chem. 272,* 15656–15660.

Bjelakovic G. and Nikolova D. and Gluud L.L. and Simonetti R.G. and Gluud C. (2007). Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *JAMA, 297*, 842–57.

Bjelakovic G. and Nikolova D. and Simonetti R.G. and Gluud C. (2004a). Antioxidant supplements for preventing gastrointestinal cancers. *Cochrane Database Syst Rev, 4,* CD004183.

Bjelakovic G. and Nikolova D. and Simonetti R.G. and Gluud C.(2004b). Antioxidant supplements for prevention of gastrointestinal cancers: a systematic review and meta-analysis. *Lancet, 364,* 1219–28.

Blankenship L.J. and Carlisle D.L. and Wise J.P. and Orenstein J.M. and Dye L.E. and Patierno S.R. (1997). Induction of apoptotic cell death by particulate lead chromate: differential effects of vitamins C and E on genotoxicity and survival. *Toxicol Appl Pharmacol 146, 2,* 270-80.

Block, G. (1991) Vitamin C and cancer prevention: the epidemiologic evidence. *Am. J. Clin. Nutr. 53,* 270–282.

Borek, C. (2004). Antioxidants and radiation therapy. *J. Nutr., 134*, 3207-3209.

Bram, S. and Froussard, P., , M .et al. (1980). Vitamin C preferential toxicity for malignant melanoma cells. *Nature,* 284: 629–631.

Brigelius-Flohe, R. and Flohe, L. (1996). Ascorbic acid, cell proliferation, and cell diferentiation in culture. *Subcell Biochem., 25*, 83-107.

Buettner G.R. (1993). The pecking order of free radicals and antioxidants: lipid peroxidation, α-tocopherol, and ascorbate. *Arch. Biochem. Biophys 300*, 535-543.

Buettner, G. R. and and Jurkiewicz, B. A. (1996). Catalytic metals, ascorbate and free radicals: combinations to avoid. *Radiat. Res,. 145***,** 532–541.

Burdon R.H. (1995). Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. *Free Rad Biol Med, 18,* 775–94.

Cadenas, S. and Barja, G. and Poulsen, H. E. and Loft, S. (1997). Oxidative DNA damage estimated by oxo8dG in the liver of guinea-pigs supplemented with graded dietary doses of ascorbic acid and a-tocopherol. *Carcinogenesis 18,* 2373–2377.

Cameron, E. and Campbell, A.(1974). The orthomolecular treatment of cancer. II. Clinical trial of high-dose ascorbic acid supplements in advanced human cancer. *Chem. Biol. Interact*, 9, 285–315.

Cameron, E. and Pauling, L. (1978). Supplemental ascorbate in the supportive treatment of cancer: reevaluation of prolongation of survival times in terminal human cancer. *Proc. Natl. Acad. Sci. USA, 75*, 4538-4542.

Carr, A,. and Frei, B., (1999). Does vitamin C act as a pro-oxidant under physiological conditions? *FASEB J. 13*, 1007-1024.

Caraballoso M. and Sacristan M. and Serra C. and Bonfill X.(2003). Drugs for preventing lung cancer in healthy people. *Cochrane Database Syst Rev, 2,* CD002141.

Carlisle D.L. and Pritchard D.E. and Singh J. and Owens B.M. and Blankenship L.J. and Orenstein J.M, Patierno S.R. (2000). Apoptosis and P53 induction in human lung fibroblasts exposed to chromium(VI): effect of ascorbate and tocopherol. *Toxicol Sci, 55*, 60-68.

Chen Q. and Espey M.G. and Krishna M.C. and Mitchell J.B. and Corpe C.P. and Buettner G.R. and Shacter E. Levine M. (2005). Pharmacologic ascorbic acid concentrations selectively kill cancer cells: action as a pro-drug to deliver hydrogen peroxide to tissues. *Proc. Natl. Acad. Sci.* USA.,102, 13604–9.

Chiang, C.D. and Song, E.J. and Yang, V.C. and Chao, C.C. (1994). Ascorbic acid increases drug accumulation and reverses vincristine resistance of human non-small-call lung-cancer cells. *Biochem J., 301*, 759-764.

Childs, A. and Jacobs, C. and Kaminski, T. and Halliwell, B. and Leeuwenburgh .C. (2001). Supplementationw ith vitamin C and.V-acetyl-cysteinein creaseso xidativc stressin humansa ftcr an acutem uscle injury inducedb y eccentrice xercise. *Frcc Radic. Biol. Mcd., 31*, 745-153.

Clement M.V. and Pervaiz S. (1999). Reactive oxygen intermediates regulate cellular response to apoptotic stimuli. *Free Rad Res, 30,* 247–52.

Clement M.V. and Ramalingam J. and Long L.H. and Halliwell B. (2001). The in vitro cytotoxicity of ascorbate depends on the culture medium used to perform the assay and involves H_2O_2. *Antioxid Redox Signal, 3, 1*, 157-63.

Collis, C. S. and Yang, M. and Diplock, A. T. and Hallinan, T. and Rice-Evans, C. A. (1997). Effects of co-supplementation of iron with ascorbic acid on antioxidant-pro-oxidant balance in the guinea pig. *Free Rad. Res. 27*, 113–121.

Cooke M.S. and Evans M.D. and Podmore I.D. and Herbert K.E. and Mistry N. and Mistry P. and Hickenbotham P.T. and Hussieni A. and Griffiths H.R. and Lunec J. (1998). Novel repair action of vitamin C upon in vivo oxidative DNA damage . *FEBS Lett 439*, 363–367.

Creagan, E.T. and Moertel, C.G. and O'Fallon, J.R. and Schutt A.J. and O'Connell, M.J. and Rubin, J. and Frytak, S. (1979). Failure of high-dose vitamin C (ascorbic acid) therapy to benefit patients with advanced cancer. A controlled trial. *N. Engl. J. med*, 301, 687-690.

Cross, C. E. and O'Neill, C. A. and Reznick, A. Z. and Hu, M. L. and Marcocci, L. and Packer, L. and Frei, B. (1993).Cigarette smoke oxidation of human plasma constituents. *Ann. NY Acad. Sci. 686*, 72–89.

Crott J.W and Fenech M. (1999). Effect of vitamin C supplementation on chromosome damage, apoptosis and necrosis ex vivo. *Carcinogenesis, 20*, 1035–1041.

Dabbagh, A. J. and and Frei, B. (1995). Human suction blister interstitial fluid prevents metal ion-dependent oxidation of low density lipoprotein by macrophages and in cell-free systems. *J. Clin. Invest. 96***,** 1958–1966.

Dasgupta, A. and Zdunek, T. (1992) *In vitro* lipid peroxidation of human serum catalyzed by cupric ion: antioxidant rather than prooxidant role of ascorbate. *Life Sci. 50***,** 875–882.

Dawkins, R.(1982/1999). The Extended Phenotype: The long Reach of the Gene, Oxford, New York: Oxford University Press; 1999.

Deng X.S. and Tuo J.S. and Poulsen H.E. and Loft S. (1998). Prevention of oxidative DNA damage in rats by Brussels sprouts. *Free Radic Res*., 28, 323–33.

Denk, P.O. and Knorr, M. (1998). In vitro effect of ascorbic acid on the proliferation of bovine scleral and Tenon's capsule fibroblasts. *Eur. J. Opthalmol, 8*, 37-41.

Deutsch J.C. (1998). Ascorbic acid oxidation by hydrogen peroxide. *Anal. biochem, 255*, 1-7.

Diaz, Z.and Colombo, M.and Mann, K.K. et al. (2005). Trolox selectively enhances arsenic-mediated oxidative stress and apoptosis in APL and other malignant cell lines. *Blood*, 105, 1237–1245.

Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids (2000). A report of the panel on dietary antioxidants and related comounds, Subcomitees on upper reference levels of nutrients and interpretation and uses of dietary reference intakes, and the standing comitee on the scientific evaluation of dietary reference intakes, Food and Nutrition Board, Institute of Medicine, National Academy Press, Washington, D.C. From internet: http: books.nap.edu/openbook.php? record_id=9810

Doroshow, J.H. (2006). Redox modulation of chemotherapy-induced tumor cell killing and normal tissue toxicity. *J. Natl. Cancer Inst*, 98, 223–225.

Dreosti, I.E. (1991). Free radical pathology and the genome. In: Dreosti, IE (Eds.), *Trace elements, micronutrients, and free radicals* (149-171).New Jersey, The Humana Press.

Drouin, R. and Rodriguez, H. and Gao, S. W. and Gebreyes, Z. and O'Connor, T. R. and Holmquist, G. P. and Akman, S. A. (1996). Cupric ion/ascorbate/hydrogen peroxide-induced DNA damage: DNA-bound copper ion primarily induces base modifications. *Free Rad. Biol. Med. 21*, 261–273 39.

Enstrom, J. (2008). *The epidemiology of vitamin C*. Elsevier.

Enstrom, J. and Kanim, L. and Klein, M.A. (1992). Vitamin C intake and mortality among a sample of the United States population. *Epidcmiology 3*, 194-202.

Ertekin, M.V. and Sezen O. (2007). Radioprotective effects of antioxidants. In: *Leading edge antioxidants*. Editor: Pangalossi, H.V., Nova Science Publishers, Inc., New York, 89-144.

Fiala, E. S. and Sodum, R. S. and Bhattacharya, M. and and Li, H. (1996). Epigallocatechin gallate, a polyphenolic tea antioxidant, inhibits peroxynitrite-mediated formation of 8-oxodeoxyguanosine and 3-nitrotyrosine. *Experientia 52*, 922–926.

Fischer-Nielsen, A. and Poulsen, H. E., and Loft, S. (1992). 8-Hydroxydeoxyguanosine Hydroxydeoxyguanosine in vitro: effects of glutathione, ascorbate, and 5-aminosalicylic acid. *Free Rad. Biol. Med. 13***,** 121–126 40.

Food and Agriculture Organization/ World Health Organization (2004):Fruit and Vegetables for Health: Report of a Joint FAO/WHO Workshop, 1-3 September 2004, Kobe, Japan.

Food and Drug Administration. November 1-3. (1993). FDA Conference on Antioxidant Vitamins and Cancer and Cardiovascular Disease. Washington. DC: Office of Special Nutritionals, 1993(transcript).

Fraga, C. G. and Motchnik, P. A. and Shigenaga, M. K. and Helbock, H. J. and Jacob, R. A. and Ames, B. N. (1991). Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. *Proc. Natl. Acad Sci. U. S. A. 88,* 11003–11006.

Fraga, C.G. and Motchnik, P.A. and Wyrobek, A.J. and Rempel, D.M. and Ames B.N. (1996). Smoking and low antioxidant levels increase oxidative damage to sperm DNA. *Mutat Res, 351,* 199–203.

Frei B.and England L.and Ames B.N. (1989). Ascorbate is an outstanding antioxidant in human blood plasma. *Proc. Natl. Acad. Sci. USA, 86*,6377–6381.

Frei, B. (1994). Reactive oxygen species and antioxidant vitamins: mechanisms of action. *Amer. J. Med, 97*, 5-13.

Frei, B. and Stocker, R. and Ames, B.N. (1988). Antioxidant defenses and lipid peroxidation in human blood plasma. *Proc. Natl. Acad. Sci. U S A. 85***,** 9748–9752.

Gale, C,R. and Martyn, C.N. and Winter, P.D. and Cooper, C. (1995). Vitamin C and risk of death from stroke and coronary heart disease in cohort of elderly people. *Br. Med. J. 310*, 1563-1566.

Gey KF, and Stähelin HB, and Eichholzer M. (1993) Poor plasma status of carotene and vitamin C is associated with higher mortality from ischemic heart disease and stroke: Basel Prospective Study. *Clin. Investig* 71(1):3-6.

Gonzalez, M.J.and Miranda-Massari, J.R.and Mora, E.M.and Guzman, A.and Riordan, N.H.and Riordan, H.D.and Casciari, J.J.and Jackson, J.A.and Roman-Franco A. (2005). Orthomolecular oncology review: ascorbic acid and cancer 25 years later. *Integr Cancer Ther*, 4, 32–44.

Grad, J.M.and Bahlis, N.J.and Reis, I., et al. (2001). Ascorbic acid enhances arsenic trioxide-induced cytotoxicity in multiple myeloma cells. *Blood*, 98, 805–813.

Green, M. H. L. and Lowe, J. E. and Waugh, A. P. W. and Aldridge, K. E. and Cole, J. and Arlett, C. F. (1994). Effect of diet and vitamin C on DNA strand breakage in freshly-isolated human white blood cells. *Mutat. Res. 316*, 91–102.

Griffiths, H.R. and Lunec J. (2001). Ascorbic acid in the 21st century – more than a simple antioxidant. *Environmental Toxicology and Pharmacology, 10*, 173–182.

Halliwell B. and Gutteridge J. 1999. Free radicals in biology and medicine, 3nd edition, Oxford: Clarendon Press.

Halliwell B. (1999). Vitamin C: poison, prophylatic or panacea? *Trends Biochem. Sci, 24*, 255-9.

Halliwell B. (2000). The antioxidant paradox. *Lancet, 355*, 1179-1180.

Halliwell B. (2000). Vitamin C and genomic stability. *Mutat Res, 475*, 29-35.

Halliwell B. (2000).Why and how should we measure oxidative DNA damage in nutritional studies? How far have we come? *Am. J. Clin. Nutr*., 72, 1082–7.

Halliwell B.and Whiteman M. (1997). Antioxidant and prooxidant properties of vitamin C. In: Packer L, Fuchs J, eds. *Vitamin C in Health and Disease.* New York: Marcel Dekker; 1997; Pp. 59–73.

Halliwell, B. and and Gutteridge, J. M. C. (1986). Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. *Arch. Biochem. Biophys., 246*, 501–514.

Halliwell, B. (1996) Vitamin C: antioxidant or pro-oxidant *in vivo*? *Free Rad. Res.* 25**,** 439–454.

Hampton, M.B. and Orrenius S. (1998). Redox regulation of apoptotic cell death. *Biofactors, 18***,** 1–5.

Harris A.L. (2002). Hypoxia: a key regulatory factor in tumour growth. *Nat. Rev. Cancer*., 2, 38–47.

Hawkes, J. and Wang, E.and Cochran, G.M. and Moyzis, R. (2007). Recent Acceleration of Human Adaptive Evolution. – *Proceedings of the National Accademy of Sciences of the United States of America*, 104(52), 20753-20758.

Herbert V. and Shaw S, Jayatilleke E. and Stopler-Kasdan T. (1994). Most free-radical injury is iron-related: it is promoted by iron, hemin, holoferritin and vitamin C. and inhibited by Desferoxamine and apoferritin. *Stem Cells, 12*, 289-303.

Herbert V. (1993). Dangers of iron and vitamin C supplements. *J. Am. Diet Assoc, 93*, 526-7.

Herbert, V. (1994). The antioxidant supplement myth. *Am. J. Clin. Nutr., 60,* 157–158.

Hoffer, L.J.and Levine, M.and Assouline, S.and Melnychuk, D.and Padayatty, S.J.and Rosadiuk, K.and Rousseau, C.and Robitaille, L. and Miller WH Jr. (2008).Phase I clinical trial of i.v. ascorbic acid in advanced malignancy. *Ann. Oncol*, 19(11):1969-74.

Hu, M. L. and Shih, M. K. (1997). Ascorbic acid inhibits lipid peroxidation but enhances DNA damage in rat liver nuclei incubated with iron ions. *Free Rad. Res*. 26, 585–592.

Hwang, M.T. and Ferraio, T. and Ho, C.T. (1994) Cancer chemoprevention by phytochemicals in fruits and vegetables: an overview. *ACS Symp. Ser,* 546, 2-16.

Ionescu J.G. and Merk, M, and Dowes F (1998) Clinical application of redox potential testing in the blood. *Syllabus of 33rd AAEM Annual Meeting, Baltimore, USA*, p503-512.

Ionescu J.G. : Simple chemiluminescence assays for reactive oxygen species in different clinical conditions. Proceedings of the Free Radical Symposium Taiwan, June 6-13, 2002.

Ionescu JG. (2005a) New evidence-based therapies for cancer. Proceedings of the 17th Int. Symposium on Integrative Medicine, p.1–21, Tenerife, Spain, June.

Ionescu JG. (2005b) Transition metals and cancer. Presented at the 12th MELISA Study Group Conference, Prague, 10th–11th September.

Ionescu, J.G.and Novotny, Jand Stejskal, Vand Latsch, Aand Blaurock-Busch, Eand Eisenmann-Klein, M (2006) Increased levels of transition metals in breast cancer tissue. *Neuro Endocrinol Lett* 27 Suppl 1, 36-9.

Ionescu, J.G. (2007a) Heavy Metal Accumulation in Malignant Tumours as Basis for a New Integrative Therapy Model. *Anti-Aging Therapeutics (Eds.: Klatz R. and Goldman R.), Vol IX,* Chapter 27, 189-201.

Ionescu, J.G. (2007b) Klinische Relevanz der Redox und chemoluminiszenz Bestimmungen bei Allergien, Haut- und Umwelterkankungen. in „Säueren-Basen-Schlacken", Marktl W, Reiter B, Ekmekcioglu C (Eds.) Springer Verlag, Wien/New York, p. 73-81.

Jackson J.A. and Roman-Franco A. (2005). Orthomolecular oncology review: ascorbic acid and cancer 25 years later. *Integr. Cancer Ther*. 4, 32–44.

Jacobs, A (1977) Low Molecular Weight Intracellular Iorn Transport Compounds. *Blood*, 50, 433-439.

Jenner, A. and England, T. G. and Aruoma, O. I. and Halliwell, B. (1998). Measurement of oxidative DNA damage by gas chromatographymass spectrometry: ethanethiol prevents artifactual generation ofoxidized DNA bases. *Biochem. J. 331***,** 365–369.

Kang S.A. and Gang Y.J. and Park M. (1998). In vivo dual effects of vitamin C on paraquat-induced lung damage: dependence on released metals from the damaged tissue. *Free Rad Res, 28*, 93–107.

Kassouf, W.and Highshaw, R.and Nelkin, G.M. et al. (2006). K3 sensitize human urothelial tumors to gemcitabine. *J Urol*, 176, 1642–1647.

Kurbacher, C.M.and Wagner, U.and Kolster, B. et al. (1996). Ascorbic acid (vitamin C) improves the antineoplastic activity of doxorubicin, cisplatin, and paclitaxel in human breast carcinoma cells in vitro. *Cancer Lett*, 1996, 103: 183–189.

Labriola, D. and Livingston, R. (1999). Possible interactions between dietary antioxidants and chemotherapy. *Oncology, 13*, 1003-1008.

Lamson, D.W. and Brignall, M.S. (1999). Antioxidants in cancer therapy; their actions and interactions with oncologic therapies. *Altern. Med. Rev, 4*, 304-329.

Lay P.A. and Levina A. (1998). Activation of molecular oxygen during the reactions of Chromium (VI/V/IV) with biological reductants: Implications for chromium-induced genotoxicities. *J. Am. Chem. Soc, 120*, 6704-6714.

Lee, B. M. and Lee, S. K. and Kim, H. S. (1998). Inhibition of oxidative DNA damage, 8-OHdG, and carbonyl contents in smokers treated with antioxidants (vitamin E, vitamin C, b-carotene and red ginseng). *Cancer Lett. 132*, 219–227.

Lenton K.J. and Therriault H. and Fulop T. and Payette H. and Wagner J.R. (1999.) Glutathione and ascorbate are negatively correlated with oxidative DNA damage in human lymphocytes. *Carcinogenesis*, *20*, 607–613.

Leo C.and Giaccia A.J.and Denko N.C. (2004). The hypoxic tumor microenvironment and gene expression. Semin Radiat Oncol., 14, 207–14.

Leung, P.Y. and Miyashita, K. and Young M. and Tsao, C.S. (1993). Cytotoxic effect of ascorbate and its derivatives on cultured malignant and nonmalignant cell lines. *Anticancer Res*, 13, 475-480.

Levine, Mand Daruwala, R Cand Park, J Band Rumsey, S Cand Wang, Y (1998). Does vitamin C have a pro-oxidant effect?. *Nature (London) 395,231-232, author reply 232*.

Levine, M.,and Rumsey, S.C.,and Daruwala, R. et al. (1999). Criteria and recommendations for vitamin C intake. *JAMA*, 281, 1415–1423.

Levine, M. and Conry-Cantilena, C. and Wang, Y. and Welch, R. W. and Washko, P. W. and Dhariwal, K. R. and Park, J. B. and Lazarev, A. and Graumlich, J. F. and King, J. and Cantilena, L. R. (1996). Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. *Proc. Natl. Acad. Sci. USA. 93*, 3704–3709.

Li Y. and Schellhorn HE. (2007). New developments and novel therapeutic perspectives for vitamin C. *J. Nutr*. Oct, 137, 2171-84.

Lode HN, and Bruchelt G, and Zinsser D, and Baader SL, and Rieth AG, and Schade UF, et al. (1994) Ascorbic acid induces lipid peroxidation on neuroectodermal SK-N-LO cells with high endogenous ferritin content and loaded with Mab-ferritin immunoconjugates. *Anticancer Res*; 14(5A): 1903–1906.

López-Torres, M.and Barja, G. (2008). Lowered methionine ingestion as responsible for the decrease in rodent mitochondrial oxidative stress in protein and dietary restriction: Possible implications for humans. *Biochemica et Biophysica Acta* 1780, 1337-1347.

Lusini L, and Tripodi SA,and Rossi R,and Giannerini F,and Giustarini D,and del Vecchio MT, and Barbanti G,and Cintorino M, andTosi P, andDi Simplicio P. (2001) Altered glutathione anti-oxidant metabolism during tumor progression in human renal-cell carcinoma. *Int. J. Cancer.* 91(1):55-9.

May J.M.and Cobb C.E.and Mendiratta S.and Hill K.E. andBurk R.F. (1998). Reduction of the ascorbyl free radical to ascorbate by thioredoxin reductase. *J. Biol. Chem 273*, 23039–23045.

McCall, M.R. and Frei, B. (1999). Can antioxidant vitamins materially reduce oxidative damage in humans?, *Free Radic. Biol. Med.* 26**,** 1034–1053.

McKersie B.D. (1996). Oxidative stress. Dept of Crop science, University of Guelph [online]. 1996 [8.02.2004]. Avaible form: URL: http://www.agronomy.psu.edu/Courses/ AGRO518/Oxygen.htm#activation (8.2.2004)

Miller E.R. and Pastor-Barriuso R. and Dalal D. and Riemersma R.A. and Appel L.J. and Guallar E. (2005). Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann. Intern. Med, 142*, 37–46.

Miller, D.M. and Buettner, G. and Aust, S.D. (1990). Transition metals as catalysts of autooxidation reactions. *Free Radic Biol Med, 8,* 95-108.

Mirvish S. and Grandjean A. and Reimers K. and Connelly B. (1998). Effect of ascorbic acid dose taken with a meal on nitrosoproline excretion in subjects ingesting nitrate and proline. *Nutr. Canc, 31,* 106-110.

Moertel, C.G. and Fleming, T.R. and Creagan, E.T. and Rubin, J. and O'Conell M.J. and Ames M.M. (1985). High-dose vitamin C versus placebo in the treatment of patients with advanced cancer who have had no prior chemotherapy. A randomized doubleblind comparison. *N. Engl. J. Med., 312*, 137-141.

Morel O, and Jesel L, and Hugel B, and Douchet MP, and Zupan M, and Chauvin M, and Freyssinet JM, and Toti F. (2003) Protective effects of vitamin C on endothelium damage and platelet activation during myocardial infarction in patients with sustained generation of circulating microparticles. *J. Thromb Haemost* 1(1):171-7

Murata, A. and Morishige, F. and Yamaguch, H. (1982). Prolongation of survival times of terminal cancer patients by administration of large doses of ascorbate. *Int. J. Vit Nutr. Res. Suppl, 23*, 103-113.

Myint PK, and Luben RN, and Welch AA,and Bingham SA,and Wareham NJ,and Khaw KT. (2008) Plasma vitamin C concentrations predict risk of incident stroke over 10 y in 20 649 participants of the European Prospective Investigation into Cancer Norfolk prospective population study. *Am. J. Clin. Nutr.* 87(1):64-9.

Nam CM, and Oh KW, andLee KH, andJee SH, andCho SY, andShim WH, andSuh I. (2003) itamin C intake and risk of ischemic heart disease in a population with a high prevalence of smoking. *J. Am. Coll Nutr.* 22(5):372-8.

National Research Council. (1992). Diet nutrition and cancer. *Na tional Academy Press*, Washington, DC.

Nemoto, S. and Otsuka, M. and Arakawa, N. and (1997). Effect of high concentration of ascorbateo n catalasea ctivity in cultured cells and tissueso f guineap igs. J. Nutr. *Sci. Vitaminol., 43,* 297-203.

Nyyssönen K,and Parviainen MT,and Salonen R,and Tuomilehto J,and Salonen JT. (1997) Vitamin C deficiency and risk of myocardial infarction: prospective population study of men from eastern Finland. *BMJ* 314(7081):634-8.

O'Brien T. and Mandel H.G. and Pritchard D.E. and Patierno S.R. (2002). Critical role of Cr-DNA interactions in the formation of Cr-induced polymerase arresting lesions. *Biochemistry, 41*, 12529-12537.

Okunieff P. (1991). Interactions between ascorbic acid and the radiation of bone marrow, skin, and tumor. *Am. J. Clin. Nutr, 54*, 1281-1283.

Osganians, .K. and Stampfer, M.J. and Rimm, U., Spiegelmand D.and Hu, F.B. and Manson, J.E. and Willett, W,C. (2003). Vitamin C and risk of coronary heart disease in women. *J. Am. Coll. Cardiol, 42*, 246-252.

Otero, P. and Viana, M. and Herrera, E. and and Bonet, B. (1997). Antioxidant and prooxidant effects of ascorbic acid, dehy- droascorbic acid and flavonoids on LDL submitted to different degrees of oxidation. *Free Rad. Res. 27*, 619–626.

Pajk Žontar, T.and Rezar, V. and Levant, A. and Salobir, J. (2006). Efficiency of various nutrients on reduction for oxidative stress in pigs as model for humans; In Gašperlin, L. and Žlender, B. (eds.): *Carcinogenic and anticarcinogenic food components*: *24th Food Technology* days 2006 dedicated to Prof. F. Bitenc. – Ljubljana: University of Ljubljana, Biotechnical faculty, pg.199-206.

Panayiotidis M. and Collins A.R. (1997). Ex vivo assessment of lymphocyte antioxidant status using the comet assay. *Free Rad Res, 27*,533–537.

Paolini M. and Pozzetti L. and Pedulli G.F. and Marchesi E. and Cantelli-Forti G. (1999). The nature of prooxidant activity of vitamin C. *Life Sci 23, 64*, 273-278.

Park J.B. and Levine M. (1996). Purification, cloning and expression of dehydroascorbic acid-reducing activity from human neutrophils: Identification as glutaredoxin. *Biochem J, 315*, 931–938.

Peterkofsky, B. and Prather, W. (1977). Cytotoxicity of ascorbate and other reducing agents towards cultured fibroblasts as a result of hydrogen peroxide formation. *J Cell Physol, 90*, 61-70.

Podmore, I. D. and Griffiths, H. R. and Herbert, K. E. and Mistry, N. and Mistry, P. and Lunec, J. (1998). Vitamin C exhibits pro-oxidant properties. *Nature (London) 392*, 559.

Pohl H. and Reidy J.A. (1989). Vitamin C intake influences the bleomycin-induced chr mosome damage assay: Implications for detection of cancer susceptibility and chromosome breakage syndromes. *Mutat Res, 224*, 247–252.

Poljsak, B. and, Gazdag, Z., and Pesti, M., and Farkas, N., and Plesnicar, S., and and Raspor, P. (2002). The role of antioxidant vitamins on chromium(VI) induced damage. *Toxicology Letters*,144(1),144.

Poljsak, B. and, Gazdag, Z., and Jenko-Brinovec, S., and Fujs, S., and Pesti, M., and Belagy, J., and Plesnicar, S., and and Raspor, P. (2005). Pro-oxidative vs antioxidative properties of ascorbic acid in chromium(VI)-induced damage: an in vivo and in vitro approach. *Journal of Applied Toxicology*, 25(6),535-548.

Poljsak, B,, and Raspor, P. (2008). The antioxidant and pro-oxidant activity of vitamin C and trolox in vitro: a comparative study. *J. Appl. Toxicol*. 28(2):183-8.

Poljšak, B. and Erjavec, M.and Likar, K. and Pandel Mikuš, R. (2006). The use of vitamin and mineral supplements in nutrition. *Obzor. Zdr*. N., 40, 1-10.

Porkkala-Sarataho, E. and Salonen, J.T. and Nyyssonen, K. and Kaikkonen, J. and Salonen, R. and Ristonmaa, U. and Diczfalusy, U. and Brigelius-Flohe, R. and Loft, S. and Poulsen, H.E. (2000). Long term effects of vitamin E, vitamin C and combined supplementation on urinary 7-hydro-8- oxo-2_-deoxyguanosine, serum cholesterol oxidation products, and oxidation resistance of lipids in nondepleted men. *Art. Thromb. Vasc. Biol. 20*, 2087.

Porter W.L. (1993). Paradoxical behaviour of antioxidants in food and biological systems. *Toxic Ind Health*, 9, 93-122.

Prieme, H. and Loft, S. and Nyyssonen, K. and Salonen, J. T., Poulsen, H. E. (1997). No effect of supplementation with vitamin E, ascorbic acid, or coenzyme Q10 on oxidative DNA damage estimated by 8-oxo-7,8-dihydro-29-deoxyguanosine excretion in smokers. *Am. J. Clin. Nutr*. 65, 503–507.

Quievryn G. and Peterson E.and Messer J. and Zhitkovich A. (2003). Genotoxicity and mutagenicity of chromium(VI)/ascorbate-generated DNA adducts in human and bacterial cells. *Biochemistry 42*, 1062-1070.

Ramirez J, and Flowers NC. (1980) Leukocyte ascorbic acid and its relationship to coronary artery disease in man. *Am J. Clin. Nutr*. 33(10):2079-87.

Rehman A. and Collis, C.S. and Yang. M. and Kelly, M. and Diplock, A.T. and Halliwell, B. and Fuce-Evans, C. (1998). The effects of iron and vitamin C cosupplemenlation on oxidative damage to DNA in healthy volunteers. Biochem. *Biophys. Res. Commun, 246*, 293-298.

Rietjens, I. and Boersma, M. and de Haan, L and et al. (2001). The pro-oxidant chemistry of the natural antioxidants vitamin C, vitamin E, carotenoids and flavonoids. *Environ Toxicol Pharmacol, 11*, 321-333.

Sakagami, H. and Satoh, K. and Kochi, M. (1997). Comparative study of antitumor action between sodium 5,6- benzylidene-L ascorbate and sodium ascorbate (minireview). *Anticancer Res*, 17, 4451-4462.

Sakagami, H. and Satoh, K. and Hakeda, Y. and Kumegawa, M. (2000). Apoptosis-inducing activity of vitamin C and vitamin K. *Cell Mol Biol, 46*, 129-143.

Salganik, R.I. (2001). The benefits and hazards of antioxidants: controlling apoptosis and other protective mechanisms in cancer patients and the human population. *J. Am. Coll Nutr 20*, 464–72.

Sarna,S. and Bhola,R. K. (1993). Chemo-immunotherapeutical studies on Dalton's lymphoma in mice using cisplatin and ascorbic acid: synergistic antitumor effect in vivo and in vitro. *Arch. Immunol. Ther. Exp*. 1993; 41: 327–333.

Sarma, L. and Kesevan, P.C. (1993). Protective effects of vitamin C and E against gama-ray induced chromosomal damage in mouse. *Int. J. radiol. Biol, 63*, 759-764.

Satoh K. and Ida Y. and Hosaka M. and Arakawa H. and Maeda M. and Ishihara M. and Kunii S. and Kanda Y. and Toguchi M. and Sakagami H. (1998). Induction of apoptosis by cooperative action of vitamins C and E. *Anticancer Res, 18*, 4371-4375.

Scarpa, M. and Stevanato, R. and Viglino, P. and Rigo, A. (1983). Superoxide ion as active intermediate in the autoxidation of ascorbate by molecular oxygen. Effect of superoxide dismutase. *J. Biol. Chem. 258,11*, 6695-7.

Schofield CJ, and Ratcliffe PJ. (2004). Oxygen sensing by HIF hydroxylases. *Nat. Rev. Mol. Cell Biol*., 5, 343–54.

Schwartz JL. (1996). The dual roles of nutrients as antioxidants and prooxidants: their effects on tumor growth. *J. Nutr*., 126 1221S-1227S.

Selman, C., and McLaren, J., and Meyer, C., and Duncan, J., and Redman, p., and Collins, A., Duthie, G., Speakman, J. (2006). Life-long vitamin C supplementation in combination with cold exposure does not affect oxidative damage or lifespan in mice, but decreases expression of antioxidant protection genes. In: *Mechanisms of ageing and development*, Volume 127, Issue 12, pp. 897-904.

Sestili, P.and Brandi, G.and Brambilla, L. et al. (1996). Hydrogen peroxide mediates the killing of U937 tumor cells elicited by pharmacologically attainable concentrations of ascorbic acid: cell death prevention by extracellular catalase or catalase from cocultured erythrocytes or fibroblasts. *J. Pharmacol. Ex.p Ther*, 277, 1719–1725.

Shinke T, andShite J, andTakaoka H, andHata K, andInoue N, andYoshikawa R, andMatsumoto H, andMasai H, andWatanabe S, andOzawa T, andOtake H, andMatsumoto D, andHirata K, andYokoyama M. (2007) Vitamin C restores the contractile response to dobutamine and improves myocardial efficiency in patients with heart failure after anterior myocardial infarction. *Am. Heart J*. 154(4), 645.e1-8.

Shklar, G. and Schwartz, J.L. (1994). A common pathway for the destruction of cancer cells: experimental evidence and clinical implications (review). *Int. J. Oncol., 4*, 213-224.

Sies H.and Stahl W. (1995). Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. *Am. J. Clin. Nutr. 62*,1315S–1321.

Simopoulos A.P. and Herbert V. and Jacobson B. (1993). Genetic nutrition: designing a diet based on your family medical history. New York: Macmillan.

Singh J, and Carlisle DL, andPritchard DE, andPatierno SR. (1998) Chromium-induced genotoxicity and apoptosis: relationship to chromium carcinogenesis. *Oncol. Rep*. 5(6):1307-18.

Song, E.J.and Yang, V.C.and Chiang, C.D. and Chao, C.C. (1995). Potentiation of growth inhibition due to vincristine by ascorbic acid in a resistant human non-small cell lung cancer cell line. *Eur. J. Pharmacol*, 292, 119–125.

Stearns, D.M. and Kennedy, L.J. and Courtney, K.D. and Giangrande, P.H, Phieffer, L.S, Wetterhahn, K.E. (1995). Reduction of chromium(VI) by ascorbate leads to chromium-DNA binding and DNA strand breaks in vitro. *Biochemistry 34*, 3, 910-9.

Stich H.F., and Karim J., Koropatnick J.,and Lo L. (1976). Mutagaenic action of ascorbic acid. *Nature*, 260, 722–4.

Sugiyama M. and Tsuzuki K. and Hidaka T. and Ogura R. and Yamamoto M. (1991). Reduction of chromium(VI) in Chinese hamster V-79 cells. *Biol. Trace Elem. Res 30,1*, 1-8.

Sugiyama M. and Tsuzuki K. and Ogura R. (1991). Effect of ascorbic acid on DNA damage, cytotoxicity, glutathione reductase, and formation of paramagnetic chromium in Chinese hamster V-79 cells treated with sodium chromate(VI). *J. Biol. Chem. 266,6*, 3383-3386.

Sugiyama M. (1991). Effects of vitamins on chromium(VI) induced damage. *Environ. Health Perspect* 92:63-70.

Swain J.A, Darley-Usmar V. and Gutteridge J.M.C. (1994). Peroxynitrite releases copper from caeruloplasmin: implications for atherosclerosis. *FEBS Lett, 342*, 49–52.

Taper, H.S. and Keyeux, A. and Roberfroid, M. (1996). Potentiation of radiotherapy by nontoxic pretreatment with combined vitamin C and K3 in mice bearing solid transplantable tumor. *Anticancer Res, 16*, 499-504.

Taper, H.S.and Jamison, J.M.and Gilloteaux, J., et al. (2004). Inhibition of the development of metastases by dietary vitamin C:K3 combination. *Life Sci*, 75, 955–967.

Telang S., and Clem A.L., and Eaton J.W., and Chesney J. (2007). Depletion of ascorbic acid restricts angiogenesis and retards tumor growth in a mouse model. *Neoplasia* 9, 47–56.

Tetef, M.and Margolin, K.and Ahn, C., et al. (1995). Mitomycin C and menadione for the treatment of lung cancer: a phase II trial. *Invest New Drugs*,13, 157–162.

Tewfik, F.A. and Tewfik, H.H. and Riley, E.F. (1982). The influence of ascorbic acid on the growth of solid tumors in mice and on tumor control by X-irradiation. *Int. J. Vita Nutr. Res Suppl, 23*, 257-263.

Tijskens, P. Discovering the Future: Modelling Quality Matters. – Wageningen University, 2004.

Vaupel P. (2004). The role of hypoxia-induced factors in tumor progression. *Oncologist*., 9, 10–7.

Verrax, J.and Stockis, J.and Tison, A., et al. (2006). Oxidative stress by ascorbate/menadione association kills K562 human chronic myelogenous leukaemia cells and inhibits its tumour growth in nude mice. *Biochem. Pharmacol*, 72, 671–680.

Vivekananthan DP, and Penn MS, and Sapp SK,and Hsu A, and Topol EJ. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. *Lancet* (2003) 361:2017–23.

Voogd, A., and Sluiter W., and van Eijk H. G., and Koster, J.K. (1992) Low Molecular Weight Iron and the Oxygen Paradox in Isolated Rat Hearts. *J. Clin. Invest.*, 90, 2050-2055.

Vojdani A, and Bazargan M, and Vojdani E, and Wright J. New evidence for antioxidant properties of vitamin C. *Cancer Detect Prev*. (2000) 24(6):508-23.

Watson, J. D., and Berry, A. DNA: *The Secret of Life*. Ljubljana: Modrijan, 2007.

Witenberg B. and Kalir H.H. and Raviv Z. and Kletter Y. and Kravtsov V. and Fabian I. (1999). Inhibition of ascorbic acid of apoptosis induced by oxidative stress in HL-60 myeloid leukemia cells. *Biochem. Pharmacol 57*, 823-832.

Witenberg, B. and Kletter, Y. and Kalir, H.H. and Raviv, Z. and Fenig, E. and Nagler, A. and Halperin, D. and Fabian, I. (1999). Ascorbic acid inhibits apoptosis induced by X irradiation in HL 60 myeloid leukemia cells. *Radiat Res, 152*, 468-478.

Wittes, R.E. (1985). Vitamin C and cancer. *N. Engl. J. Med*., 312, 178-179.

Yaman M, and Atici D, and Bakirdere S, and Akdeniz I. (2005) Comparison of trace metal concentrations in malign and benign human prostate. *J. Med. Chem.* 48(2):630-4.

Yeh CC, and Hou MF,and Wu SH,and Tsai SM,and Lin SK,and Hou LA,and Ma H, and Tsai LY. (2006) A study of glutathione status in the blood and tissues of patients with breast cancer. *Cell Biochem. Funct* 24(6):555-9.

Zaidi, S.M. and Al-Qirim, T.M. and Banu, N. (2005). Effects of antioxidant vitamins on glutathione depletion and lipid peroxidation induced by restraint stress in the rat liver. *Drugs R D*, 6, 157-165.