Klin Wochenschr (1991) 69:1123–1134

Klinische Wochenschrift

Free Radicals in Chemical Carcinogenesis*

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Summary. During the past decade, remarkable progress has been made in our understanding of cancer-causing agents, mechanisms of cancer formation and the behavior of cancer cells. Cancer is characterized primarily by an increase in the number of abnormal cells derived from a given normal tissue, invasion of adjacent tissues by these abnormal cells, and lymphatic or blood-borne spread of malignant cells to regional lymph nodes and to distant sites (metastasis). It has been estimated that about 75–80% of all human cancers are environmentally induced, 30–40% of them by diet. Only a small minority, possibly no more than 2% of all cases, result purely from inherent genetic changes. Several lines of evidence confirm that the fundamental molecular event or events that cause a cell to become malignant occur at the level of the DNA and a variety of studies indicate that the critical molecular event in *chemical carcinogenesis* is the interaction of the chemical agent with DNA. The demonstration that DNA isolated from tumor cells can transfect normal cells and render them neoplastic provides direct proof that an alteration of the DNA is responsible for cancer. The transforming genes, or oncogenes, have been identified by restriction endonuclease mapping. One of the characteristics of tumor cells generated by transformation with viruses, chemicals, or radiation is their reduced requirement for serum growth factors. A critical significance of electrophilic metabolites of carcinogenes in *chemical carcinogenesis* has been demonstrated. A number of "proximate" and "ultimate" metabolites, especially those of aromatic amines, were described. The "ultimate" forms of carcinogens actually interact with cellular constituents to cause neoplastic transformation and are the final metabolic products in most pathways. Recent evidence indicates that free radical derivatives of chemical carcinogens may be produced both metabolically and nonenzymatically during their metabolism. Free radicals carry no charge but do possess a single unpaired electron, making the radical extremely reactive. That such forms may be important in the introduction of neoplastic transformation by chemicals comes from two lines of evidence. (1) Various molecules that inhibit the formation of free radicals, many of which are termed antioxidants, can inhibit the carcinogenic action of a variety of chemical carcinogens. (2) There are relatively specific metabolic reactions of certain chemical carcinogens, particularly of polycyclic hydrocarbons, for which it has been shown to proceed through free radical intermediates. In conclusion, free radical processes with *direct effects on DNA* can be proposed for a variety of human and animal carcinogens. As already mentioned, part of the indirect evidence supporting a role for free radicals and active species of oxygen in carcinogenesis is the inhibitory effects of antioxidants in a number of encouraging but yet inconclusive, epidemiological studies and ongoing prospective trials of antioxidant treatment in humans (chemoprevention). Cancer chemoprevention research takes leads from epidemiologic and laboratory research and develops them through in vitro and in vivo preclinical research and initial human studies into randomized controlled clinical trials. The most commonly used chemopreventive agents are retinoids, β-carotene, and vitamins. Some of the most exciting developments in the control of cancer over the next decade will very likely result from

* Dedicated to Prof. Dr. med. Dr. h.c. Hans Dierck Waller on the occasion of his 65th birthday

Abbreviations: DNA = deoxyribonucleic acid; PAH = polycyclic aromatic hydrocarbons

Manuscripts published in this issue were the matter of a Symposium held at the University of Ulm, April 24–27, 1991

this chemopreventive approach, with application not only to prevention of the first or primary malignancy, especially in high risk populations, but also to clinical situations traditionally considered to be in the domain of chemotherapeutic strategies, such as adjuvant treatment after definitive therapy of a primary cancer.

Key words: Free radicals – Carcinogenesis – Antioxidants – Chemoprevention

Cancer Incidence and Development

Total cancer death rates per year vary from country to country. They are available for twenty countries which all have major populations with nationwide reporting systems and report to the World Health Organization. For meaningful comparisons between countries, cancer death rates were age-adjusted to a standard age distribution. Cancer is the second leading cause of death in the United States and accounts for 10% of the total cost of illness. At present 910000 new cancers are diagnosed every year and it has been estimated that one of every four Americans will develop some form of cancer. When comparing cancer-related mortality figures from 1950 to 1982 in the U.S. population, there has been a slow and steady increase in age-adjusted mortality in all race and sex groups combined. The sharp and continuing rise in deaths from lung cancer, nearly all from cigarette smoking, has substantially affected mortality rates from all cancers combined. The increased cancer death rate is also associated with the increased average life span, with more people living to an age where cancer risk is highest. The situation in Germany is comparable with the U.S. The absolute cancer mortality increased twofold between 1955 and 1988. In 1988, for example, 169171 persons died of cancer (Becker et al. 1989; Dix 1989; Newell et al. 1989).

During the past decade, remarkable progress has been made in our understanding of cancer-causing agents, mechanisms of cancer formation and the behavior of cancer cells. Normally, worn-out tissues are replaced and injuries are repaired. Quite often, cells change to a precancerous stage but the body's immune system detects this change and destroys the dangerous abnormality. Occasionally, certain cells undergo an abnormal change without detection by the immune system and begin a progress of uncontrolled growth and spread. These cells may grow into masses of tissues (tumors) and may be either benign or malignant (cancerous). Cancer invades and destroys normal tissues and, if left untreated, is likely to spread throughout the body, usually resulting in death. The beginning of uncontrolled growth and cancer results from external factors, combined with a hereditary disposition for cancer. A number of tumor initiators and promoters have been identified in the diet and in the environment (Ames 1983, 1987; Higginson 1988; Palmer and Bakshi 1987; Weisburger et al. 1987; Cullen et al. 1990). It has been estimated that about 75–80% of all human cancers are environmentally induced, 30–40% of them by diet. Only a small minority, possibly no more than 2% of all cases, result purely from inherent genetic changes.

Pathobiology of Cancer

Cancer is characterized primarily by an increase in the number of abnormal cells derived from a given normal tissue, invasion of adjacent tissues by these abnormal cells, and lymphatic or blood-borne spread of malignant cells to regional lymph nodes and to distant sites (metastasis). Insight into the complex events that lead from normal cellular growth to neoplasia, invasion, and metastasis is crucial for the development of effective diagnostic and therapeutic strategies. Cancer may affect any organ in the body; 12 major forms and more than 50 minor forms of the disease have been identified. An interplay of environmental and genetic factors is thought to give rise to neoplasia. As already mentioned, it is estimated that environmental factors contribute significantly to more than 70% of human cancers. Genetic, or host, factors are also important. For example, one rare genetic disorder, multiple polyposis of the colon, will lead to cancer in all affected persons unless they undergo a prophylactic colectomy. Far more commonly, genetic factors exert a complex effect and produce a much lower, but significant, increase in cancer risk. Although environmental factors may predominate in some situations and genetic influences in others, it is the interaction of these two elements that determines the development of neoplasia (Fig. 1). Smoking is the major cause of lung cancer (Hammond and Horn 1984). 93% of the 807 patients with lung cancer seen at one institution between 1980 and 1985 were cigarette smokers. Although lung cancer develops in about 10% of individuals with a history of 40 pack-years of smoking, it does not occur in 90% of such smokers, which suggests that genetic and other factors contribute to the risk of lung cancer. Pharmacogenetic evidence in-

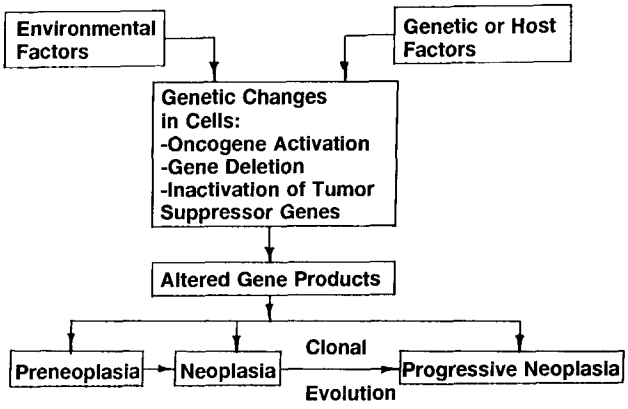

Fig. 1. Interaction between environmental and genetic factors giving rise to neoplasia

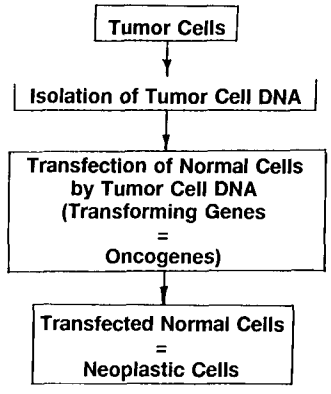

Fig. 2. DNA isolated from tumor cells and inserted into normal cells (transfection) can transform the recipient cells to the neoplastic phenotype

dicates that a person's capacity to transform or inactivate carcinogens present in cigarette smoke may be the manifestation of genetic risk factors.

Several lines of evidence confirm that the fundamental molecular event or events that cause a cell to become malignant occur at the level of the DNA. For example, when a normal cell is experimentally transformed to a neoplastic cell, all daughter cells carry the neoplastic phenotype, which indicates that the defect is inherited. In addition, certain diseases, such as xeroderma pigmentosum, that are characterized by defective DNA repair mechanisms are associated with a high incidence of epithelial carcinomas and melanoma of skin exposed to ultraviolet light. Hence, DNA damage in affected individuals produces a marked increase in cancer risk. Other studies indicate that the critical molecular event in *chemical carcinogenesis* is the interaction of the chemical agent with DNA (Pitot 1989). Finally, the demonstration that DNA isolated from tumor cells can transfect normal cells and render them neoplastic provides direct proof that an alteration of the DNA is responsible for cancer (Der et al. 1982; Parada et al. 1982) (Fig. 2). Clinical data and molecular biologic studies indicate that cancer is a multistep process that begins with minor preneoplastic changes and may under certain conditions progress to neoplasia (Land et al. 1983; Bishop 1987). The neoplastic lesion may evolve clonally and develop an increasing capacity for invasion, growth, metastasis, and heterogeneity (Nicolson et al. 1987).

The molecular aspects of the neoplastic event are becoming increasingly defined. As mentioned above, DNA isolated from tumor cells and inserted into normal cells, a process termed transfection, can transform the recipient cells to the neoplastic phenotype. The transforming genes, or oncogenes, have been identified by restriction endonuclease mapping. Also, the discovery that many cellular oncogenes have their homologues in retroviral oncogenes has permitted the development of cDNA probes. Such probes have identified more than 20 oncogenes and have helped to define their molecular biology (Bishop 1987).

Two questions have emerged from oncogene studies: (1) how are oncogenes, which are latent in normal cells (termed proto-oncogenes) turned on, or activated (Fig. 3), and (2) how do the oncogene products transform a normal cell into a malignant one?

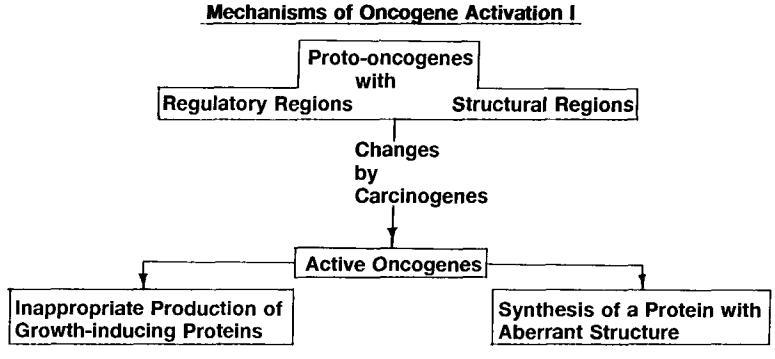

Fig. 3. Mechanisms proposed to account for the activation of cellular oncogenes

Table 1. Mechanisms of oncogene activation II

(1)	Oncogene amplification → Uncontrolled replication of the oncogene
(2)	Oncogene mutation (point mutations involving the change of a single nucleotide in the oncogene) → Increase in transcription or generation of an abnormal gene product
(3)	Chromosomal translocation (reciprocal translocation that juxtaposes a promoter sequence next to the oncogene)

Table 2. Oncogene products and probable functions

(1)	Small polypeptides, which enter the nucleus and may affect cell proliferation
(2)	Enzymes (e.g. tyrosine kinase), which may phosphorylate molecules such as cytoskeletal proteins and cause the cell to evolve neoplastic phenotype
(3)	Cellular growth factors and receptors for growth factors → autocrine stimulation → paracrine stimulation

At least three mechanisms of oncogene activation have been described: (1) oncogene amplification, which increases the number of genes and therefore the amount of gene product, (2) oncogene mutation, which causes either an increase in transcription or the product of a slightly abnormal gene product, and (3) reciprocal translocations between genetic material, as demonstrated first by cytogenetic studies, that juxtapose oncogenes and promoter or enhancing sequences and thereby increase oncogene transcription (Table 1). Because carcinogens such as ultraviolet light, x-rays, various chemical agents, and tumor viruses can experimentally induce gene amplification, mutation, and genetic translocation, it appears that they may produce their carcinogenic effects by activating cellular oncogenes. Although activated oncogenes clearly are key elements in the multistep process of carcinogenesis, they can be found in only 15% to 30% of human tumors (Bishop 1987), and then only in somatic and not in germ cells. The discovery of tumor suppressor genes (or termed growth suppressor genes), for which inactivating mutations elicit tumorigenesis, has added a new dimension to our understanding of neoplasia (Hollingsworth and Lee 1991). It is now becoming clear that inactivation of tumor suppressor genes may be a general phenomenon in the initiation and progression of cancer.

Several oncogene products, and their probable functions, have now been identified. The malignant phenotype depends on such products, which also provide potential targets for therapy. These proteins, which are produced by different oncogenes, include (1) small polypeptides that enter the nucleus and may affect cell proliferation, (2) enzymes, such as tyrosine kinases, that may phosphorylate critical molecules such as cytoskeletal proteins and cause the cell to evolve toward a neoplastic phenotype, and (3) cellular growth factors and receptors for growth factors, which may produce or maintain the neoplastic phenotype in the parent cell (autocrine stimulation) or in adjacent cells in the microenvironment (paracrine stimulation) (Table 2). Indeed, oncogenes' expression may be regulated by growth factors.

One of the characteristics of tumor cells generated by transformation with viruses, chemicals, or radiation is their reduced requirement for serum growth factors. Growth in medium supplemented with reduced serum has often been used as a method of primary selection for tumor cells. Accordingly, it has long been reasoned that an important stage in tumor development might be the release of a premalignant cell from dependence on one or more exogenous growth factors. This could occur if a cell aberrantly synthesize a growth factor which stimulates the growth of the same cell – a situation which has been termed autocrine growth (Sporn and Roberts 1985). Such autonomous cells would then escape normal growth control, which could in turn lead to tumor development.

Role of Free Radicals in Carcinogenesis

A critical significance of electrophilic metabolites of carcinogenes in *chemical carcinogenesis* has been demonstrated. A number of "proximate" and "ultimate" metabolites, especially those of aromatic amines, were described. The "ultimate" forms of carcinogens actually interact with cellular constituents to cause neoplastic transformation and are the final metabolic products in most pathways. In addition to the electrophilic intermediates comprising the structures of the ultimate forms of chemical carcinogens, recent evidence also indicates that free radical derivatives of chemical carcinogens may be produced both metabolically and nonenzymatically during their metabolism. Free radicals carry no charge but do possess a single unpaired electron, making the radical extremely reactive. That such forms may be important in the introduction of neoplastic transformation by chemicals comes from two lines of evidence. (1) Various molecules

that inhibit the formation of free radicals, many of which are termed antioxidants, can inhibit the carcinogenic action of a variety of chemical carcinogens (Kahl 1984). (2) There are relatively specific metabolic reactions of certain chemical carcinogens, particularly of polycyclic hydrocarbons, for which it has been shown to proceed through free radical intermediates. Marnett (1987) has described the co-oxygenation of polyunsaturated fatty acids with polycyclic aromatic hydrocarbons, leading to the formation of the ultimate diol epoxide form during metabolic reactions that convert polyunsaturated fatty acids to prostaglandins. Wise and coworkers (1984) have presented evidence indicating that this pathway is important in the genesis of aromatic amine-induced bladder cancer in dogs, in contrast to the enzymatic formation of the ultimate carcinogen in the liver. Further, free radicals may play a role in enhancing or promoting the development of cancer. All these results together demonstrate that the majority of chemical carcinogens must be metabolized within the cell before they exert their carcinogenic activity. This finding explains how a substance that is not carcinogenic for one species may be carcinogenic for another, the result depending on the metabolic capacities present within the species itself. However, not all chemical carcinogens require intracellular metabolism to become ultimate carcinogens, for example, direct alkylating agents like nitrogen mustard.

Our knowledge about free radical chemistry in biological systems was primarily acquired from experiments with irradiated solutions containing nucleic acids, DNA, nucleus, whole cells or organisms. Now, we are trying to determine if the insights gained from radiation biology and radiation carcinogenesis are applicable to chemical carcinogenesis. It appears highly likely that there are processes central to the causation of certain cancers which can be ascribed to free radical reactions. As a corollary it is highly unlikely that free radical reactions are involved in the development of all cancers. A major factor in evaluating the role of free radicals in *chemical carcinogenesis* is the multipotential nature of the carcinogen. Chemicals inducing the release of free radicals also have other potential reactions. In an in vitro study it is possible to quantify the rates for each of the potential reactions and to determine which one is dominant. In biological systems, however, this is difficult, in part due to the complexity of the systems. More important are the chemical rate constants for free radicals and their subsequent reactions. They must be interpreted in relation to cellular defense mechanisms and to the biological step, which is most crucial in determining the disease outcome.

Studies on the mechanism of benzene hematotoxicity (Latriano et al. 1986; Witz et al. 1985) focussed on the possibility that the opening of the benzene ring by hydroxyl radicals produces α, β-unsaturated aldehydes and related products responsible for bone marrow effects and the development of leukemia. This is a quantitatively minor chemical pathway, originally considered to account for no more than 2% of total benzene metabolites. Other potential pathways for benzene-induced DNA damage and leukemia might involve a direct or indirect radical attack on DNA through relatively major metabolites such as hydroxylated intermediates (Eastmond et al. 1987; Sadler et al. 1988; Sawahata et al. 1985). There is evidence that hepatic metabolism have a part in bone marrow toxicity (Sammett et al. 1979). However, it is implausible that a short-lived free radical or related active species could travel from liver to bone marrow. Accordingly, examinations of chemical rate constants for the formation or reactivity of active species possibly derived from benzene in the liver does not unequivocally provide an answer for bone marrow toxicity and the development of leukemia.

Localization of reactions must be considered as an intracellular and an interorgan phenomenon. In vitro studies of the genotoxic effects of free radical generating reaction mixtures are often performed by adding DNA to the mixture. Extrapolation of these findings to in vivo conditions give rise to at least three questions: (1) will short-lived free radicals be generated in the cell in sufficient proximity to DNA so that reaction is likely; (2) will normal cellular defense mechanisms be able to protect DNA in vivo; (3) will the relatively homogenous nature of the in vitro condition lead to free radical chain lengths which are longer than under the heterogenous chemical condition in the whole cell.

For free radicals acting from the outside of the cell, the cell membrane will initially be attacked possibly followed by lipid peroxidation. Damage to DNA caused by lipid peroxidation has been ascribed to the following circumstances: the action of free radicals derived from the decomposition of peroxidized lipid, of lipid hydroperoxides, of carbonyl derivates, and through a second message mechanism resulting in clastogenic activity (Demopoulos et al. 1980; Emerit 1987). It is unlikely that either free radicals or lipid hydroperoxides derived from oxidative decomposition of cell membranes can travel to the nucleus, particularly in view of efficient cellular defense mechanisms. It must be

considered that only a sublethal, but not a lethal damage of the cell can be responsible for the initiation of cancer. Additionally, a variety of non-radical-mediated mechanisms may exists translating membrane processes into DNA alterations (McConkey et al. 1988; Kozumbo et al. 1987a, b).

A role for free radicals in *chemical carcinogenesis* has been suggested for cigarette smoke. Cigarette smoke is a complex mixture of several thousand chemicals, of which more than 40, including polycyclic aromatic hydrocarbons (PAH), aza-arenes, aromatic amines, N-nitrosamines, aldehydes and metals, have demonstrable carcinogenic activity in experimental animals (Lin 1990; Hoffman and Hecht 1990) and/or activity in short-term assays for genotoxicity (Claxton et al. 1989). Many of these carcinogens exert their biological activity through the covalent interaction of reactive metabolites with cellular DNA. The production of superoxide, hydroxyl radicals, and hydrogen peroxide in smoke has recently been reported. Radicals in smoke derived from quinone components of tar are capable of activating polycyclic aromatic hydrocarbons (PAH) to a carcinogenic intermediate (Pryor et al. 1983a, b).

In conclusion, free radical processes with *direct effects on DNA* can be proposed for a variety of human and animal carcinogens. A number of pathways in the causation of cancer by those carcinogens are possible. (1) Free radicals can be involved in a metabolic step that results in an ultimate chemical carcinogen. (2) The chemical itself or one of its metabolites can be converted into a radical, which directly reacts with DNA. (3) The compound through its unintentional reactions or through "normal" metabolic pathways may cause the formation of free radicals which attack DNA. (4) Following addition to DNA, the adduct can itself generate free radicals in proximity to genetic material (Emerit 1987; Marnett 1987; Hsie 1987; Pryor 1987).

Conclusions are based on the assumption that the mechanism of chemical carcinogenesis is primarily through reaction with DNA. However, the concept of tumor promotion is now established experimentally by a number of different approaches, using isolated organs and laboratory animals (Fig. 4). Basically, a single subcarcinogenic dose of a known genotoxic carcinogen is given (tumor initiator). This treatment is followed by giving

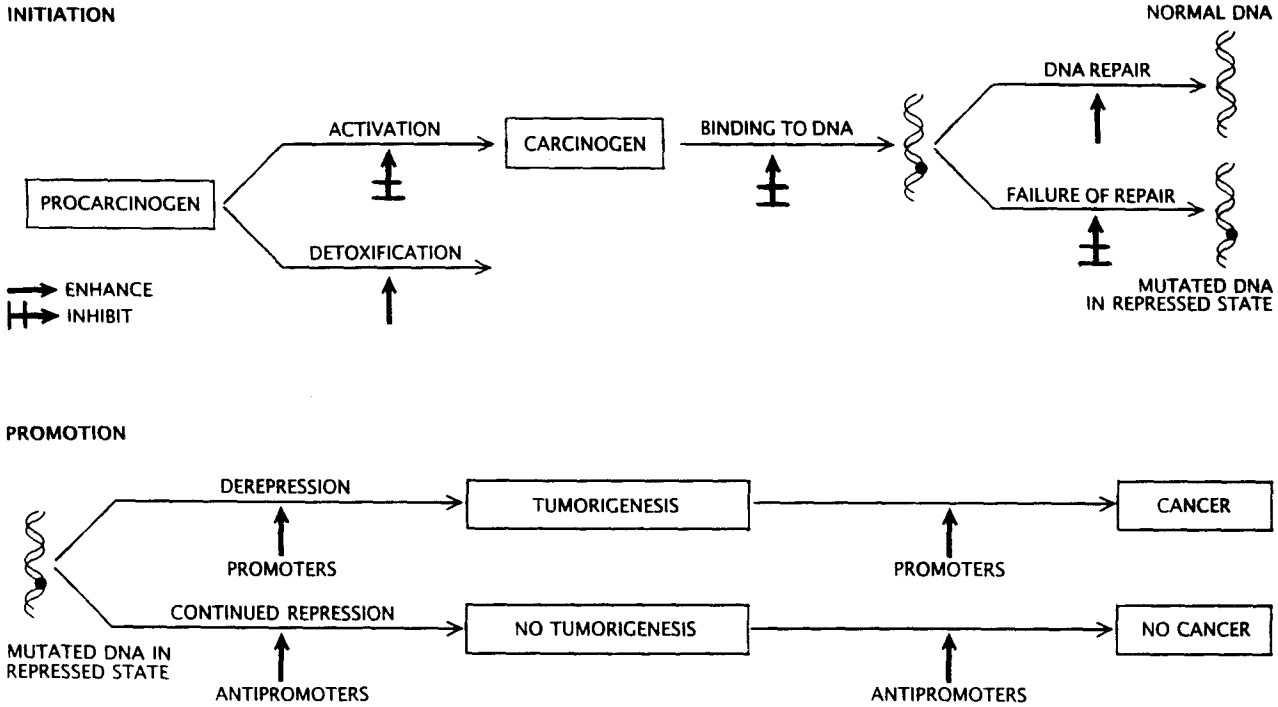

Fig. 4. Influence of dietary factors on the initiation and promotion of carcinogenesis. Cancer can be initiated when a *procarcinogen* is activated and binds to DNA, giving rise to a mutation in the DNA, presumably often affecting an *oncogene*. The mutation may or may not be repaired. Steps in activation, binding and DNA repair may be enhanced (→) or inhibited (⊢→) by dietary components as vitamin A, β-carotene, vitamins C and E, and selenium. An unrepaired mutation may transform cells to an abnormal state, but it remains repressed – a tumor does not develop and there is no progression to cancer – except in the presence of *promoters*. Antipromoters such as vitamin A tend to maintain repression and inhibit cancer growth. From: Scientific American (1987)

Table 3. Free radicals and reactive oxygen species in chemical carcinogenesis

Initiation
1. Activation of procarcinogens
2. Binding of carcinogen to DNA
3. Direct damage to DNA without binding
4. Formation of genotoxic radical following binding to DNA
Promotion
1. Process leading to enhanced cellular expression of somatic mutation
2. Process leading to suppression of extracellular inhibitors of growth

From: Goldstein and Witz (1990)

Table 4. Free radicals and reactive oxygen species in tumor promotion and progression

1. Free radical generating compounds, such as organic peroxides, are tumor promoters and progressors
2. Promoters stimulate the endogenous production of reactive oxygen species
3. Reactive oxygen generating systems mimic the action of tumor promoters in cell cultures
4. Tumor promoters provoke rapid and sustained changes in cellular antioxidant enzyme activities
5. Antioxidants inhibit tumor promotion and progression

From: Goldstein and Witz (1990)

repetitive doses of an agent (tumor promotor), which by itself will not produce cancer (cocarcinogen). The role for free radicals and active states of oxygen in this concept of tumor promotion is supported by studies showing that free radical generating compounds act as promoters, that free radical scavengers can act as promotion inhibitors (anticarcinogens) (Ames 1983; Cerutti 1985; Perchellet and Perchellet 1989), and that endogenous cellular antioxidant defenses undergo changes upon promoter treatment (Table 3). Part of the indirect evidence supporting a role for free radicals and active species of oxygen in carcinogenesis is the inhibitory effects of antioxidants in a number of encouraging but yet inconclusive, epidemiological studies and ongoing prospective trials of antioxidant treatment in humans (chemoprevention) (Higginson 1988; Boone et al. 1990; Greenwald et al. 1990; Wattenberg 1990; Dorgan and Schatzkin 1991).

Some of the earliest and most thoroughly studied tumor promoters have been the phorbol esters present in croton oil. The inflammatory properties of these potent tumor promoters have long been recognized. However, the lack of correlation between the tumor promoting ability and inflammatory activity of the various phorbol esters had appeared to rule out a role for inflammatory activity in tumor promotion. Interferential evidence suggestive of a role of free radicals in tumor promotion, as well as studies demonstrating that antiprotease compounds that inhibit tumor promotion also inhibited the production of superoxide anion radical by PMA-activated phagocytic cells (Goldstein et al. 1979), led some workers to reexamine the correlation between the tumor promoting abilities of phorbol esters with one specific aspect of the inflammatory process: the production of active oxygen species by phagocytic cells. The excellent correlation observed (Witz et al. 1980; Goldstein et al. 1981) led to the hypothesis that free radicals might play a role in tumor promotion (Cerutti 1985; Emerit 1987; Marnett 1987). Some of the evidence supporting a role for free radicals and active states of oxygen in tumor promotion is outlined in Table 4. Compounds such as benzoyl peroxide are tumor promoters (Slaga et al. 1981); organic peroxides and active oxygen species mimic certain effects of tumor promoters in cell systems; tumor promoters affect cellular antioxidant enzyme activities; and, various antioxidants inhibit tumor promotion and progression (Barak et al. 1983; Weitzman et al. 1985; Kensler and Taffe 1986; Perchellet and Perchellet 1989).

As summarized in Table 3, there are two basic ways that free radicals may be involved in tumor promotion: (1) through growth of the cancer cells by enhancing the expression of the somatic mutation or other growth-promoting processes between initiation and the development of a clinically recognizable tumor; or, (2) by interference in the extracellular processes that normally inhibit cancer cell growth. It must be emphasized that tumor promoters such as phorbol esters have many different effects on cells. Hence, proof of a role for free radicals in tumor promotion, particularly in human cancers, is still lacking (Goldstein and Witz 1990).

Antioxidants as Anticarcinogens

With the aging of the population and the continued fall in rates of mortality from cardiovascular diseases, cancer will emerge after the year 2000 of being the leading cause of death in the U.S. and Europe. Unfortunately, dramatic therapeutic successes in the treatment of cancer reached a plateau in the mid-1970s, and advances since then have been incremental. Whether the remarkable progress in our understanding of the biologic and

genetic causes of normal and transformed cellular growth in the past 15 years will be translated into substantial therapeutic benefit remains to be demonstrated. Alternatives to therapy of late disease need to be developed for the control of cancer. Chemoprevention, or the chemical prevention of cancer formation, is one of such novel approach (Meyskens 1990). The expression of tumor transformation in vitro and in vivo can be inhibited by a variety of compounds. Normal dietary constituents and pharmacologic agents may be candidates for chemopreventive activity (Boone et al. 1990; Garewal and Meyskens 1991). Clearly, one of the top priorities in cancer prevention remains elimination of known carcinogens, the most important of which is tobacco. However, complete elimination of carcinogens is not likely to be socially acceptable or achievable. Therefore, other strategies must also be developed and applied.

As initially mentioned, there is substantial evidence that many dietary factors influence the incidence of cancer in humans. Some nutrients increase cancer incidence by acting as carcinogen, others decrease cancer incidence by acting as anticarcinogens (Carr 1985) (Fig. 4). Nutritional anticarcinogens may function in the following ways: (1) inhibition of tumor initiation by altering cell functions; (2) uptake of active forms of carcinogens and preventing them from reaching target sites; (3) alteration of the body's defense system; and, (4) inhibition of progression of previously initiated cancers. Information is relatively limited concerning concentrations of nutritional anticarcinogens that are effective in preventing cancer in humans. The different cells, which become cancerous as well as the number of agents which can cause human cancer implicit that preventive concentrations may be effective for one cancer but not for another.

A number of antioxidants' functions have relevance in the consideration of their role in cancer prevention and control. Antioxidant micronutrients, including the carotenoids, vitamin C (ascorbic acid), vitamin E (α-tocopherol), selenium, zinc, copper, iron, and manganese, are one of the body's primary defenses against free radicals and reactive oxygen species. Carotenoids, vitamin C, and vitamin E trap free radicals and reactive oxygen species, whereas selenium, zinc, copper, iron, and manganese are essential components of antioxidant enzymes (Machlin and Bendich 1987).

Carotenoids comprise a group of more than 500 naturally occuring pigments that are powerful antioxidants and efficient scavengers of reactive oxygen species (Di Mascio et al. 1989). Carotenoids also stimulate the immune system, although the mechanism for this action is unclear. Approximately 10% of carotenoids, including β-carotene, can be metabolized to retinol and have effects that are not intrinsic to carotenoids but are due to their provitamin A activity (Bendich and Olson 1989). Retinol has no antioxidant function, but controls the growth, alteration and function of body tissues. Vitamin C is a water-soluble antioxidant. In addition to trapping free radicals and reactive oxygen species, it reduces nitrite. This reaction blocks the formation of nitrosamines and nitrosamides, compounds that induce tumors in experimental animals and possibly in humans (Lin 1990). Vitamin C also stimulates the immune system and may protect against the development of cancer by enhancing immune surveillance (Mirvish 1986; Anderson et al. 1990; Bendich 1990). Vitamin E is the major lipid-soluble antioxidant found in cell membranes, where it protects against lipid peroxidation (Machlin and Bendich 1987). Like vitamin C, vitamin E reduces nitrite, inhibiting the production of nitrosamines and nitrosamides (Mirvish 1986). Vitamin E may also play a role in the immune system, potentially the immune response (Bendich 1990; Tengerdy 1990). Selenium is an essential constituent of the enzyme glutathione peroxidase, which reduces peroxides before they can attack intracellular membranes. However, glutathione peroxidase activity is probably not the primary mechanism by which selenium protects against cancer. Other proposed mechanisms include inhibition of DNA synthesis and cell proliferation and stimulation of the immune system (Medina 1986; Spallholz et al. 1990).

Results of cell and animal research suggest that antioxidants alter cancer incidence and growth by acting as anticarcinogens. In limited epidemiological human studies a dietary deficiency and low blood concentration of antioxidants is conversely correlated with the incidence of certain cancers. Therefore, the question rises how good the epidemiological evidence is in support of a cancer-enhancing effect of diets and a cancer-inhibiting effect for dietary fiber, vitamins A, C and E, β-carotene and selenium. For most of the substances the evidence is limited. Although many and varied epidemiological studies have been made, none of them fully satifies the criteria epidemiologists employ in drawing inferences about causes from statistical associations. The epidemiological evidence for anticancer effects from vitamin A or its dietary precursor β-carotene, comes almost exclusively from the comparison of cancer patients with healthy people. The findings pertain mainly lung cancer; to a lesser

extend they also encompass cancer of the colon, stomach, bladder, oesophagus and oral cavity. There is literally no evidence for a protective effect of other vitamins, with the exception of a possible association between the intake of vitamins C and E and reduced rates of gastric cancer.

An overview and some selected studies on antioxidants and cancer follow with the intention to demonstrate that antioxidants show promise as cancer prevention agents, alone or in combination. Additional studies are underway to investigate the role of antioxidants in prevention and control of cancer. There are 28 studies funded by the National Cancer Institute (NCI) of the United States currently in progress on the association between antioxidants, cancer prevention and cancer risk.

Some epidemiologic evidence of a protective effect of *vitamin C* for non-hormone-dependent cancers is strong. Of the 46 such studies in which a dietary vitamin C index was calculated, 33 found statistically significant protection, with high intake conferring approximately a twofold protective effect compared with low intake. Of 29 additional studies that assessed fruit intake, 21 found significant protection. For cancers of the oesophagus, larynx, oral cavity, and pancreas, evidence for a protective effect of vitamin C or some component in fruit is strong and consistent. For cancers of the stomach, rectum, breast, and cervix there is also strong evidence. Several recent lung cancer studies found significant protective effects of vitamin C or of foods that are better sources of vitamin C than β-carotene. It is likely that ascorbic acid, carotenoids, and other factors in fruits and vegetables act jointly (Block 1991).

Serum α-tocopherol concentrations were studied for its prediction of cancer in a cohort of 36265 adults in Finland. During a mean follow-up of 8 years, cancer was diagnosed in 766 persons. The concentrations of serum α-tocopherol were determined from stored serum samples taken from these cancer patients and from 1419 matched control subjects. Individuals with a low α-tocopherol concentration had about a 1.5-fold risk of cancer compared with those with higher concentrations. The strength of the association between serum α-tocopherol concentration and cancer risk varied for different cancer sites and was strongest for some gastrointestinal cancers and for the combined group of cancers unrelated to smoking. The association was strongest among nonsmoking men and among women with low levels of serum selenium (Knekt et al. 1991).

In 1971–1973 at a third examination of the so-called Basel Study started in 1959, the major antioxidant vitamins and carotene were measured in the plasma of 2974 men. A subsample and their families were reinvestigated in 1977–1979. During the 12-year observation period (1973–1985) 553 men died, 204 of cancer (lung 68, stomach 20, colon 17, all other malignancies 99). Significant lower mean carotene concentrations were found for all cancers, bronchus cancer, and stomach cancer (all $P<0.01$) compared with the 2421 survivors. The relative risk of subjects with low carotene ($<$ 0.23 μmol/L) was significantly elevated ($P<0.05$) for lung cancer (Cox's model). Higher risks were noted for all cancers ($P<0.01$) if both *carotene and retinol* were low (Stähelin et al. 1991).

The association of *serum retinol, the carotenoids β-carotene and lycopene, and tocopherol* with the risk of cancer was investigated in a nested case-control study. For the study, serum obtained in 1974 and 1975 from 25802 persons in Washington County, MD, was used. Prediagnostic samples from 436 cancer cases and 765 matched control subjects have been assayed. Serum β-carotene and tocopherol concentrations showed a strong protective association with lung cancer. Low concentrations of serum lycopene were strongly associated with pancreatic cancer. Serum concentrations of the nutrients in 103 men who developed prostate cancer were compared with concentrations in 103 control subjects matched for age and race. Although no significant associations were observed with β-carotene, lycopene, or tocopherol, the data suggest an inverse relationship between serum retinol and risk of prostate cancer (Hsing et al. 1990; Comstock et al. 1991).

The anticancer role for *selenium* was originally based on the findings that concentrations of selenium in soil, forage crops and blood were inversely related to the risk of breast and colon cancer in various cities and regions of the U.S. International studies revealed a similar relation. The strength and consistency of these associations, however, are weak in some cases. For example, New Zealand has one of the lowest levels of selenium intake (50 μg per day) and a breast-cancer mortality rate of 30 per 100000 population per year, whereas the U.S. has a selenium intake of about 120 μg per day and almost the same rate of deaths from breast cancer (27 per 100000). Such inconsistencies do not rule out a role for selenium, but they suggest that other factors may be more dominant. As the above discussion indicates, epidemiologists essentially look at the results of experiments in nature and work backward to causes. Because only relatively crude methods are available for measuring food consumption, particularly what people have

eaten in years past, the best an epidemiologists can do is to demonstrate strong and consistent correlations or associations, such as the one between dietary fat and breast cancer. Statistical associations can only imply causation, however, they cannot prove it. An experimentalist, on the other hand, works forward from causes to results and thereby provides the second major source of evidence bearing on the relation between diet and cancer. A drawback of this work is that results of laboratory experiments involving rodents must be treated with caution, particularly when the findings are being extrapolated in an effort to assess effects on human beings.

In general, one can assume that low intake of vegetables, fruits, and carotenoids is consistently associated with increased risk of lung cancer in both prospective and retrospective studies. In addition, low concentrations of β-carotene in serum or plasma are consistently associated with the subsequent development of lung cancer. The simplest explanation is that β-carotene is protective. Since retinol (preformed vitamin A) is not related in a similar manner to lung cancer risk, β-carotene appears to function through a mechanism that does not require conversion into vitamin A. However, the importance of other carotenoids and other constituents of vegetables and fruit has not been adequately explored. Both prospective and retrospective studies suggest that vegetable and fruit intake may reduce the risk of cancer of the mouth, pharynx, larynx, oesophagus, stomach, colon, rectum, bladder, and cervix. But because of fewer studies and less consistency among studies, the epidemiologic evidence is at present less persuasive than for lung cancer (Ziegler 1991).

Cancer chemoprevention research takes leads from epidemiologic and laboratory research and develops them through in vitro and in vivo preclinical research and initial human studies into randomized controlled clinical trials (Malone 1991). Nutritional intervention trials are important tools in the search for efficient cancer prevention strategies. They can be divided into two types of trials; chemoprevention in which intervention is a defined chemical agent or a micronutrient, and diet trials in which intervention is a change in dietary habits. The most commonly used chemopreventive agents are retinoids, β-carotene, and vitamins. Study designs include single agent randomization, combination of agents and complete factorial designs. The chemoprevention trials are directed to general cancer prevention or focus on target organs (colon, lung, oesophagus, cervix, bladder, and skin). The diet intervention studies include volunteers from the general population; populations at high risk for cancer because of occupation, lifestyle, or place of residence; persons with previously treated cancers; and persons with preneoplastic lesions. Endpoints in these studies include overall incidence of cancer, incidence of specific cancers, rate of regression or progression of preneoplastic changes, and changes in cellular or biochemical parameters. Although several completed chemopreventive studies indicate that certain micronutrients can prevent neoplastic growth, the follow-up period is still too short for most nutritional intervention studies to determine whether their preventive strategies are effective. Study of premalignant lesions offers a comparatively expedient approach to identifying and evaluating the efficacy of the cancer chemopreventive components of foods. Some recent findings suggest roles for β-carotene and/or vitamin C in reversing or reducing the risk of cervical dysplasia and oral leukoplakia. There are some indications that vitamin C and β-carotene may reduce the risk of atrophic gastritis and gastric cancer. The results of two large randomized clinical trials of chemoprevention were recently presented (Greenberg et al. 1990; Hong et al. 1990). They have been carefully designed and impeccably conducted, but they led to different conclusions. β-carotene did not lower the rate of development of new basal-cell or squamous-cell cancers of the skin in subjects with previous skin cancers. In contrast, isotretinoin did lower the rate of development of second regional tumors in patients with a previous oral cancer. Why these opposite results? The most obvious explanation is that β-carotene and isotretinoin represent two quite different inhibitors of carcinogenesis. In most model systems β-carotene is a weak anticarcinogen, whereas isotretinoin is a potent inhibitor of cancer formation. However, since β-carotene has recently been demonstrated to reverse a preneoplastic condition in humans – a step rather late in the pathway to cancer – other explanations should be sought (Garewal et al. 1990). Additional epidemiological and molecular biology studies and clinical intervention trials using premalignant lesions as the marker of specific cancer risks should become an important component of future research in the area of cancer chemoprevention.

Some of the most exciting developments in the control of cancer over the next decade will very likely result from this chemopreventive approach, with application not only to prevention of the first or primary malignancy, especially in high risk populations, but also to clinical situations traditionally considered to be in the domain of chemotherapeutic strategies, such as adjuvant treatment after de-

finitive therapy of a primary cancer (Garewal and Meyskens 1991).

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Redaktion: Klinische Wochenschrift, Editor in Chief, Prof. Dr. N. Zöllner, Medizinische Poliklinik der Universität München, Pettenkoferstr. 8a, W-8000 München 2
Schriftleitung: Prof. Dr. N. Zöllner, Medizinische Poliklinik der Universität München, Pettenkoferstr. 8a, W-8000 München 2
Anzeigen: E. Lückermann, C. Hänzi, Springer-Verlag, Heidelberger Platz 3, W-1000 Berlin 33
Druckerei: Universitätsdruckerei H. Stürtz AG, Beethovenstr. 5, W-8700 Würzburg

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