International Journal of Occupational Medicine and Environmental Health, Vol. 13, No 3, 233–245, 2000

REVIEW PAPERS

THE ROLE OF REACTIVE OXYGEN SPECIES IN THE DEVELOPMENT OF MALIGNANCIES

JOLANTA GROMADZIŃSKA and WOJCIECH WĄSOWICZ

Department of Toxicology and Carcinogenesis
The Nofer Institute of Occupational Medicine
Łódź, Poland

Key words: Reactive oxygen species, Free radicals, Antioxidants, Carcinogenesis, Vitamins A, C and E, Selenium

Abstract. Numerous studies on animal models and cell cultures confirm the role of free radicals/reactive oxygen species and oxidative damages in the process of the initiation and promotion of neoplastic diseases. Also epidemiological studies attribute an important role to disturbances in the pro- and antioxidative potential in the development of malignancies and highlight the essential function of antioxidants to protect the cells from the attack of free oxygen radicals.

The current literature data on the parameters of the anti- and prooxidant status in patients with malignant diseases and their role in pathogenesis of the disease is reviewed in this article.

REACTIVE OXYGEN SPECIES

Endogenous sources

Reactive oxygen species (ROS) are products of partial reduction of molecular oxygen (mono-, di-, or trielectron reduction), and/or its excited form – singlet oxygen – can be produced (Fig. 1). They are formed as a result of regular metabolic transformations, such as electron transfer in the mitochondrial respiratory chain, oxygen burst during phagocytosis, synthesis of eicosanoids or reactions catalysed by some oxygenases and oxidases (44). An important source of ROS are also transformations of xenobiotics associated with P450 cytochrome. This cytochrome metabolises a number of various substances, including drugs and aromatic hydrocarbon derivatives present, *inter alia* in smog and tobacco smoke.

Address reprint requests to Dr J. Gromadzińska, Department of Toxicology and Carcinogenesis, The Nofer Institute of Occupational Medicine, P.O. Box 199, 90-950 Łódź, Poland.

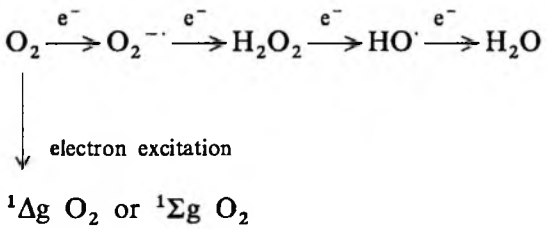

Fig. 1. Univalent stepwise reduction and excitation of molecular oxygen. e^- – electron.

Excessive production of abnormal transformations of reactive oxygen species exerts harmful effects on cellular metabolism. The intensity of their formation is often increased by the presence of transition metals, such as iron or copper (47). Table 1 gives examples of free radicals and reactive oxygen species. Reactive oxygen species do not usually exhibit high specificity in relation to substrates, and can therefore react with many compounds present in the cell. Excessive generation of ROS may cause dangerous damage in nucleic acids, proteins, lipids or carbohydrates (7,43).

Table 1. Examples of oxygen free radicals and related active species

	Name	Chemical formula
Radicals	Superoxide	$O_2^{-\cdot}$
	Hydroxyl radical	$HO^{\cdot}$
	Hydroperoxy radical	$HOO^{\cdot}$
	Alkoxyl radical	$LO^{\cdot}$
	Lipid peroxyradical	$LOO^{\cdot}$
	Nitric oxide	$NO^{\cdot}$
	Thiyl radical	$RS^{\cdot}$
Nonradicals	Hydrogen peroxide	H_2O_2
	Singlet molecular oxygen	$^1O_2$
	Hypochlorous acid	HOCl
	Peroxynitrite	$ONOO^-$
	Lipid hydroperoxides	LOOH

An important source of ROS, associated with the metabolism of every organism, is the oxidative stress resulting from inflammatory processes. Activated leukocytes produce superoxide radicals, H_2O_2 and hyperchlorous acid. Activated phagocytes release a whole complex of cytokins, which may penetrate the cell nucleus. In the presence of ROS and other mediators of inflammatory processes, they activate oncogenes with concurrent inactivation of suppressor genes. This mechanism of DNA modification is probably responsible for the replication of c-fos protooncogene. The induction of the carcinogenic process by chronic inflammation has been proved in the case of mesothelioma caused by asbestos fibres (13).

Exogenous sources of free radicals

A number of exogenous factors, impossible to eliminate from the surrounding environment, are responsible for oxidative stress (Table 2). Tobacco smoke and some dust containing metals, especially transition metals, have been proved to generate reactive oxygen species. The presence of some chemical substances, such as nitrosoamines or polycyclic aromatic hydrocarbons, increases the ability of ROS to induce carcinogenic processes (13). Under certain conditions, if the body is exposed to promotors, this initiation may lead to the development of neoplasm (45).

Table 2. Sources of reactive ROS and metabolic pathways involved in oxidative cell damage (modified after 20)

Sources of ROS in humans:		Metabolic pathways involved in oxidative cell damage:
• Ionising radiation		• Increase in radical-generating enzymes (e.g. xanthine oxidase)
• Ultraviolet		• Activation of phagocytes
• Ultrasound		• Activation of phospholipase, cyclooxygenase, lipoxygenase
• Chemicals (e.g. drugs, diesel exhaust, tobacco smoke)	→	• Destruction (breakdown) of antioxidants
• Infections		• Release of free metal ions from sequestreted proteins
• Hyperoxia		• Release of heme proteins
• Toxins		• Disruption of mitochondrial electron transport chain

DAMAGE OF MACROMOLECULES BY REACTIVE OXYGEN SPECIES

Oxidative protein degradation can cause modification of amino acid chain and prosthetic groups of enzymes, especially metals in active site: Fe^{+2}, Cu^{+2}, which are susceptible to attack of $O_2^{\cdot}$ and H_2O_2. Degradation of proteins results in aggregation or fragmentation of polypeptide chains. Some of amino acids (including tyrosine, histidine, cysteine and methionine) are particularly sensitive to reactive oxygen species modification. Oxidative damage of proteins lead to rapid loss of their biological activity. Oxidatively modified proteins are shown to activate proteolytic enzymes and become even more susceptible to proteolysis (17). 670-kDa proteolytic complex macroxyproteinase degrades oxidatively modified proteins and prevents their accumulation and aggregation in the cells (33).

The coupled double bonds in polyunsaturated fatty acids make lipids sensitive to oxidation. The reaction of these compounds with reactive oxygen species and fragmentation of their derivatives, results in the formation of alkanes, alkenes, 4-hydroxyalkanes, aldehydes, ketones, dienes, hydroxyl-, and epoxide derivatives of fatty acids, as well as of modified phospholipids. Intermediate products are alkoxyl radicals and peroxyl radicals. Aldehydes formed as a result of breakdown of lipid peroxides, including malondialdehyde, are particularly dangerous. Their half-life

period is relatively long and they can react with cell lipids and proteins far from the site where they were formed (12). As a consequence of peroxidation of membrane phospholipids, the membrane becomes rigid, loses its channel function, and finally fails to preserve membrane integrity.

Reactive oxygen species react with all components of DNA molecule: purine and pyrimidine base, and deoxyribose backbone. Chemical damage of DNA is always concomitant with structural damage of DNA-double helix. While many active oxygen species-related DNA base modifications result in a replicative block, some may induce point mutations by misreading base during the replication (13). Reactive oxygen species may also cause chromosomal aberrations and disturb the exchange of sister chromatids (9). Comparative studies of DNA damage, induced by hydroxyl radicals in human breast cancer cells obtained during biopses, demonstrated that in the patients with metastases its level was doubled. The direct relationship between DNA modification induced by ROS and the process of carcinogenesis has not been proved, but it is suggested that ROS reacting with DNA, can activate oncogenes and/or inactivate antioncogenes. The initiation, the first stage of carcinogenesis, requires continuous modification of the genetic material in the cell. It is estimated that the number of oxidative attacks on one human cell accounts for about 10,000 a day. DNA damage is removed by specific and non-specific repair processes (13). However, some of these damages, remain not repaired and their number increases with age.

OXIDATIVE STRESS AND ANTIOXIDANT DEFENCE

The cells of aerobic organisms possess defence mechanisms for protecting them from ROS. These mechanisms may be classified as follows (38):

1. isolation of generation sites of reactive oxygen species;
2. inhibition of propagation phase of reactive oxygen species;
3. scavenging of reactive oxygen species;
4. repair of the damage caused by reactive oxygen species.

The compounds which can inhibit oxidation processes are referred to as antioxidants. Superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px), glutathione transferase, and ceruloplasmin (Cp) belong to enzymatic antioxidants, while α-tocopherol, β-carotene (provitamin A), ascorbic acid (vitamin C), glutathione, uric acid and bilirubin to low-molecular weight non-enzymatic ones. The microelements (selenium, zinc and copper) bound to the active site or occurring as structural elements of antioxidative enzymes also play an important role. Another group is formed by proteins combined with transition metals: albumin (Fe, Cu), transferrin (Fe), ferritin (Fe), and ceruloplasmin (Cu) (2,28,36). Table 3 overviews some antioxidants and their biological activity.

In normal cells, there is a balance between the pathways of ROS formation and protective systems, due to which they are metabolised. The condition caused by the disturbed balance between the process of generating reactive oxygen species and oxidation processes lead to oxidative stress. The disturbance of the balance with prevailing pro-oxidative processes is associated with many diseases and pathologies (22).

Table 3. Protection against ROS damage

	Antioxidant	Biological function
Direct protection against ROS	Superoxide dismutase (Mn, Cu, Zn)	Catalyses dismutation of $O_2^{-\cdot}$
	Glutathione peroxidase (Se)	Reduces organic peroxides and H_20_2 in low concentration
	Catalase (Fe)	Metabolises H_20_2
Non-specific reductions system	Glutathione	Cosubstrate in numerous enzymatic reactions, e.g. GSH-Px, GST, scavenger of $O_2^{-\cdot}$, $^1O_2$, HO
	Vitamin C	Scavenger of oxygen radicals
Protection against lipid peroxidation	GSH-Px	Reduces phospholipid peroxides and free fatty acid peroxides
	Vitamin E	Radical chain breaking, lipid-soluble
	β-carotene	Scavenger of singlet oxygen
Sequestration of metals	Transferrin	Binds iron
	Lactoferrin	Binds iron
	Ferritin	Binds iron
	Ceruloplasmin	Binds copper, oxidizes Fe^{+2} to Fe^{+3}
	Metalothionein	Binds metals
Repair enzymes	DNA repair enzymes	
	Macroxyproteinases	Oxidises protein turnover
	Glutathione transferase	Conjugation of aldehydes

REACTIVE OXYGEN SPECIES AND CANCER INDUCTION

Malignancies fall within the category of diseases whose pathogenesis is postulated to be related to the ROS effects. Their intensified production, deficiency of antioxidants or impairment of the repair mechanism result in the increased processes responsible for the development of malignancies (7,49). The potential role of ROS in the pathogenesis of the upper respiratory tract malignancies is confirmed by the fact that normal epithelial lining fluid contains mainly proteins which perform anti-oxidative functions and low-molecular weight antioxidants identical with those present in blood, but at a higher concentration (11).

If the development of a neoplasm is considered to be a long-term process, a temporary oxidative stress cannot be treated as a cause of the disease. It may, however, be responsible for primary changes in the genetic material, the structure of the cell membrane, and expression of cytokins — oncogenes, possible agents of initiating or promoting carcinogenesis (13).

The model of the neoplastic disease development presented in 1980, indicates permanent changes in DNA molecule induced by ROS as a result of cellular metabolism on the one hand, and by continuous exposure to ionising radiation on the other. Most of these changes are recognised and removed by the cell. However, if the body is in contact with promotors, this initiation change may lead to the development of the disease (46). Many promotors act by generating ROS. There is evidence that organic peroxides may also function as promotors of neoplasm (23).

Investigations of the mechanisms responsible for the development of neoplastic diseases showed that antioxidants may modify the action of the majority of tumour promotors. It has been shown that in the skin cells exposed to 12-o-tetradecanoilphorbol acetate, the activity of superoxide dismutase, glutathione reductase and catalase is strongly inhibited (48). The only antioxidative enzyme with increased activity is glutathione peroxidase (36). It has been demonstrated that the products of lipid peroxidation, mainly malondialdehyde, may act at the initiation and promotion stages of the neoplastic tumour growth. The compounds which are considered to be promotors of the tumour growth: phenols, phorbol esters and halogens also induce damage of the cellular membrane through lipid peroxidation (38).

In a number of experiments, enzymatic and non-enzymatic antioxidants have been introduced to the system along with simultaneous initiation of carcinogenesis. It has been indicated that only the presence of antioxidants at a proper concentration and at the moment of the promotor action, may inhibit the development of the carcinogenic process (50). Administration of these compounds at a later stage is either of no importance or may even intensify the neoplastic process, probably along the pathway independent of the effects of reactive oxygen species. Therefore, it is likely that proper concentration/activity of antioxidants during the whole lifetime of the cell is a key point in the process of preventing carcinogenesis. Table 4 presents exogenous sources of ROS involved in carcinogenic processes.

Table 4. Exogenous sources of ROS involved in carcinogenesis (modified after 13)

Sources	ROS	Exposure-related cancer
Tobacco smoke	$NO^{\cdot}$, $HO^{\cdot}$	Bronchogenic carcinoma
Ultraviolet light	$HO^{\cdot}$, $LO^{\cdot}$, $LOO^{\cdot}$	Melanoma and other skin cancers
Dietary fatty acids	$LOO^{\cdot}$, LOOH	Colorectal cancer, breast cancer
Transitional metal ions (Fe, Cu)	$HO^{\cdot}$	Colorectal cancer
Ethanol	LOOH	Hepatocellular carcinoma, breast cancer
Inflammation:	'respiratory burst' $O_2^{-\cdot}$, $HO^{\cdot}$, $^1O_2$	
Asbestos		Lung mesothelioma
Schistosoma haematobium infections		Bladder cancer
Liver viral infections		Hepatocellular carcinoma

ANTIOXIDANT TRACE ELEMENTS AND TRACE ELEMENTS BOUND PROTEINS IN NEOPLASTIC DISEASE

Selenium

Current studies of the carcinogenic mechanisms seem to confirm anticarcinogenic effects of selenium (Se). In tissue cultures, the inhibited growth of neoplastic cells, under the influence of various Se compounds, has been observed (15). Studies on chemically induced malignancies in animals have shown that in rats

with mammary gland cancer, induced by dimethylbenzanthracene, the number of developing malignancies decreased with the increase of Se concentration in the diet (32). It is believed that Se inhibits the development of neoplasm in animals, such as colon cancer induced by dimethylhydrazine, skin cancers induced by crotonic oil and benz(a)piren, and liver neoplasm due to the effect of dimethylaminobenzene and aflatoxin (41). Anticarcinogenic effect of Se compounds administered in drinking water was observed in UV-induced skin cancer in mice (5). A similar influence of selenium compounds, provided in food and drinking water, has also been found in many studies of neoplasm induced by oncoviruses. In mice, the incidence of mammary gland cancer induced by MuMTV-S oncovirus has been found significantly lower in the group supplemented with Se than in the group with a diet poor in selenium (31). Some epidemiological surveys indicate a possible anticarcinogenic effect of Se in humans. In the 1960s, a negative correlation was found between mortality caused by many types of malignancies and Se occurrence in different geographical regions (42). The results of the USA investigations suggest that there is a relationship between the amount of Se consumed and the incidence of cancer of some sites (10). In patients with neoplastic diseases, the concentration of Se in blood is often lower than in healthy people. Epidemiological studies in a group of 190 people, carried out in Finland, pointed out to a higher risk of lung cancer in subjects with a very low concentration of Se in blood plasma (< 45.5 μg/l, p for trend 0.046) (26). In patients with colorectal adenoma ($n = 48$), plasma selenium concentration below the median was associated with higher risk of cancer (OR – 3.79, 95% CI,1.02 – 15.17), but in another study ($n = 139$) an increased risk of colorectal cancer related to the highest quartile of plasma Se concentration (OR – 1.8, 95% CI, 0.9 – 4.0) was found (53). It has also been observed that in a group of cancer patients, a higher blood plasma Se concentration sometimes correlates with a milder course of the disease (24).

It is presumed that chemical protection provided by Se may consist in its ability to inhibit the activity of microsomal enzymes responsible for the generation of some types of carcinogens or in the prevention of enzymatic conversion (at the cellular level) of pro-carcinogens into carcinogens. A possible anticarcinogenic effect of Se by regulating the rate of cell division and protein synthesis is also considered. A high Se concentration decreases the level of reduced glutathione (GSH) and glutathione reduced/glutathione oxidised ratio (GSH/GSSG). The proper GSH/GSSG ratio is one of the important factors which regulate the division of the cells. A decrease in this ratio delays G1, S and G2 phases of cellular growth cycle, and thus, lowers the rate of cell proliferation. Selenium also affects the pace of protein synthesis. Some Se derivatives of glutathione, such as selenodiglutathione (GSSeSG), inhibit protein synthesis by blocking initiation factor 2 and elongation factor (10). Since most carcinogenic substances become activated in free radical reactions with DNA, anticarcinogenic effect of Se may be limited to disturbing free radical modification of nucleic acids. This hypothesis seems to be confirmed by intracellular distribution of Se: the highest concentration occurs in the nucleus, and the lower in cytosol, mitochondrial and microsomal fractions (58).

Investigating the role of Se in the pathogenesis of neoplasm, it is important not only to establish its supply and concentration in the body, but also to determine its biological activity, expressed as the activity of selenoenzymes, primarily GSH-Px.

In humans, a positive correlation is found between the concentration of Se and GSH-Px activity within the concentration range up to 100 μg/l of blood. GSH-Px activity does not increase above this level of Se. It is agreed that Se concentration, at which a plateau of GSH-Px activity is observed, is sufficient for this microelement to perform its biochemical function (39). GSH-Px decomposes the organic peroxides and H_2O_2 formed as a result of free radical reactions, and thus, protects cellular membrane lipids from structural modifications. It is presumed that maintaining the integrity of cellular membrane is one of the mechanisms protecting from induction and development of malignant neoplasm.

The relationship between GSH-Px activity and its anticarcinogenic effect has not been explicitly proved. The mortality of patients with gastric cancer has been found to be related to the content of fat in the diet and inversely related to Se concentration and GSH-Px activity in blood (3,59).

Patients with neoplastic diseases often exhibit lower activity of GSH-Px in blood plasma and morphotic components of blood than healthy people. It has been found in the case of cancer of stomach, colon, breast (40) and lungs (59). However, many authors do not find this relationship, for instance in breast cancer patients (55).

Zinc

The presence of zinc (Zn) is found in over 300 metaloenzymes, as well as in many non-enzymatic metaloproteins and biopolymers, which perform catalytic, structural and regulatory functions. Zinc takes part in metabolism of nucleic acid and biosynthesis of protein, also as a necessary component of DNA and RNA polymerase (54). Deficiency of zinc may cause disturbances in immune processes of the body. Zinc ions are necessary for blastic transformation of lymphocytes (34). The association between Zn and the carcinogenic process is complex and not explicit. Some reports indicate that Zn deficiency may lead to weakening of cell resistance and thus contribute to the initiation of a neoplastic disease (52). On the other hand, the deficiency of this element inhibits the tissue growth, including neoplastic tissues, because it takes part in activation of enzymes necessary for DNA replication and biosynthesis of proteins (18).

Copper

The role of copper (Cu) in the body is associated with activation of a number of enzymes involved in the oxidation-reduction processes, e.g. cytochrome oxidase, o-diphenol oxidase (tyrosinase), lisyl oxidase, superoxide dismutase and ceruloplasmin. Bound to the structure of ceruloplasmin, it regulates transportation of iron, facilitates oxidation of iron and its transfer from ferritin to transferrin (51).

The role of Cu in the process of carcinogenesis is not clear. Some epidemiological studies indicate an increased risk of a neoplastic disease in people with low Cu concentration in blood (27). Other results suggest an association between high concentration of Cu in blood serum and the incidence of cancer of breast, cervix, lungs, digestive tract, as well as of malignant melanoma, lymphoma and leukemia (1,8). It has sometimes been observed that the concentration of Cu is higher in the neoplastic tissue than in the surrounding healthy tissue.

Both of the above discussed microelements are integral components of superoxide dismutase. Copper ion, which is reduced and oxidised in the enzyme active site, plays an important role in the reaction of dismutation of superoxide radicals, catalysed by SOD. The probable role of Zn is limited to providing structural stability of the protein in the region of the active site (4). SOD activity depends on the body concentration of metals present in the active site of the enzyme. The investigations on animal models have revealed an evident decrease in the activity of the enzyme in cases of Cu deficiency. Similar relationship has been observed in humans who consumed food with a very low content of this element. This enzyme activity increases if an additional amount of the microelement is supplied (35). Higher SOD activity is observed in conditions that are probably initiated and developed due to free radical mechanisms. The system regulating SOD biosynthesis is sensitive to agents responsible for the increased concentration of superoxide anion, including paraquat, ozone and others (21). During inflammation, including gastritis, the reaction of the body to the increased generation of active oxygen species may synthesise additional amounts of antioxidant enzymes. The activity of SOD in the mucous membrane of the stomach is significantly higher in the patients with different conditions of atrophic gastritis than in healthy subjects (3). No explicitly consistent tendencies have been found in the studies of SOD activity in blood of patients with malignant diseases. It has been observed that SOD activity in red blood cells of children differs, depending on the localisation of the neoplasm (56). In women with breast cancer, SOD activity in erythrocytes is significantly lower than in the reference group (19). Similarly, in lung cancer patients, the activity of erythrocytic SOD is significantly lower than in the control group. Moreover, a decrease in the enzyme activity, conditioned by the stage of the disease development, has been observed (30).

In blood, 60–72% of copper is bound to ceruloplasmin – a protein which constitutes a reservoir of this microelement and a source for synthesis of other copper-dependent proteins. Antioxidative Cp activity, present only in extracellular fluids, results from its ferroxidase activity: Cp catalyses oxidation of Fe^{+2} to Fe^{+3}. Moreover, by catalysing oxidation reactions, it enables incorporation of iron into transferrin and decreases the concentration of a powerful pro-oxidative agent (6). Antioxidative properties of Cp also result from its ability to combine with Cu ions, which are strong catalysts of pro-oxidative processes. Cp is one of the proteins of an acute phase reaction. A considerable increase in its activity is observed in inflammatory conditions and in neoplastic diseases (e.g cervical and ovarian carcinomas as well as breast cancer) (29,57).

ANTIOXIDANT VITAMINS

The most common substance showing antioxidant properties is vitamin E (α-tocopherol). Due to its lipophilic nature it is associated with cellular membranes and other hydrophobic structures. The biological role of vitamin E consists in the prevention of the damage of cellular structures by breaking the chains of reactions, leading to peroxidation of polyunsaturated fatty acids. Vitamin E may inhibit oxidation processes at the stage of initiation or propagation by reacting with organic peroxyl radicals and it is transformed into vitamin E radical. This radical exhibits relatively low activity and is soon regenerated at the cost of the antioxidative

potential of other antioxidants (glutathione, ascorbic acid, quinone compounds) (2). Vitamin C and β-carotene prevent autooxidation of α-tocopherol. Vitamin C is an effective antioxidant in the hydrophilic environment, other vitamins act in the lipid environment. Thus the mixture of antioxidant vitamins may be more effective than individual ones in protection of cell structures against oxidative damage (37). Actions of antioxidant vitamins are summarised in Table 5.

Table 5. Actions of antioxidant vitamins (modified after 37)

- Scavenge oxygen free radiclas
- Stimulate humoral and cellular immunity
- Reduce promotion of mutagens
- Prevent oxidation and formation of some active carcinogens (e.g. nitrosoamine, benzo(a)pyrene)
- Vitamin C and carotenoids reduce *in vitro* growth of cancer cells
- Reduce chemical- and radiation-induced mutations
- Modulate cell signalling pathways and affect some oncogene synthesis

In vitro studies have shown that the addition of tocopherol derivatives or β-carotene to fibroblast cultures reduces the extent of chemically- and radiation-induced transformation. The authors suggest that vitamins may prevent certain mutations and thus inhibit the process of carcinogenesis. At the same time, there is evidence that the addition of individual antioxidative vitamins at low concentrations to tissue cultures may have a procarcinogenic effect. On the other hand, it has been found that the addition of a mixture of four vitamins: vitamin C, α-tocopherol, retinoic acid and β-carotene, inhibits significantly the growth of human melanoma cell culture and human parotid carcinoma cells (37).

DIETARY ANTIOXIDANT VITAMINS AND CANCER RISK

The comparison of dietary habits of different sub-populations in the USA (Adventists of the Seventh Day, vegetarians, Mormons) with the incidence and localisation of malignant neoplasms showed that different amounts of consumed animal fat, carbohydrates and vitamins, mainly vitamins A and C are correlated with the incidence of certain types of malignancies. It has been suggested that consumption of high amounts of antioxidative vitamins may have an anticarcinogenic effect. A long-term epidemiological study on large groups of patients have not explicitly proved that the diet supplementation with antioxidative vitamins decreases the incidence of neoplastic diseases (25). However, some reports have confirmed that combined supplementation with vitamins A, C and E may decrease the risk of chemically induced cancer. In populations at a particularly high risk of malignant diseases, supplemented with a mixture of vitamins A, C and E in amounts 2 – 3 times higher than RDA (Recommended Dietary Allowances), the incidence of cancer was by 13% and mortality by 10% lower. The results were particularly promising when the diet was rich in fresh vegetables and fruit containing these vitamins. The risk of lung cancer was 2.6 times higher in the population of non-smokers who consumed low amounts of yellow and red vegetables and fruits than in those with diet rich in these products (95% CI 0.8 – 8.3) (4).

The risk of oral and oesophagus cancers in relation to dietary intake of glutathione, estimated in a similar way, decreases in individuals who prefer a diet rich in glutathione consumed in fruits and vegetables (16). It has been demonstrated that the concentration of vitamin E in blood plasma of lung cancer patients is statistically lower than in healthy people (14).

The results described here indicate the relationship between active oxygen species and neoplastic diseases. It would be an oversimplification to associate the changes in activity/concentration of any antioxidant with pathogenesis of neoplasm. However, at the present stage, none of the hypotheses should be rejected, as the mechanisms responsible for neoplastic growth have not as yet been clarified despite many pronged studies.

REFERENCES

1. Altintas A, Vardar MA, Gonlusen F et al. Copper, zinc and magnesium tissue and serum levels in patients with cervical carcinoma. Eur J Gynaecol Oncol 4: 278–281, 1995.
2. Bast A, Guidi RM, Haenen M, Cees J, Doelman A. Oxidants and antioxidants: State of art. Am J Med 91: 2S–13S, 1991.
3. Beno I, Volkova K, Stachurova M. Gastric mucosal antioxidant activity in patients at increased risk of gastric cancer. Neoplasma 5: 315–319, 1993.
4. Bordo D, Djinovic K, Bolognesi M. Conserved patters in the Cu,Zn superoxide dismutase family. J Mol Biol 238: 366–386, 1994.
5. Burke KE, Combs GF Jr, Gross EG, Bhuyan KC, Abu-Libden H. The effects of topical and oral L-selenomethionine on pigmentation and skin cancer induced by ultraviolet irradiation. Nutr Cancer 17: 123–137, 1992.
6. Carver FJ, Farb DI, Frieden E. The effect of albumin, ceruloplasmin and other serum constituents on Fe(II) oxidation. Biol Trace Elem Res 4: 1–19, 1982.
7. Clemens MR. Free radicals in chemical carcinogenesis. Klin Wochenschr 69: 1123–1134, 1991.
8. Coates RJ, Weiss NS, Daling JR, Rettmer LR, Warnick GR. Cancer risk in relation to serum copper levels. Cancer Res 49: 4353–4357, 1989.
9. Collins AR. Oxidative DNA damage, antioxidants and cancer. BioEssays 21: 238-246, 1999.
10. Combs GF Jr, Gray WP. Chemopreventive agents: Selenium. Pharmacol Ther 79: 179–192, 1998.
11. Cross CE, Van der Vliet A, O'Neil ChA, Louie S, Halliwell B. Oxidants, antioxidants and respiratory tract lining fluids. Environ Health Perspect 102 (Suppl. 10): 185–191, 1994.
12. Dargel L. Lipid peroxidation – a common pathogenic mechanism. Exp Toxic Pathol 44: 169–181, 1992.
13. Dreher D, Junod AF. Role of oxygen free radicals in cancer development. Eur J Cancer 32A: 30–38, 1996.
14. Erhola M, Nieminen MM, Kellokumpu-Lehtinen P, Metsa-Ketela T, Poussa T, Alho H. Plasma peroxyl radical trapping capacity in lung cancer patients: a case-control study. Free Radic Res 26: 439–447, 1997.
15. Fico ME, Poirier KA, Watrach AM, Milner JA. Differential effects of selenium on normal and neoplastic canine mammary cells. Cancer Res 46: 3384–3385, 1986.
16. Flagg EW, Coates RJ, Jones DP et al. Dietary glutathione intake and risk of oral and pharyngeal cancer. Am J Epidemiol 139: 453–465, 1994.
17. Goldstein S, Czapski G, Cohen H, Meyerstein D. Free radicals induced peptide damage in the presence of transition metal ions. A plausible pathway for biological deleterious processes. Free Radic Biol Med 1: 11–18, 1994.
18. Goncharova EI, Rossman TG. A role for metallothionein and zinc in spontaneous mutagenesis. Cancer Res 54: 5318–5323, 1994.

19. Gromadzińska J, Skłodowska M, Wołkanin P, Wąsowicz W, Zambrano Quispe O, Płużańska A. Lipid peroxide levels and antioxidant enzyme activities in blood of breast cancer patients. J Clin Chem Biochem Nutr 13: 127–135, 1992.
20. Halliwell B, Gutteridge JMC, Cros CE. Free radicals, antioxidants and human disease: where are we now? J Lab Clin Med 119: 598–620, 1992.
21. Ichikawa I, Kiyama S, Yoshioka T. Renal antioxidant enzymes: their regulation and function. Kidney Int 45: 1–9, 1994.
22. Kehrer JP. Free radicals as mediators of tissue injury and disease. Crit Rev Toxicol 23: 21–48, 1993.
23. Kehrer JP, Lund LG. Cellular reducing equivalents and oxidative stress. Free Radic Biol Med 1: 65–75, 1994.
24. Kiremidjian-Schumacher L, Roy M, Glickman R et al. Selenium and immunocompetence in patients with head and neck cancer. Biol Trace Elem Res 73: 97–111, 2000.
25. Knekt P, Jarvinen R, Seppanen R et al. Dietary antioxidants and the risk of lung cancer. Am J Epidemiol 134: 471–479, 1991.
26. Knekt P, Marniemi J, Heliovaara M, Aroma A. Is low selenium status a risk factor for lung cancer? Am J Epidemiol 148: 975–982, 1998.
27. Kok FJ, van Duijn CM, Hoffman A. Serum copper and zinc and the risk of death from cancer and cardiovascular disease. Am J Epidemiol 128: 352–357, 1988.
28. Krinski NJ. Mechanism action of biological antioxidants. Proc Soc Exp Biol Med 200: 248–254, 1992.
29. Linder MC, Hazegh-Azam M. Copper biochemistry and molecular biology. Am J Clin Nutr 63: 791S–811S, 1996.
30. Martin-Mateo MC, Molpeceres LM, Ramos G. Assay for erythrocyte superoxide dismutase in patients with lung cancer and effects on pollution and smoke trace elements. Biol Trace Elem Res 60: 215–226, 1997.
31. Medina D, Lane HW, Shepherd F. Effects of selenium on mouse mammary tumorigenesis and glutathione peroxidase activity. Anticancer Res 1: 377–383, 1981.
32. Medina D, Shepherd F. Selenium mediated inhibition of mouse mammary tumorigenesis. Cancer Lett 8: 241–245, 1980.
33. Nakazawa H, Genka C, Fujishima M. Pathological aspects of active oxygens/free radicals. Jap J Physiol 46: 15–32, 1996.
34. Odeh M. The role of zinc in acqurired immunodeficiency syndrome. J Int Med 231: 463–469, 1992.
35. Olivares M, Uauy R. Copper as an essential nutrient. Am J Clin Nutr 63: 791S–796S, 1996.
36. Perchellet JP, Perchellet EM, Orten DK, Schneider BA. Inhibition of the effects of 12-o-tetradacanoylphorbol-13-acetate on mouse epidermal glutathione peroxidase and ornitine decarboxylase activities by glutathione level-raising agents and selenium containing compounds. Cancer Lett 26: 283–287, 1985.
37. Prąsad KN, Hovland AR, Cole WC, Prasad JE. Vitamin supplements and modification in diet and lifestyle: essential ingredients for a cancer prevention strategy. In: Cancer and Nutrition, eds. KN Prasad, WC Cole. IOS Press, London, 21–36, 1998.
38. Rangen U, Bulkley GB. Prospects for treatment of free radical-mediated tissue injury. Br Med Bull 3: 700–718, 1993.
39. Rea HM, Thompson CD, Campbell DR, Robinson MF. Relation between erythrocyte selenium concentrations and glutahione peroxidase (E.C. 1.11.1.9) activities of New Zealand residents and visitors to New Zealand. Brit J Nutr 42: 201–208, 1979.
40. Schrauzer GN. Selenium. Mechanistic aspects of anticarcinogenic action. Biol Trace Elem Res 32: 51–61, 1992.
41. Shamberger RJ. Selenium metabolism in man and animals, In: Frontiers in Bioinorganic Chemistry, ed. AV Xavier. Wienheim, 152–159, 1986.
42. Shamberger RJ, Frost DV. Possible protective effect of selenium against human cancer. Can Med Assoc 100: 682–683, 1969.
43. Sies H. Strategies of antioxidant defense. Eur J Biochem 215: 213–219, 1993.
44. Sies H, de Groot H. Role of reactive oxygen species in cell toxicity. Toxicol Lett 64: 547–551, 1992.

45. Singh SV, Haque AK, Ahman H, Medh RD, Awasthi YC. Glutathione S-transferase isoenzymes in human lung tumours. Carcinogenesis 9: 1681–1685, 1988.
46. Slaga TJ, Fischer SM, Nelson K, Gleason GL. Studies on the mechanism of skin tumor promotion. Proc Natl Acad Sci (USA) 77: 3659–3663, 1980.
47. Slater TF. Free radicals in medicine. Free Radic Res Commun 7: 119–190, 1989.
48. Solanki V, Rana TRS, Slaga TJ. Diminution of mouse epidermal superoxide dismutase and catalase activities by tumor promotors. Carcinogenesis 2: 1141–1146, 1981.
49. Sun Y. Free radicals, antioxidant enzymes and carcinogenesis. Free Radic Biol Med 8: 583–599, 1990.
50. Tompson JA, Bolton JL, Malkinson AM. Relationship between the metabolism of butylated hydroksytoluene (BHT) and lung tumor promotion in mice. Exp Lung Res 17: 439–453, 1991.
51. Turnlund JR. Copper nutriture, bioavailability, and the influence of dietary factors. J Amer Diet Assoc 88: 303–310, 1988.
52. Van Wouwe JP, Van Zulen L. Changes in zinc values in children during malignant disease. Biol Trace Elem Res 30: 197–204, 1991.
53. Vinceti M, Rovesti S, Bergomi M, Vivoli G. The epidemiology of selenium and human cancer. Tumori 86: 105–118, 2000.
54. Walsh CT, Sandstead HH, Prasad AS, Newberne PM, Fraker PJ. Zinc: Health effects and research priorities for the 1990s. Environ Health Persp 102 (suppl. 2): 5–46, 1994.
55. Wąsowicz W, Gromadzińska J, Skłodowska M, Wołkanin P, Zambrano Quispe O, Płużańska A. Selenium concentrations and glutathione peroxidase activities in blood of breast cancer women. Trace Elem Med 4: 168–172, 1993.
56. Wąsowicz W, Kantorski J, Perek D, Popadiuk S. Concentration of zinc and zinc-copper superoxide dismutase activity in red blood cells in normals and children with cancer. J Clin Chem Clin Biochem 27: 413–418, 1989.
57. Wirth PL, Linder MC. Distribution of copper among components of human serum. J Natl Cancer Inst 75: 277–284, 1985.
58. Yu BP. Cellular defenses against damage from reactive oxygen species. Physiol Rev 1: 139–162, 1994.
59. Zachara BA, Marchaluk-Wiśniewska E, Maciąg A, Pepliński J, Skokowski J, Lambrecht W. Decreased selenium concentration and glutathione peroxidase activity in blood and increase of these parameters in malignant tissue of lung cancer patients. Lung 175: 321–332, 1997.

Received for publication: July 31, 2000
Approved for publication: September 4, 2000