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Role of oxidative DNA damage in cancer initiation and promotion

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Normal aerobic metabolism produces huge amounts of potentially dangerous oxidants, controlled by a variety of antioxidant systems. An imbalance between the generated and exogenously inflicted oxidants and the oxidant system is termed oxidative stress. Even without oxidative stress, i.e. under normal physiological conditions, the damage to vital cellular micromolecules, such as DNA, is extensive, amounting to hundreds of hits per cell per day. More than one hundred different oxidative modifications in DNA have been described. The hydroxylation of guanine in the 8-position is the most frequent and most mutagenic lesion described. The 8-hydroxylation of guanine leads to lack of base pairing specifically and misreading of the modified base and adjacent residues. The modifications to DNA are so frequent that extensive and specific repair is needed for survival. Indeed, multiple repair enzyme systems to mediate and remove/repair oxidative DNA modification are described. Within DNA, hot-spots of oxidative modification and subsequent mutation have been described, and some specificity appears as compared to other agents that can lead to modification of DNA, i.e. aflatoxin and benzo[a]pyrene. Numerous publications from epidemiology and intervention studies with antioxidants point at oxidative modification as an important factor in cancer development at certain sites. Yet, direct evidence linking oxidative DNA modification to cancer has not been published. With regard to antoxidant prevention of cancer no effective single substance has so far been identified.

Key words: antioxidants, cancer, DNA repair, mutations, oxidative DNA damage

Oxidative stress

Under physiological conditions normal aerobic metabolism gives rise to active and potentially dangerous oxidants in cells and tissues. Pathophysiologically, inflammation, ischaemia/reperfsion, radiation, lifestyle factors, and exposure to foreign compounds may give rise to oxidants. The various flavo- and heme proteins involved in the cellular energy processes often 'leak' electrons resulting in generation of reactive oxygen species derived from molecular oxygen. It has been estimated that of the total oxygen consumed a few per cent result in reactive oxygen species, maybe higher in the liver than in other organs (Chance *et al*, 1979). The deleterious effect of these oxidants is usually controlled by several antioxidant systems, an imbalance between the generated and exogenously inflicted oxidants, and the antioxidant systems is termed oxidative stress. The oxygen radicals and reactive oxygen species are potentially deleterious to the cell because interactions with cellular (macro)molecules may produce

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secondary and tertiary free radicals, *eg* from amino acids, ascorbic acid, glutathione, lipids (Freeman and Crapo, 1982). Paradoxically, such interactions may also work as antioxidant functions (Ames *et al*, 1993).

The modifications to vital cellular macromolecules such as DNA by reactive oxygen species are quite extensive, amounting to about 10^5 hits to DNA per cell per day (Fraga *et al*, 1990). In relation to cancer development, DNA modifications are considered particularly relevant (Ames *et al*, 1995; Poulsen and Loft, 1995).

A plethora of modifications from oxidative stress have been described totalling more than 100 different forms of damage (von Sonnentag, 1987; Dizdaroglu, 1993; Demple and Harrison, 1994). The levels reported from the various laboratories using different techniques span several orders of magnitude and artefacts have been suggested (Halliwell and Dizdaroglu, 1992; Halliwell, 1993; Ravanat *et al*, 1995). This controversy will not be discussed further in the present paper.

The lines of evidence between oxidative DNA modifications/damage and cancer range from details on the chemistry of oxidative DNA modification, detailed molecular evidence of the consequences of oxidative DNA modification, to indirect observational evidence, epidemiological evidence, and evidence from intervention with antioxidant supplementation, and dietary changes.

Chemistry of oxidative DNA modification

More than 100 modifications in DNA from reactive oxygen species have been described (von Sonnentag, 1987; Dizdaroglu, 1992) including different modifications of purines, pyrimidines, deoxyribose and single-strand breaks, double-strand breaks and intrastrand cross-links. Much of the evidence comes from *in vitro* experimentation and from studies of the radiation chemistry of DNA and nucleotides. Often the primary damages are unstable and further products arise from hydrolysis and rearrangements. The modifications arise from the classical reactive oxygen species single oxygen($^1O_2$), superoxide anion ($O^{\bullet -}$), hydrogen peroxide (H_2O_2), the hydroxyl radical ($OH^{\bullet}$), peroxyl ($RO_2^{\bullet}$), alkoxyl ($RO^{\bullet}$) and other species that are easily converted into radicals such as hypochlorite (HOCl) and peroxynitrite ($ONOO^-$). These reactive oxygen species, of which some are free radicals, are collectively termed ROS. Increasing interest have been given to reactive nitrogen species, termed RNS, and a collective term ROS/RNS has been suggested (Wiseman and Halliwell, 1996).

It is beyond doubt that the ROS/RNS can modify DNA and low steady-state levels have been detected in nuclear DNA from human cells and tissues (Wiseman and Halliwell, 1996, and the review and references therein). However, while ROS-mediated modifications have been described extensively, descriptions of RNS-induced modifications are less detailed. It was demonstrated *in vitro* that peroxynitrate reacts with guanine, forming 8-nitroguanine (Yermilov *et al*, 1995a), which, however, is spontaneously removed at physiological conditions *in vitro* with a half-life of 4 h, resulting in AP sites (Yermilov *et al*, 1995b). 8-Aminoguanine can also be produced by *in vivo* administration of the hepatocarcinogen 2-nitropropane, which also induces an increase in 8-oxodG, mainly by oxygen species formed during the metabolism of the carcinogen, but possibly also by deamination of 8-aminodeoxyguanosine or rearrangement and hydrolysis of a 7-aminoguanosine intermediate (Sodum *et al*, 1993).

In isolated bacteriophage PM2 exposed to peroxynitrite or to SIN-1 which generates peroxynitrite, NO, and superoxide, a high number of Fpg sensitive modifications was found (Epe *et al*, 1996) indicating that a single reactive intermediate rather than peroxynitrate itself is responsible for the damage. This is also supported by the finding with calf thymus DNA *in vitro* where peroxynitrite-induced formation of 8-oxodG could be inhibited by hydroxyl radical scavengers (Inoue and Kawanishi, 1995). Presumably peroxynitrate can be regarded as a transport form for hydroxyl radicals. Regardless of the mechanism, the combined action of oxidative stress and chronically elevated NO production is a hallmark of chronic infections and could be one mechanism which could explain why chronic infection increases the risk of certain cancers (Liu and Hotchkiss, 1995). Evidence from infections with *Helicobacter* and experimental hepatitis supports this notion (Togashi *et al*, 1993; Young *et al*, 1996).

Consequences of oxidative DNA modifications

In humans, the multiplicity of oxidative modifications of DNA and their consequences have not been described exhaustively. However, an array of investigations *in vitro* and in cells demonstrate that, for example, C-8 hydroxylation of guanine leads to a lack of base pairing specificity and misreading of the modified base and adjacent residues (Kuchino *et al*,

1987) when looking at DNA synthesis on oligodeoxynucleotide templates. Later reports contrast this finding and showed incorporation of dCMP and dAMP selectively opposite 8-oxodG (Shibutani *et al*, 1991), and the discrepancy was suggested to be related to differences in the DNA polymerase used. The consequence of formation of amino- and nitroguanine (or - adenine) is dominantly depurination leading to AP sites (deRojas-Walker *et al*, 1995; Tamit *et al*, 1996;, pairing and mutational consequences were not elucidated.

The genome contains 3×10^9 base pairs which gives a total of about 30,000 8-hydroxylated guanine bases per cell. The most conservative estimation of the rate of guanine oxidation from the urinary excretion of 8-oxodG is about 14–60 nmol/70 kg (200–600 pmol per kg BW (Loft *et al*, 1992)), amounting to about 158–504 hits per day. If repair suddenly failed completely this would mean a rapid accumulation of oxidized hits in DNA, with a doubling time of 60 to 190 days. In a study of 8-oxodG in human brain the accumulation rate in the nuclear DNA was about 10-fold higher in older people (79 years) compared to that in younger subjects (42 years), and was even higher for mitochondrial DNA (Mecocci *et al*, 1993). In studies of 8-oxodG in tissues from humans in a broad age range no sign of major accumulation is apparent (Bergtold *et al*, 1990; Bartsch *et al*, 1991; Loft and Poulsen, 1996). Similarly, animal experiments have shown minimal and only organ specific accumulation of 8-oxodG in DNA (Hirano *et al*, 1996). After ionizing radiation the increase in urinary excretion of thymine glycol and 8-oxodG had passed within 24 h in humans (Bergtold *et al*, 1990; Bergtold and Simic, 1991) whereas excess 8-oxodG was removed from mouse liver DNA after approximately 90 min (Kasai *et al*, 1986).

Given the high rate of oxidative damage, presumably at least five times the rate of 8-oxodG formation and because of other types of damage (Demple and Harrison, 1994; Jaruga and Dizdaroglu, 1996), repair is a necessity. Assuming an oxidative damage rate of 2,500 for each base per 24 h, it would take about 8 years to oxidize 1% of the bases in the genome. Considering that oxidation is located in certain hot spots (Hoebee *et al*, 1993) such a high number of modifications appears far beyond the limit for survival.

Indeed, there are multiple repair enzyme systems to mediate the removal and repair of oxidative damage from DNA and to help counteract the cytotoxic, mutagenic, and carcinogenic effects (Dimple and Harrison, 1994), whether the reactive oxygen species originate from cellular metabolism, ionizing radiation, oxidation of xenobiotica, lifestyle factors, genetic factors or other known or unknown sources. Free-radical attack on DNA produces many different modifications and this constant and significant threat is counteracted by enzymes that attack specific types of oxidative damage. Some of these enzymes recognize a range of substrates. The knowledge about DNA repair mechanisms is increasing rapidly: since 1993 about 3,500 papers concerning DNA repair have been indexed by MedLine. The repair process is now clearly divided into several steps performed by several, and sometimes alternative, protein functions. The sequence is damage recognition, lesion demarcation and unwinding, dual incision, release of damaged nucleotide, gap filling and DNA synthesis (Bohr *et al*, 1993, and reviews and references therein). Of particular interest are three general mechanisms: direct repair, base-excision repair, and nucleotide-excision repair. These repair systems act on damaged DNA; in addition, other and distinctly different systems act on mismatches generated by replication by enzymes that perform different base- and nucleotide-excisions.

Glycosylase activity results in excretion of the oxidatively modified base, *eg* 8-oxoGua into urine, and nucleotide/nucleoside excision in the excretion of the nucleoside (Shigenaga *et al*, 1989) and complete excretion into urine (Loft *et al*, 1995). A wide range of different glycosylase activities has been described. The repair protein Fgp-glycosylase is of particular interest because of its apparent specificity towards oxidatively modified DNA bases and abasic sites (Tchou *et al*, 1991; Tchou and Grollman, 1995). Nucleotide/nucleoside excision repair (Sancar, 1995) results in fast (Loft *et al*, 1995) excretion of the oxidatively modified nucleoside into urine (Shigenaga *et al*, 1989) and appears also in human cells (Nagashima *et al*, 1997). The various repair enzymes seem to have a broader specificity than previously expected (Demple and Harrison, 1994).

As indicated in the previous section oxidative DNA damage will lead to misreading of DNA templates, and if it persists for a long time this will lead to formation of an incorrect protein, which may or may not show a changed function. However, repair is rapid and efficient as argued earlier in this paper and this mechanism presumably is without much importance for the development of cancer. More important, studies using the reversion assay and the

forward mutation assay show a high frequency of G → T and A → C substitutions, indicating that mutagenic replication of 8-oxodG as the template causes G → T substitutions, and misincorporation of 8-oxodG as substrate causes A → C substitution. This mutation is found with particularly high frequency in the hotspot codons 248/249 of the human tumour suppressor gene p53 after ROS treatment (Hussain *et al*, 1994). Although not as specific as initially hypothesized a mutational pattern appears in p53 (Figure 1) from various chemical insults.

Indirect observational evidence

Numerous observations have been published concerning the relation between the metabolic rate of various mammalian species and the cumulative incidence of cancer (Cutler 1991; Ames *et al*, 1993), whereas the urinary excretion of oxidative DNA repair products correlates (Figure 2) with the specific metabolic rate (Fraga *et al*, 1990; Loft *et al*, 1993). Cumulative cancer incidence increases with a power function of age (Ross *et al*, 1982) and since it also relates to the specific oxygen consumption across species it has been hypothesized that oxygen consumption is a major determinant for human cancer. In support of this hypothesis, an increased incidence of some types cancer has been found in hyperthyroid women (Goldman *et al*, 1990), as well as an association between leanness and low body mass index and lung cancer (Knekt *et al*, 1991; Kabat and Wynder, 1992).

Within this context we have demonstrated (Figure 3) that human urinary excretion of the DNA repair product 8-oxodG varies about 7-fold (Loft *et al*, 1992), and that in normal weight healthy women there is a high correlation between individual oxygen uptake and urinary excretion of 8-oxodG (Loft *et al*, 1994). These observations open up the possibility of establishing, in humans, a relation between oxidative stress to DNA and cancer, from nested case–control studies, for example. Such studies are initiated but no data are yet available. Although not indicative of a causal relationship these data are in line with the notion that oxidative stress to DNA could be linked to cancer development. Interestingly, the cancer types that appear particularly related to vegetable-antioxidant intake in humans are related to epithelial surfaces (Block 1991, 1992). It could thus be hypothesized that the combination of constant proliferating cells and oxidative stress to DNA are major determinants for cancer development.

Evidence from descriptive epidemiology

Another line of evidence stems from relations between antioxidant content in human food and cancer incidence. This type of evidence is based on the antioxidant properties of antioxidant vitamins that are considered to quench the deleterious effects of reactive oxygen species, thereby reducing the oxidative modifications of DNA. Quite consistent results have been given in epidemiological studies

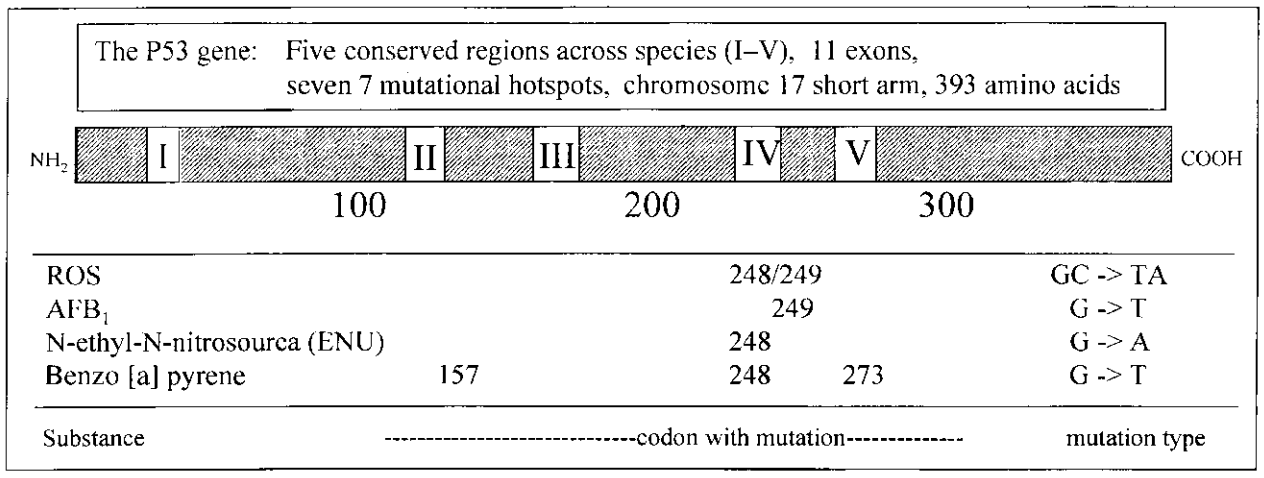

Figure 1. Graphic depiction of tumour supressor gene p53 for mutational hotspots (Aguilar *et al*, 1994; Amstad *et al*, 1994; Cerutti *et al*, 1994; Ziegler *et al*, 1994; Denissenko *et al*, 1996) for reactive oxygen species (ROS), aflatoxin (AFB1), *N*-ethyl-*N*-nitrosourea (ENU), and benzo[a]pyrene.

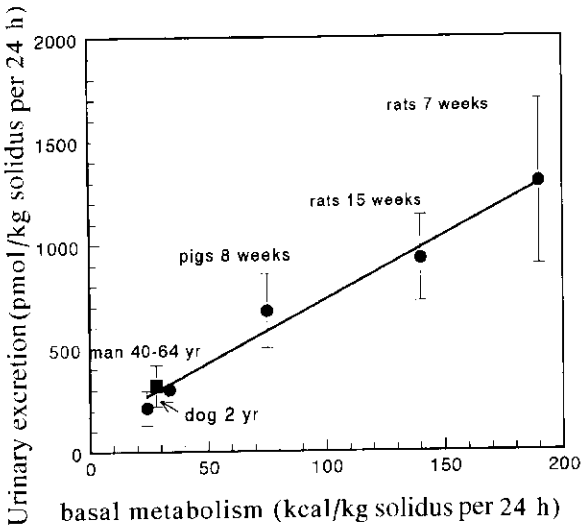

Figure 2. Relationship between basal metabolism and urinary excretion of the repair product of 8-hydroxylation of guanine in DNA (adapted from Loft *et al*, 1993).

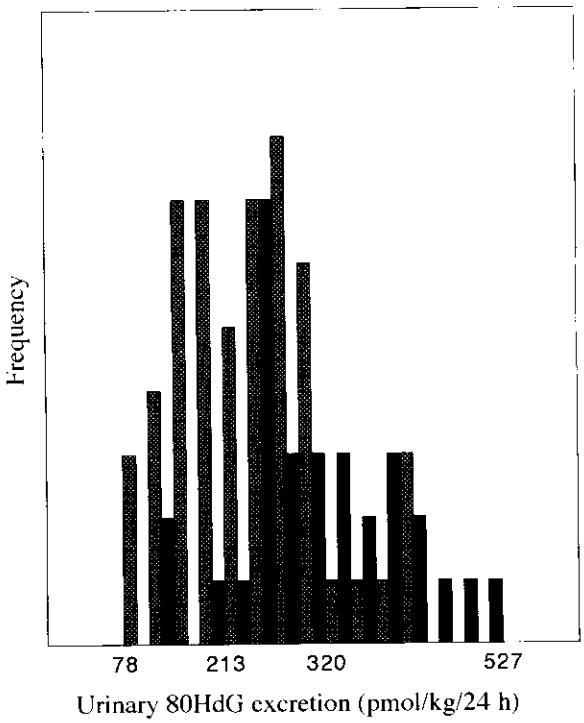

Figure 3. Urinary excretion of the repair product 8-oxodG from DNA oxidation in a random population sample. The black bars indicate the frequency of non-smokers; and the white bars, the frequency of smokers, related to urinary 24 h excretion of 8-oxodG. The numbers on the *x*-axis indicate the ranges (outer numbers) and the means of non-smokers and smokers (inner numbers). (The figure is adapted from Loft *et al*, 1992.)

linking high intake of food rich in antioxidant vitamins to reduced cancer incidence, particularly for epithelial cancers in the upper body (Block *et al*, 1992; Ames *et al*, 1995). It is noteworthy that this association can be refound at baseline in a controlled trial, which, however, could not confirm an effect after substitution with low dose α-tocopherol and high dose β-carotene (Heinonen *et al*, 1994).

Evidence from intervention with antioxidant supplementation and dietary changes

Of the two major types of intervention trial, one relies on measuring hard endpoints and one on intermediate markers. The first large intervention trial performed in China, the Linxian study (Blot *et al*, 1993), showed a reduction in the risk of stomach cancer after supplementation with a combination of β-carotene, vitamin E and selenium, the relative risk for total cancer being 0.87, CI = 0.75–1.00. The next published trial, the ATBC trial conducted in Finland (Heinonen *et al*, 1994) unexpectedly showed an 18% (95% CI, 3–36%) higher incidence of lung cancer among the men who received β-carotene than those who did not. In the CARET study (β-carotene and retinol efficacy trial) the finding of a 46% (95% CI, 7–100%) increased risk of lung cancer after treatment with β-carotene and retinol (Omenn *et al*, 1996) led to its cessation 21 months earlier than planned, with 5 years further follow-up. On the other hand, the American physician's health study (Hennekens *et al*, 1996), conducted over 12 years, and thereby the longest intervention trial performed, showed neither benefit nor harm in terms of incidence of malignant neoplasms, cardiovascular diseases, or death from all causes. In these intervention trials the focus has mainly been on β-carotene. In the ATBC trial no effects were found from the moderate 20 mg dose of α-tocopherol and in the American physician's health study 25,000 IU retinol showed no effect. A number of studies are ongoing with the traditional antioxidants β-carotene and α-tocopherol, but the results are not available at present.

Investigations such as the above-cited controlled intervention trials with hard end-points are extremely costly and suffer from a number of difficulties. These include: no dose–response information; the influence from unknown factors due to the long observation times necessary; the lack of appropriate details concerning whether the trial is a primary or secondary prevention trial; the selection of a study population where no preventive effect is possible; selection of the

wrong antioxidant(s); and the impossibility of exploiting synergistic effects of several antioxidants. The biomarker approach, where one or more events in the carcinogenic process is measured, has been suggested (Sculte and Perera, 1992) as a mechanism-based approach to overcome some of the difficulties in hard end-points primary intervention trials with regard to cancer.

Such a biomarker approach measurement is the urinary excretion of repair products of DNA, damage *eg* 8-oxodG, which has been used to estimate the effects of antioxidant supplementation. These studies are in accordance with the intervention trials cited above, as no change was found in the DNA oxidation rate after double-blinded intervention with β-carotene (van Poppel *et al*, 1995). In agreement, β-carotene had no influence on DNA damage, as reflected by sister chromatid exchanges (van Poppel *et al*, 1995), but on the other hand micronuclei in sputum were reduced in the β-carotene treated group of smokers (van Poppel *et al*, 1992). DNA damage is assumed to be a prerequisite for the mutations that are a necessary, but not the ultimate, step in the carcinogenic process (Loft and Poulsen, 1996, and references therein).

A controlled intervention trial with daily doses of vitamin C 500 mg, α-tocopherol 200 mg (both antioxidants), coenzyme Q10 or placebo did not reveal any change in the rate of DNA oxidation, as measured by urinary excretion of 8-oxodG (Prieme *et al*, 1997). Although vitamin C seems to have little or no overall effect, it is of interest that supplementation with vitamin C clearly reduced oxidative DNA modification in sperm cells, as measured by their 8-oxodG content (Scadding *et al*, 1988; Fraga *et al*, 1991). Another intervention study where vitamin C (100 mg), α-tocopherol (280 mg), and β-carotene were given to smokers showed reduced oxidative damage in lymphocyte DNA, as measured by the Comet assay. In addition, the lymphocytes showed increased resistance to *in vitro* challenge with hydrogen peroxide (Duthie *et al*, 1996). The Comet assay, single-cell gel electrophoresis, is believed to specifically determine endogenous damage to pyrimidines, *eg* in lymphocytes, but also includes strand breaks and maybe other types of damage to DNA (Duthie *et al*, 1996). In experimental settings the Comet assay correlates with the measurement of 8-oxodG (Deng *et al*, 1997).

In non-smokers, replacement of non-cruciferous vegatables with the brassica vegetable Brussels's sprouts, 300 g per day, led to a 30% decrease in the rate of oxidative DNA damage, as measured by urinary excretion of 8-oxodG (Verhagen *et al*, 1995), supporting the notion that more than one antioxidant is necessary to reduce spontaneous endogenous DNA damage, or that even other substances or combination of substances could be responsible for this effect.

Conclusion

The large bulk of evidence points at active principles in dietary fruit and vegetables, the intake of which is associated with reduced risk of certain cancers, particularly cancers from the epithelial surfaces in the upper part of the body. Mechanistic and epidemiological evidence supports this notion, and the antioxidants β-carotene, α-tocopherol, and ascorbic acid have for a long time been the prime candidates for this effect. Regarding β-carotene, several large scale clinical intervention trials have clearly demonstrated that the cancer preventive effects can not be related to β-carotene as a single substance. The epidemiological evidence repeatedly and consistently associate a diet rich in fruit and vegetables with low cancer incidence. Nevertheless, it is not known whether changing the diet also changes the risk of cancer. With regard to α-tocopherol the controlled intervention studies are still too few and inconclusive to determine if it possesses cancer preventive effects, and controlled intervention studies with vitamin C are not available. The intervention studies with single or two antioxidants are in line with controlled intervention studies using biomarkers for oxidative DNA damage, and a single small intervention study supports the cancer protective role of brassica vegetables.

There appears little doubt that fruit and vegetables contain active principles that have a potential for preventing certain forms of cancer. It has not been possible to identify a single substance, but the possibility that β-carotene is such a substance has, essentially, been ruled out. The active principle, whether it is a single substance or a combination of substances, remains obscure.

References

Aguilar F, Harris CC, Sun T, Hollstein M, Cerutti P (1994). Geographic variation of p53 mutational profile in nonmalignant human liver. *Science* **264:** 1317–9.

Ames BN, Shigenaga MK, Hagen TM (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci, USA* **90:** 7915–22.

Ames BN, Gold LS, Willett WC (1995). The causes and prevention of cancer. *Proc Natl Acad Sci, USA* **92:** 5258–65.

Amstad P, Hussain SP, Cerutti P (1994). Ultraviolet B light-induced mutagenesis of p53 hotspot codons 248 and 249 in human skin fibroblasts. *Mol Carcinogen* **10:** 181–8.

Bartsch H, Petruzelli S, De Flora S *et al* (1991). Carcinogen metabolism and DNA adducts in human lung tissues as affected by tobacco smoking or metabolic phenotype: a case–control study on lung cancer. *Mut Res* **250:** 103–14.

Bergtold DS, Simic MG (1991). Hydroxy radical in radiation dosimetry and metabolism: dietary caloric effect. *Prog Clin Biol Res* **372:** 21–32.

Bergtold DS, Berg CD, Simic MG (1990). Urinary biomarkers in radiation therapy of cancer. *Adv Exp Med Biol* **264:** 311–6 .

Block G (1991). Vitamin C and cancer prevention: the epidemiologic evidence. *Am J Clin Nutr* **53:** 270S–82S.

Block G (1992). The data support a role for antioxidants in reducing cancer risk. *Nutr Rev* **50:** 207–13.

Block G, Patterson B, Subar A (1992). Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutr Cancer* **18:** 1–29.

Blot WJ, Li J-Y, Taylor PR *et al* (1993). Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. *J Natl Cancer Inst* **85:** 1483–92.

Bohr VA, Wassermann K, Kraemer KH (1993). *DNA Repair Mechanisms*. Alfred Benzon Symposium No. 35. Munksgaard, Copenhagen.

Cerutti P, Hussain P, Pourzand C, Aguilar F (1994). Mutagenesis of the H-ras protooncogene and the p53 tumor suppressor gene. *Cancer Res* **54(Suppl):** 1934–8.

Chance B, Sies H, Boveris A (1979). Hydroperoxide metabolism in mammalian organs. *Physiol Rev* **59:** 527–605.

Cutler RG (1991). Human longevity and aging: possible role of reactive oxygen species. *Ann NY Acad Sci* **621:** 1–28.

Demple B, Harrison L (1994). Repair of oxidative damage to DNA: enzymology and biology. *Ann Rev Biochem* **63:** 915–48.

Deng X, Tuo J, Poulsen HE, Loft S (1997). 2-Nitropropane induced DNA damage in rat bone marrow. *Mut Res* **1391:** 165–9.

Denissenko MF, Pao A, Moon-shong T, Pfeifer GP (1996). Preferential formation of benzo[a]pyrene adducts at lung cancer mutational hotspots in p53. *Science* **274:** 430–2.

deRojas-Walker T, Tamir S, Ji H, Wishnok JS, Tannenbaum SR (1995). Nitric oxide induces oxidative damage in addition to deamination in macrophage DNA. *Chem Res Toxicol* **8:** 473–7.

Dizdaroglu M (1992). Oxidative damage to DNA in mammalian chromatin. *Mut Res* **275:** 331–42.

Dizdaroglu M (1993). Chemistry of free radical damage to DNA and nucleoproteins. In *DNA and Free Radicals* (eds Halliwell B, Aruoma OI); Ellis Horwood Ltd, Chichester, UK, pp. 19–39.

Duthie SJ, Ma A, Ross MA, Collins AR (1996). Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. *Cancer Res* **56:** 1291–5.

Epe B, Ballmaier D, Roussyn I, Briviba K, Sies H (1996). DNA damage by peroxynitrite characterized with DNA repair enzymes. *Nucl Acids Res* **24:** 4105–10.

Fraga C, Shigenaga MK, Park J-W, Degan P, Ames BN (1990). Oxidative damage to DNA during aging: 8-hydroxy-2'-deoxyguanosine in rat organ DNA and urine. *Proc Natl Acad Sci, USA* **87:** 4533–7.

Fraga CG, Motchnik PA, Shigenaga MK, Helbock HJ, Jacob RA, Ames BN (1991). Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. *Proc Natl Acad Sci, USA* **88:** 11003–6.

Freeman BA, Crapo JD (1982). Biology of disease. Free radicals and tissue injury. *Lab Invest* **47:** 412–26.

Goldman MB, Monson RR, Maloof F (1990). Cancer mortality in women with thyroid disease. *Cancer Res* **50:** 2283–9.

Halliwell B (1993). Oxidative DNA damage: meaning and measurement. In *DNA and Free Radicals* (eds Halliwell B, Aruoma OI); Ellis Horwood Ltd, Chichester, UK, pp. 67–79.

Halliwell B, Dizdaroglu M (1992). The measurement of oxidative damage to DNA by HPLC and GC/MS techniques. *Free Rad Res Commun* **16:** 75–87.

Heinonen OP, Albanes D *et al* (1994). The effect of vitamin E and β-carotene on the incidence of lung cancer and other cancers in male smokers. *New Engl J Med* **330:** 1029–35.

Hennekens CH, Buring JE, Manson JE *et al* (1996). Lack of long-term supplementation with β-carotene on the incidence of malignant neoplasms and cardiovascular disease. *New Engl J Med* **334:** 1145–9.

Hirano T, Yamaguchi R, Asami S, Iwamoto N, Kasai H (1996). 8-Hydroxyguanine levels in nuclear DNA and its repair activity in rat organs associated with age. *J Gerontology* **51:** B303–7.

Hoebee B, Braun JE, Lutgerink JT *et al* (1993). Mutational specificity of oxidative DNA damage. *Mutat Res* **299:** 165–82.

Hussain SP, Aguilar F, Cerutti P (1994). Mutagenesis of codon 248 of the human p53 tumor suppressor gene by *N*-ethyl-*N*-nitrosourea. *Oncogene* **9:** 13–8.

Inoue S, Kawanishi S (1995). Oxidative DNA damage induced by simultaneous generation of nitric oxide and superoxide. *FEBS Lett* **371:** 86–8.

Jaruga P, Dizdaroglu M (1996). Repair of products of oxidative DNA base damage in human cells. *Nucl Acids Res* **24:** 1389–94.

Kabat GC, Wynder EL (1992). Body mass index and lung cancer risk. *Am J Epidemiol* **135:** 769–74.

Kasai H, Crain PF, Kuchino Y, Nishimura S, Ootsuyama A, Tanooka H (1986). Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. *Carcinogenesis* **7:** 1849–51.

Knekt P, Heliovaara M, Rissanen A *et al* (1991). Leanness and lung-cancer risk. *Int J Cancer* **49:** 208–13.

Kuchino Y, Mori F, Kasai H *et al* (1987). Misreading of DNA templates containing 8-hydroxydeoxyguanosine at the modified base and at adjacent residues. *Nature* **327:** 77–9.

Liu RH, Hotchkiss JH (1995). Potential genotoxicity of chronically elevated nitric oxide: a review. *Mut Res* **339:** 73–89.

Loft S, Poulsen HE (1996). Cancer risk and oxidative DNA damage in man. *J Molec Med* **74:** 297–312.

Loft S, Vistisen K, Ewertz M *et al* (1992). Oxidative DNA-damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Carcinogenesis* **13:** 2241–7.

Loft S, Fischer-Nielsen A, Jeding IB, Vistisen K, Poulsen HE (1993). 8-Hydroxydeoxyguanosine as a biomarker of oxidative DNA damage. *J Toxicol Environ Health* **40:** 391–404.

Loft S, Astrup A, Buemann B, Poulsen HE (1994). Oxidative DNA damage correlates with oxygen consumption in humans. *FASEB J* **8:** 534–7.

Loft S, Larsen PN, Rasmussen A *et al* (1995). Oxidative DNA damage after transplantation of the liver and small intestine in pigs. *Transplantation* **59:** 16–20.

Mecocci P, MacGarvey U, Kaufman AE *et al* (1993). Oxidative damage to mitochondrial DNA shows marked age-dependent increases in human brain. *Ann Neurol* **34:** 609–16.

Nagashima M, Sasaki A, Morishita K *et al* (1997). Presence of human cellular protein(s) that specifically binds and cleaves 8-hydroxyguanine containing DNA. *Mutat Res* **383:** 49–59.

Omenn GS, Goodman GE, Thornquist MD *et al* (1996). Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *New Engl J Med* **334:** 1150–5.

Poulsen HE, Loft S (1995). Early biochemical markers of effects: enzyme induction, oncogene activation and markers of oxidative damage. *Toxicol* **101:** 55–64.

Prieme H, Loft S, Nyyssvnen K, Salonen JT, Poulsen HE (1997). No effect of supplementation with vitamin E, ascorbic acid or coenzyme Q10 on oxidative DNA damage estimated by 8-oxo-7,8-dihydro-2′-deoxyguanosine excretion in smokers. *Am J Clin Nutr* **65:** 503–7.

Ravanat J-L, Turesky RJ, Gremaud E, Trudel LJ, Stader RH (1995). Determination of 8-oxoguanine in DNA by gas chromatography–mass spectrometry and HPLC–electrochemical detection: overestimation of the background level of the oxidized base by the gas chromatography–mass spectrometry assay. *Chem Res Toxicol* **8:** 1039–45.

Ross MH, Lustbader ED, Reddy MK, Lalwani ND (1982). Dietary practices of early life and spontaneous tumors of the rat. *Nutr Cancer* **3:** 150–67.

Sancar A (1995). Excision repair in mammalian cells. *J Biol Chem* **270:** 15915–8.

Scadding GK, Ayesh R, Brostoff J *et al* (1988). Poor sulphoxidation ability in patients with food sensitivity. *Br Med J* **297:** 105–7.

Sculte PA, Perera FP (1992). *Molecular Epidemiology. Principles and Practices*. Academic Press Inc, San Diego.

Shibutani S, Takeshita M, Grollman AP (1991). Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. *Nature* **349:** 431–4.

Shigenaga MK, Gimeno CJ, Ames BN (1989). Urinary 8-hydroxy-2′-deoxyguanosine as a biological marker of *in vivo* oxidative DNA damage. *Proc Natl Acad Sci, USA* **86:** 9697–701.

Sodum RS, Nie G, Fiala ES (1993). 8-Aminoguanine: a base modification produced in rat liver nucleic acids by the hepatocarcinogen 2-nitropropane. *Chem Res Toxicol* **6:** 269–76.

Tamit S, deRojas-Walker T, Wishnok JS, Tannenbaum SR (1996). DNA damage and genotoxicity by nitric oxide. *Methods Enzymology* **269:** 230–43.

Tchou J, Grollman AP (1995). The catalytic mechanism of Fpg protein. Evidence for a Schiff base intermediate and amino terminus localization of the catalytic site. *J Biol Chem* **270:** 11671–7.

Tchou J, Kasai H, Chung MH, Grollmann AP, Nishimura S (1991). 8-Oxoguanine (8-hydroxyguanine) DNA glycosylase and its substrate specificity. *Proc Natl Acad Sci, USA* **88:** 4690–4.

Togashi Y, Kasai H, Hosokawa M, Takeichi N (1993). Prevention of spontaneous hepatocellular carcinoma in Long–Evans cinnamon rats with hereditary hepatitis by the administration of D-penicillamine. *Hepatology* **18:** 614–20.

van Poppel G, Kok FJ, Hermus RJ (1992). Beta-carotene supplementation in smokers reduces the frequency of micronuclei in sputum. *Br J Cancer* **66:** 1164–8.

van Poppel G, Poulsen H, Loft S, Verhagen H (1995). No influence of beta carotene on oxidative DNA damage in male smokers. *J Natl Cancer Inst* **87:** 310–1.

Verhagen H, Poulsen HE, Loft S *et al* (1995). Reduction of oxidative DNA damage in humans by Brussels sprouts. *Carcinogenesis* **16:** 969–70.

von Sonnentag C (1987). *The Chemical Basis for Radiation Biology*. Taylor & Francis, London.

Wiseman H, Halliwell B (1996). Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochem J* **313:** 17–29.

Yermilov V, Rubio J, Becchi M, Friesen MD, Pignatelli B, Ohshima H (1995a). Formation of 8-nitroguanine by the reaction of guanine with peroxynitrite *in vivo*. *Carcinogenesis* **16:** 2045–50.

Yermilov V, Rubio J, Ohshima H (1995b). Formation of 8-nitroguanine in DNA treated with peroxynitrite *in vitro* and its rapid removal from DNA by depurination. *FEBS Lett* **376:** 207–10.

Young HS, Chung MH, Lee WK, *et al* (1996). Increased oxidative DNA damage in *Helicobacter pylori*-infected human gastric mucosa. *Cancer Res* **56:** 1279–82.

Ziegler A, Jonason A, Leffell D, *et al* (1994). Sunburn and p53 in the onset of skin cancer. *Nature* **372:** 773–6.