Carcinogenic and Anticarcinogenic Food Components

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Carcinogenic and Anticarcinogenic Food Components
Chemical and Functional Properties of Food Components Series

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Preface

Fifty years of research on carcinogenesis have identified multiple cancer risk factors in the human environment, including food. Attempts to counteract carcinogenesis have led to discoveries of many components of plant foods that display biochemical and biological activities capable of preventing cancer. The term “nutraceuticals” has been proposed to describe those food components that exhibit health-promoting properties. Anticarcinogenic food components have become the subject of interest of a relatively new cancer research field called cancer chemoprevention. Although many data on carcinogenic and anticarcinogenic food components have been collected, our understanding of causal mechanisms linking diet and cancer is still evolving. As a result, evaluations of official dietary recommendations are needed on an ongoing basis.

This multidisciplinary book has been designed to provide a broad spectrum of information on both cancer-causing and cancer-preventing food components. Highly qualified and internationally recognized biochemists, toxicologists, epidemiologists, and food scientists have contributed chapters to this book.

Chapter 1 introduces the reader to the basic concepts of food and cancer, and the carcinogenic and anticarcinogenic potentials of food components. The subsequent Chapters 2 and 3 cover the complexities of the molecular and cellular events during the multistage process of chemical carcinogenesis as well as metabolic transformations of mutagens and carcinogens required to convert inert chemical molecules into DNA-reactive agents. In Chapters 4 through 8, a variety of dietary sources of carcinogenic compounds, their abundance in foods, and possible cancer risks are reviewed. Specifically, these chapters cover genotoxic (Chapter 4) and environmental (Chapter 6) contaminants of foods, the impact of food processing on the carcinogen content (Chapter 5), and the presumed roles of anti- and prooxidants (Chapter 7) and polyunsaturated fatty acids (Chapter 8) in carcinogenesis.

The subsequent chapters deal with the cancer-preventive potential of food components. While Chapter 9 familiarizes the reader with basic mechanisms and targets of chemoprevention, the subsequent chapters discuss anticarcinogenic food components of particular current interest. These include phenolic compounds in common spices (Chapter 10), tea and tea constituents (Chapter 11), wine polyphenols and resveratrol (Chapter 12), flavonoids of fruits and vegetables (Chapter 13), carotenoids (Chapter 14), constituents of cruciferous vegetables (Chapter 15), and phytoestrogens (Chapter 16). Chapter 17, the final chapter, provides perspective on the impact of diet on cancer prevention based on human trials and discusses future directions of research in this important field.

This book is designed for professionals employed by the food-processing industry and food scientists involved in research and educational endeavors as well as
students of food science. This book will also be of interest to nutritional and biomedical scientists involved in studies of cancer etiology and prevention.

The editors wish to thank all authors for their invaluable contributions.

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Dr. Bartoszek’s main research interests focus on interactions of antitumor drugs with DNA, chemopreventive properties of selected food components, and dietary intervention during cancer chemotherapy.

Dr. Bartoszek has also been involved with both Polish and American publishers in preparation of several textbooks for students and professionals.

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An association between food intake and life is both evident and banal. Simply, food is essential. Any level of human activity, starting from cellular metabolism (e.g., synthesis of biomolecules) through tissue physiology (e.g., muscle contraction) to organism physiology (e.g., physical labor or sports), requires energy expenditure. The main purpose of nutrition is to provide energy for current and future consumption. Otherwise, food is necessary for body growth, maintenance, and tissue repair. Such an intuitive understanding of nutrition, with no precise knowledge concerning the biology of feeding, was satisfactory for centuries. Acquiring the amount of food sufficient to survive was the main and often the only priority for an individual as well as for a group or population. Regardless of the way the food was gained, be it hunting, agriculture, war, or robbery, a shortage or abundance of food was an indication of poverty or prosperity, of a poor or fortunate life. Populations of early food-gatherers, hunters, farmers, and even well-trained eighteenth-century soldiers (“an army has to feed itself”) repeatedly faced the same problem: a shortage of food during the period preceding the next hunt or harvest. And, given the threat of famine versus that of any negative health effects connected with feeding, the former would always prevail.
A seasonal (in agriculture) or occasional (in hunting, fishery) overproduction of supplies posed the question of food storage. Some of the early techniques of meat or fish storage, such as smoking or salting, as well as those of vegetable and fruit conservation, such as drying, pickling, or souring, have stayed basically the same to the present day. Numerous food additives, mostly herbs, were also known and used in the past. Their assortment and applications varied locally, but the primary purpose was generally the same: to prolong the usability of food products. Spices imported from India, surprisingly popular in Europe during medieval times, were used not only to improve taste but also to cover an unpleasant smell and appearance of food stored for too long a time. Obviously, the present-day understanding of spices as antioxidants and scavengers of free radicals is very far from that of the past, when their use was mostly intuitive.

The above historical remarks concern the early, rather basic and primitive understanding of nutrition. Nutrition as a branch of science, exploiting the achievements of biochemistry, physiology, and recently, genetics, does not go back further than the nineteenth century. The main achievement of that time was establishing a classification of food constituents (proteins, fats, carbohydrates, and minerals), followed by determining how to estimate calorie intake. The role of vitamins as necessary constituents of the human diet was not discovered until the beginning of the twentieth century. The first and primary target of the early period of nutrition science was to propose justifiable recommendations for the required amount of food and its composition. Such recommendations, applicable and needed for group feeding of soldiers and workers, came into practical use in the United States, Canada, Great Britain, and the Soviet Union during the 1930s. However, the optimal dietary recommendations still remain an open question.

1.2 NUTRITIONAL NEEDS

To begin a scientific consideration of nutrition, some definitions are needed (Sikorski, 2002; Katan and De Roos, 2003). Nutrients are defined as constituents of food necessary for maintaining normal physiological functions. Essential nutrients are food components required for optimal health. The early dietary recommendations were expected to provide proper and definitely not suboptimal feeding; this can be described by the recently introduced term functional food, understood as a food improving the health or at least the well-being of an individual. This term, to a certain extent, refers to food technology aiming at optimization of food components in a final product without compromising sensory quality (taste, smell, color). Other important questions in nutrition studies besides the design of functional foods for a random population of consumers concern preventive diet and diet as a part of illness treatment.

Work on nutrition optimization involves different aspects and concerns both for the general population and for narrow groups. Construction of the so-called Food Guide Pyramid, performed by the U.S. Department of Agriculture, provides a good example illustrating the need for further work. The pyramid itself is a pictorial model of dietary guidelines calling for a variety of foods and informing the public...
about recommended daily proportions (http://schoolmeals.nal.usda.gov/py/pmap.htm). The general idea underlying the guidelines is to limit the daily intake of fat, replacing it by food rich in polysaccharides, and to enhance the nutritional quality by extensive consumption of fruits and vegetables. One of the recent revisions to these guidelines reflects the recognition of two different types of fats characterized by different levels of aliphatic chain saturation (butter and oil are examples of saturated and unsaturated fats, respectively). The second important revision stems from the appreciation of the function of fiber. It is broadly accepted that bread, oats, and rice can be divided according to their content of dietary fiber into regular, fiber-enhanced (more fiber), and fiber-deficient (e.g., hulled rice) products. On the other hand, results concerning the significance of fiber as an anticarcinogenic agent are still conflicting. It should be admitted that in spite of broad criticism, the Food Guide Pyramid was never officially withdrawn. Finally, there is a tendency to limit meat consumption. Thus, current research efforts aim to provide better guidelines to decision makers, those responsible for collective feeding, and individual consumers.

A relatively new extension of nutrition science reflects the recent expansion of genetics into the biomedical sciences. The genetic information (DNA sequence) specific for the species *Homo sapiens* is in principle the same for all humans. The full DNA sequence of human genome was published in April 2003. However, a number of subtle sequence variations exist that make each individual unique. Individualization of food requirements and ingestion is one of the manifestations of human population variability. The differences in DNA coding regions and in regulatory sequences are responsible for the occurrence of various forms of proteins and variable patterns of enzymatic activity. Awareness of genetic variability and the unraveling of human DNA inspired researchers to create a new field of science called *nutrigenomics* (Go et al., 2003; Müller and Kersten, 2003). Nutrigenomics is attempting to investigate a large area including identification of genes involved in the process of nutrition; estimation of interactions between nutrients and genes and their protein products; analysis of metabolic processes associated with nutrition; and recognition of genetically determined individual predispositions to develop nutrition-related diseases. Personalization of diet in relation to an individual’s genetic status seems to be an obvious target for nutrigenomics. Prevention of diet-related diseases could be a specific goal in this regard.

Studies on obesity provide another good example of an impact of gene polymorphism on nutrition. Obesity has been found to be associated with expression of the gene encoding a protein called leptin. The *ob* (obesity) gene has been identified and localized in the human genome. Ethnic differences in the distribution of *ob* gene polymorphisms and defects have been determined, and their association with genetically determined obesity confirmed (Gura, 1997). In the course of subsequent studies, it became clear that obesity is not a single-gene condition; many more genes are probably involved (Barsh et al., 2000). DNA polymorphism in another group of genes plays a role in individual differences in the sensitivity to a variety of pathogens, including carcinogens. Thus, genetically determined variability within the human population is recognizable at the nutrition level (Ahima and Osei, 2001).
1.3 CARCINOGENIC POTENTIAL OF FOOD

One of the major concerns to be analyzed in a study on carcinogenesis is exposure to exogenous carcinogens, which may be (Ames et al., 1995):

- Present in both natural and polluted human environments
- Associated with working conditions (occupational exposure)
- Attributable to lifestyle (this term primarily covers tobacco smoking or chewing and the consumption of alcoholic beverages)
- Following an intake of cytostatic agents in the course of chemotherapy (paradoxically, drugs designed to eliminate cancer cells can also generate lesions in normal cells)
- Related to diet

According to a rough estimation in a random population of people with cancer, one-third of all cancers are associated with tobacco smoking and one-third with different dietary factors.

The link between diet and cancer is rather complex and definitely not unidirectional. The majority of food products are neutral in relation to carcinogenesis. Only some exhibit pro- or anticarcinogenic properties. Obviously, an abundance of pro-carcinogenic food constituents is as harmful as a lack of anticarcinogens in the diet. The point, however, is that such a categorization is easily applicable only to a single chemical compound, while dietary products are almost exclusively complex mixtures. Hence, there are numerous situations when a single food product contains, at the same time, some substances that are harmful and some that are beneficial (Willett, 2001; Kritchevsky, 2003).

Let us consider coffee, soy sauce, and red wine as examples:

Caffeine present in coffee beans belongs to a group of so-called oxypurines, capable of intercalating into the DNA helix, which leads to a reversible DNA lesion. Methylglyoxal, another compound found in coffee beans, has been identified as the main mutagen in coffee. On the other hand, it has been shown that an extract from coffee beans exhibits antimutagenic activity against nitrosourea-induced DNA damage.

Soy sauce provides another example of such a bivalent activity. Because it is rich in scavengers of oxygen-derived radicals, soy sauce should be classified as an antimutagenic product. On the other hand, Japanese researchers have shown that soy sauce contains a furanone derivative with DNA-breaking activity; however, this activity is masked by the abundance in soy sauce of antioxidants bearing sulfhydryl groups.

The third example to be mentioned here is red wine. Obviously, it contains up to 18% of ethanol. Ethanol by itself is not mutagenic, but it can act as a cocarcinogen, increasing the genotoxicity of other substances; in addition, acetaldehyde, the first metabolite of ethanol, has the ability to interact with DNA. Furthermore, many brands of wine and beer have been shown to contain traces of one of the heterocyclic amines typically
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present in temperature-processed meat and fish and known to be strong mutagens. On the other hand, some of the phenol and alcohol compounds present in red wine have been shown to reduce the DNA damage caused by oxidation and ionizing radiation. These well-documented protective properties along with the acknowledged beneficial influence on digestion are the reason why a moderate consumption of red wine is recommended by nutritionists.

A plethora of carcinogens occurs in food. A categorization of food carcinogens according to their origin has been introduced. The first group includes natural food carcinogens and consists mainly of genotoxins occurring in plants. Their role in nature is to protect plants against fungi, insects, or animals. Examples of such genotoxins are hydrazines (present in mushrooms), safrole (spices in root beer), estragole (dried basil), and psoralens (celery) (Miller and Miller, 1976).

Another group of food carcinogens consists of genotoxic substances that can appear in food because of environmental pollution or in the course of food storage, conservation, and processing.

The term pollution is a very broad one and covers all types of contamination emitted to the ground, surface waters, and atmosphere that could be further acquired by plants, fish, and farm animals. The sources of pollution include industrial waste, diesel exhaust (mostly in the vicinity of roads), and residual amounts of pesticides (Ames et al., 1987; Evangelista de Duffard, 1996).

Dioxins and related compounds (chlorinated hydrocarbons) are of special concern (Kaiser, 2000). Broadly used in the industrial production of chloroorganic chemicals, when emitted to the environment they can easily contaminate food of land animal and fish origin. When dioxins penetrate human bodies, they exert neurotoxic effects and increase cancer risk in many organs. Public attention has been drawn to them as a consequence of several industrial accidents (the best-known example of which was an explosion in Seveso, Italy, in June 1976) that caused serious environmental contamination followed by an increase in morbidity (due to cancer and other diseases) in the population inhabiting the surrounding areas. Dioxins are extremely toxic, and most probably there is no threshold level below which they do not exert carcinogenic potential.

Another example of the effect of environmental pollution on nutrition is contamination of fish by polycyclic aromatic hydrocarbons. Fish living in a polluted aqueous reservoir (e.g., in the vicinity of a petroleum refinery) use the contaminated water to rinse their gills; this ultimately results in the deposition of polycyclic aromatic hydrocarbons in the fish body.

The final example to be mentioned is contamination of foodstuffs by heavy metals. Although some metals are essential for human nutrition, others, including arsenic, cadmium, chromium, nickel, and lead, have been found to pose a potential carcinogenic threat to humans. Arsenic (its main dietary source is fish) and perhaps cadmium appear to be the most harmful.

*Food storage/conservation* as a source of carcinogens usually reflects improper storage conditions resulting in the occurrence of mycotoxins such as aflatoxins, which are known to be hepatocarcinogens. On the other hand, a number of chemicals
are added to food to extend its storage period. Although many of them are recognized as having carcinogenic properties, they are in common use; the goal is to keep a balance between the increased risk of cancer and extended food usability (Ames et al., 1987; Ferguson, 1999). The preservation of attractive appearance and pleasant aroma of food products is also an important concern for food producers. Examples of additives with potentially carcinogenic properties include ethanol, saccharin, caramel, AF-2 (furylfuramide), nitrate (III), and nitrate (V). The latter two are used as coloring and preservation agents in meat products. Interestingly, some potentially harmful substances, such as derivatives of benzoic acid (used as conservatives of processed fruit) or the highly carcinogenic azo dyes, are banned in many countries but still in use in others. This indicates the need for international standardization of food storage and compatibility of the underlying legal regulations.

The last group of food carcinogens to be considered includes those resulting from food processing. Food can be processed in many ways, but perhaps the most frequently used is application of high temperatures (e.g., cooking). The main groups of food carcinogens resulting from high-temperature processing are polycyclic aromatic hydrocarbons (PAH), heterocyclic aromatic amines (HAA), and N-nitrosoamines (NOC):

PAH are products of the incomplete combustion of organic matter and therefore occur commonly in temperature-processed foodstuffs (Phillips, 1999). It is not difficult to predict that more PAH would be generated at high temperatures (but not high enough for complete combustion) than at low temperatures. The exact method of heat processing of food is also an important factor. Direct contact with flames (grill, barbecue) drastically increases PAH content, while boiling in water appears to generate the smallest amount of PAH.

HAA are products of the pyrolysis of food rich in proteins, especially meat and fish (Robbana-Barnat et al., 1996). HA in animals are carcinogenic primarily for the liver. In humans HA may play a role in several cancers, especially colorectal and breast cancers (Ferguson, 1999).

NOC are generated due to high temperatures in food containing nitrate (III) (cured meat, sausages, ham, smoked and pickled foods); in addition, some nitrous compounds can be converted into NOC under certain processing conditions other than high temperature.

The intake of fat and calories constitutes a separate category that does not fall into the above classification (Ames et al., 1987; Weindruch, 1996; Willett, 2001). Several studies have documented an increased risk of cancers (of the colorectum, breast, and other sites) related to these factors, but some of the results were conflicting. Experiments in laboratory animals exposed to carcinogens have shown a decreased cancer risk in animals kept on a fat- and calorie-restricted diet compared to those fed ad libitum. An extension of life span as a consequence of the restricted diet was also shown in many animal models. The beneficial effect of such dietary restriction on human health and well-being has been considered mostly in the context of coronary disease and arteriosclerosis, but a decrease of cancer risk has also been
noted. Mechanisms of the carcinogenic effect of fat consumed in high amounts are not fully clear. The following processes have been taken into account to explain carcinogenicity of fat:

1. Overproduction of reactive oxygen species paralleling lipid oxidation
2. Deregulation of hormone metabolism
3. Alterations of carcinogen metabolism in the liver, which is the organ primarily responsible for elimination of xenobiotics
4. Changes in molecular and cellular processes involving DNA synthesis, DNA repair, and cell proliferation

Food carcinogens have also been categorized according to their mode of action on the genetic material. The first category includes genotoxic agents causing DNA lesions, as well as clastogenic agents inducing chromosome aberrations. The second category, comprising tumor promoters, mostly includes agents responsible for uncontrolled cell proliferation. Based on the involvement of carcinogens in particular stages of the whole process of carcinogenesis, nutrients with carcinogenic activity can be divided into:

1. Agents initiating the process
2. Agents acting at the stage of cancer progression

1.4 ANTICARCINOGENIC ACTIVITY OF FOOD

Having listed the main categories of food-related mutagens/carcinogens, it is also necessary to acknowledge the anticarcinogenic properties of some foodstuffs (Ferguson, 1994). In general, food products rich in antioxidants and free radical scavengers are potential candidates to directly counteract carcinogenesis. Food compounds increasing the processes of detoxification and DNA repair, as well as those decreasing the metabolic activation of procarcinogens, also contribute to anticarcinogenesis, acting indirectly. Anticarcinogenic potential has been documented for fresh fruits and some vegetables such as pepper, which are rich in vitamins; cruciferous vegetables (cabbage, Brussels sprouts, broccoli, etc.), where glucosinolates are responsible for the high detoxifying activity; common spices (especially garlic, also ginger, coumarin, cinnamon, and many others); and coffee (in moderation) as well as black and green tea, with an advantage of the green tea due to a high content of polyphenols, inhibiting activation of many carcinogens.

Categorization of food carcinogens in relation to the progression of carcinogenesis brings about yet another question. Do nutrients with established carcinogenic or anticarcinogenic activity retain the same character throughout the whole process of carcinogenesis? Not many studies have addressed this question, but unexpected results of one of them demonstrated the need for caution in interpreting analogical experiments.

During the mid-1980s, the existing evidence for possible anticancer activity of \( \beta \)-carotene was considered sufficient to justify launching large-scale human intervention trials. These trials were designed to provide final support for the hypothesis that the high consumption of vegetables or fruits containing \( \beta \)-carotene (or of this com-
pound alone) is protective against some types of cancer. Studies were initiated in the United States (4- to 12-year studies completed in 1998 or earlier), China, and Finland.

The Finnish study aimed to determine the influence of dietary intake of β-carotene and vitamin E (separately and together) on the incidence of lung cancer. The study population comprised 29,133 male smokers aged 50 to 69 years. Contrary to what was expected, the group receiving vitamins showed an 18% higher lung cancer incidence and an 8% increase in total mortality. It was consequently assumed that β-carotene, while decreasing the risk of developing tobacco smoke–associated cancer, could increase progression of already-initiated lung cancers. Alternatively, genetic polymorphism was considered as a possible explanation of the Finnish results. The carriers of rare gene variants (presumably more frequent in Finland than in other populations) could have reacted to the anticarcinogenic activity of β-carotene in a different way than the majority of responders do (Charleux, 1996). The experimental attempts to explain a failure of β-carotene preventive activity do not provide a final verdict, but at least two effects have been found (Seifried et al., 2003):

1. A weak prooxidative effect of β-carotene in relation to tobacco smoke
2. An interference of β-carotene with radio- and chemotherapy, affecting treatment efficacy

The findings reported to date leave no doubt that the link between nutrition and cancer initiation should be considered in two opposing contexts: risk increase (consumption of carcinogens present in food) and prevention (intake of food with anticarcinogenic activity).

There is still another point to be raised: can dietary factors influence survival of subjects already treated for cancer? This question is being addressed through chemoprevention studies. Initially, the term “chemoprevention” was used in a narrow sense, concerning a protective activity of some foods towards cancer. Presently, chemoprevention studies and therapeutic attempts focus on three aspects: food intake significance in cancer initiation (already discussed) and cancer progression, and the influence of diet on subjects already treated for cancer, who are at risk for tumor recurrence or development of secondary tumors (De Flora et al., 2001; Sabichi et al., 2003).

Concerning the second and third aspects, there are indications that such factors as fiber, tocopherol, or β-carotene may inhibit cancer growth. At the moment, large clinical trials on the effects of fiber on colon tumor growth inhibition and on the effects of β-carotene, α-tocopherol, and vitamin A on lung cancer are in progress. The uptake of 13-cis-retinoic acid has been shown to decrease the percentage of second primary tumors in head and neck cancer; the occurrence of second primary tumors is among the main failures of treatment in this cancer. Another major focus of chemoprevention diet studies is cancer of the breast (Rock, 2003), the most common tumor site in women. Breast cancer survivors who changed their diet towards fiber-rich and low-fat food, with more fruits and vegetables, were reported to have a lower incidence of secondary cancer events and increased survival. In breast cancer, and to some extent also in head and neck cancer, the risk is also related to hormonal activity. Accordingly, another direction of chemoprevention studies involves investigating the nutritional regulation of hormones.
1.5 GENETICALLY DETERMINED VARIABILITY OF CANCER RISK

Another important topic relating to the association between food and cancer is genetic polymorphism. All humans carry the same set of genes. However, some subtle variations exist in their structure, which results in variable enzymatic activity of their protein products. The variability of enzyme activity is usually distributed in a population according to the Gauss (normal) curve. Some genes have more pronounced variants, in which case the distribution of activity is bi- or trimodal (reflecting low/medium/high activity). There are noticeable ethnic differences in the distribution of gene variants. One of the best-known examples is a shift of alcohol dehydrogenase activity to high values in Caucasians compared with low activity in the Japanese population. This results in relatively low tolerance to alcoholic beverages in Japanese people, as compared to Caucasians.

To exert their activity, carcinogens have to penetrate human bodies, cells, and finally cell nuclei to interact with genetic material. Most carcinogens require metabolic activation inside the penetrated cell. The activation of carcinogens helps them become substrates for the process of detoxication; however, at the same time, they acquire DNA-damaging activity. Carcinogen-induced DNA lesions are removed in the course of the DNA repair process. All the mentioned processes (activation, detoxication, DNA repair) are under genetic control executed by enzymes. It has been established that the activity of DNA-metabolizing and DNA-repair enzymes is extremely variable in the human population. Hence, an individual’s susceptibility to carcinogens is determined genetically, and drastic differences are observed among individuals. For some people, even a low exposure to carcinogens could be very harmful, whereas negative health effects are not observed in other people receiving much more extensive exposure (Ames, 1999; Miller et al., 2001).

The consequences of genetic polymorphism have been discussed in relation to exposure to carcinogens. It must be admitted that nutritional needs and food metabolism are also the subject of polymorphic enzymatic activity and therefore are also subject to variation among individuals.

1.6 EPIDEMIOLOGIC EVIDENCE FOR AN ASSOCIATION BETWEEN NUTRITION AND CANCER

Finally, we should pose an obvious question concerning a proof for an association between nutrition and cancer at the population level. A partial answer has already been given above; in the following section some more epidemiological evidence will be quoted. When assessing the examples below, one has to bear in mind that humans are not laboratory animals. Looking for the effects that a given diet exerts on health, we have to remember that the subjects are exposed to other carcinogens present in the polluted environment associated with their jobs, medication, and lifestyle. Hence, epidemiological studies must be very carefully designed, and their results must be interpreted with caution. With this in mind, nutrition epidemiologists aim to analyze
precisely defined, preferably large groups, characterized by strict and stable feeding habits, lack of nonnutrient exposures, and uniform ethnicity. Some epidemiological examples of connections between nutrition and carcinogenesis, illustrating the problem’s significance, are presented below.

In Kashmir, an Asian region located along the India/Pakistan border, there is a high incidence of esophageal cancer. At first glance, its association with the high consumption of tea seems rather surprising, given the recognized anticarcinogenic activity of tea. However, the way of preparing tea in Kashmir is quite specific. Instead of being hot water–extracted as in China, Europe, and America, the tea leaves are boiled in salty water for a prolonged time. Under these conditions (high temperature and the presence of salt), alkaloids present in the tea leaves are converted into N-nitrosamines, compounds that are highly carcinogenic toward the esophagus.

Another example comes from Sweden. The traditional diet in Scandinavia included a large amount of well-done red meat; in addition, consumption of fresh vegetables and fruits was rather scanty. As a result, the Swedish people have relatively high incidences of gastric and rectal cancers as compared with those in other parts of Europe. This finding triggered a rational campaign aiming to change the eating habits of the nation.

Interestingly, the highest place in world cancer statistics, both general and concerning some specific types of cancer, such as lung cancer and laryngeal cancer, is held by Hungary. Epidemiologists cannot offer any satisfactory explanation. There have been some hints about socioeconomic reasons. The high level of tobacco smoking has been considered, but given the exceptionally high consumption of pepper, which contains anticarcinogenic vitamins, the cause for the observed incidence of cancer still is not clear.

Migrant people are desirable subjects of investigations for epidemiologists. They provide several variants of dependencies, from traditional eating and food-processing habits (kept or lost in the new location), through environmental and local contamination of food, to ethnic differences in genetic polymorphism. A few examples are presented below.

The incidence of tumors associated with using cycad plants as a source of starch in some of the Japanese islands is rather high, while the cancer risk in migrants from these islands who no longer eat cycad nuts is considerably reduced. The high rate of stomach cancer in Egypt believed to be due to the presence of aflatoxins in inappropriately stored crops is not observed in Egyptian emigrants, who no longer consume contaminated crops. The connection between a high level of fat intake and the risk of developing breast cancer demonstrated for Caucasian women does not apply to Japanese women; this seems to reflect genetically determined differences in these populations.

1.7 FINAL REMARKS

Nutritionists recommend the Mediterranean diet because of the associated low risk of cancer development. The reasons for this seem clear: an abundance of fresh fruits and vegetables, preferential usage of plant oil, more fish than meat, lots of spices, and red wine; all of this is within the comprehensive nutrition recommendations.
On the contrary, German, Polish, and Czech diets are rather heavy and rich in fat. Fortunately, these diets include large amounts of cabbage, which seems to be a favorite vegetable in all these nations. Cabbage, together with broccoli, cauliflower, and Brussels sprouts, belongs to the group known as cruciferous vegetables. Their strong anticarcinogenic activity is related to their high content of glucosinolates — compounds that have an ability to stimulate carcinogen-detoxifying enzymes. Fortunately, this anticarcinogenic property of cabbage is not restricted to fresh cabbage but persists in cooked or fermented (e.g., sauerkraut) forms of this vegetable.

Also, some elements of Chinese cuisine could be recommended, e.g., short time of heat processing of meat or fish, abundant usage of spices, a high consumption of green tea.

In general, the amounts of carcinogens taken in with food are rather small. As shown by the epidemiological examples, only a persistently monotonous diet or its elements available as food supplements could be associated with an induction of diet-related cancer. Nevertheless, the question remains: what can be done to reduce the risk of nutrition-associated cancer? Nutritionists offer a very simple recommendation at this point. Its shortest version reads “a varied diet.” In the more extended version, this varied diet is described as rich in fresh vegetables and fruits, with limited usage of meat and fat (preferably vegetable oil), and with the avoidance of alcoholic beverages (except for a moderate amount of red wine).

Bon (but moderate and reasonable) appetit!

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2 Molecular Mechanisms of Carcinogenesis

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2.1 INTRODUCTION

In 1775, the London surgeon Sir Percival Pott reported a link between scrotal skin cancer in adult men and their chronic exposure in boyhood, as chimney sweeps, to soot. The significance of this first recognition of the latent nature of cancer was not realized, however, until ~1950, when occupational exposure of workers to aromatic amines in the dyestuff, textile, and rubber industries was shown to be clearly associated with an increased risk of urinary bladder cancer later in life (Gorrod and Manson, 1986). Likewise, positive associations between cigarette smoking and lung cancer (Hoffmann and Hecht, 1990), and exposure to asbestos and lung cancer and mesotheliomas (Mossman and Gee, 1989) were established. Since the latent period
between initial exposure to a carcinogen(s) and clinical manifestation of cancer may take 15 to 25 and in some cases up to 40 years, elucidation of biochemical and molecular changes involved in cancer development has been particularly difficult. Although substantial progress toward understanding the complex process of carcinogenesis has been made during the past 50 years, its intricate mystery is not yet fully unraveled.

Our current knowledge of the process of chemical carcinogenesis has been advanced by the studies in several areas:

1. Testing of hundreds of chemical compounds in laboratory animals, and in bacterial and cell culture systems, led to delineation of the structure–activity relationships within several chemical classes of compounds eliciting carcinogenic and/or mutagenic activities.
2. Studies of the metabolism of chemical carcinogens prompted discoveries of the metabolic pathways yielding electrophilic reactants capable of modifying DNA and inflicting genotoxic damage (Miller and Miller, 1981).
3. Development of two-stage carcinogenesis models in rodents, notably skin carcinogenesis in mice (Slaga, 1984) and liver carcinogenesis in rats (Pitot and Sirica, 1980), demonstrated the irreversible and permanent nature of cancer initiation by a genotoxic carcinogen and the instability or reversibility of promotion by nongenotoxic (epigenetic) compounds.

In classical terms, three stages of the carcinogenesis process are recognized: initiation, promotion, and progression, each involving multiple steps or events. While the development of cancer in animal models can be followed precisely after administration of a single genotoxic carcinogen, human populations are chronically exposed to complex mixtures of carcinogens from environmental, occupational, tobacco smoke, and dietary sources, making it much more difficult to draw a link between carcinogen exposure and carcinogenesis end points.

This review describes the key molecular and cellular mechanisms involved in the multistage process of carcinogenesis and identifies types of DNA damage induced by representative human dietary carcinogens. The complexity and further details of the carcinogenesis process, including the molecular biology of human cancer, are more comprehensively addressed in numerous recent reviews (Cunningham, 1996; Parodi and Mancuso, 1996; Minamoto et al., 1999; Balmain and Harris, 2000; Hanahan and Weinberg, 2000; Loeb and Loeb, 2000; Bertram, 2001; Brennan, 2002; Hahn and Weinberg, 2002a; 2002b; Nebert, 2002; Bignold, 2004).

### 2.2 MULTISTAGE PROCESS OF CARCINOGENESIS

The vast majority of all carcinogens, including those present in diets, require metabolic activation to become reactive toward DNA nucleophiles. It is now generally accepted that an individual’s genotype determines carcinogen-metabolizing enzyme polymorphism, and in turn “low-risk” and “high-risk” individuals with respect to the activation of carcinogens and potentially initiation of carcinogenesis (Graham et al., 1991). Chemical reactions of carcinogen-derived electrophilic species with
nucleophilic sites in DNA produce covalent adducts, thus inflicting DNA damage in normal cells (Miller and Miller, 1981). DNA adducts formed by a genotoxic compound may (Figure 2.1):

1. Undergo complete repair by DNA repair enzymes
2. Block DNA replication and transcription resulting in cell death
3. Remain unrepaired and be bypassed by DNA polymerases, resulting in permanent genetic damage in a new generation of cells

DNA replication in the presence of nonlethal DNA damage is a key requirement for the transformation of a normal cell into a preneoplastic cell and thus, initiation of carcinogenesis. Neoplastic transformation in replicating cells (chiefly the renewing stem cells in tissues) is acquired through mutation(s), which include base substitutions, deletions, translocations, or other modifications that affect the function of proteins involved in control of cell growth and differentiation. These genetic alterations conclude neoplastic transformation, which is sometimes perceived as an intermediate stage preceding promotion (Williams, 2001). Further stages of carcinogenesis, i.e., promotion of a neoplastic cell via clonal expansion to a benign neoplasm, and progression of the latter to a malignant neoplasm (Figure 2.1), involve complex cellular responses. These are prompted by alterations in the expression of cancer-related genes and function of their products.

### 2.2.1 Molecular Mechanisms of Mutagenesis and Carcinogenesis

#### 2.2.1.1 Role of DNA Damage

Chemical damage to DNA involves the formation of carcinogen–DNA adducts, which itself is not a mutagenic event. Although most DNA damage is repairable, the unrepaired carcinogen–DNA adducts can be misread by DNA polymerases, giving rise to irreversible changes in DNA sequence. The conversion of chemical damage to DNA nucleobases into heritable mutations takes place during cell division. In a normal cell, DNA polymerases generate an accurate and complete copy of the genetic information, using a parent DNA strand as a template. The incoming nucleotides are selected according to their ability to form Watson–Crick base pairs with the bases in the template strand [guanine (G):cytosine (C) and adenine (A):thymine (T)]. Major replicative DNA polymerases have the ability to proofread and remove incorrectly added nucleotides, resulting in a remarkable accuracy (fidelity) of DNA biosynthesis. The typical error rate by DNA polymerases is 1 per $10^8$ to $10^{10}$ nucleotides in *Escherichia coli* and *Drosophila*, and polymerase fidelity is even greater in mammalian cells (Johnson, 1993).

Covalent binding of carcinogen-derived electrophiles to DNA nucleobases can alter their ability to form correct Watson–Crick base pairs by changing their molecular shape and hydrogen bonding characteristics. For example, $O^6$-alkylated guanine exists as an enol tautomer, leading to a change in H-bonding pattern at N-1 and N-6. As a result, $O^6$-alkylguanine prefers to pair with T rather than with C, guanine’s normal Watson–Crick partner (Figure 2.2). Following the second round of DNA
FIGURE 2.1 Multistage carcinogenesis initiated by a genotoxic carcinogen.
replication, this leads to a change from G → A at the affected guanine (transition mutation) (Loechler et al., 1984). Other carcinogens have the ability to intercalate between DNA base pairs, leading to polymerase slippage and the formation of frameshift mutations characterized by deletion or insertion of one or more base pairs (Singer and Grunberger, 1983).

Since many bulky adducts are known to completely block primary replicative DNA polymerases, their ability to induce point mutations has been unexplained until the recent discovery of specialized lesion bypass polymerases (Johnson et al., 1999). These enzymes act cooperatively, adding nucleotides opposite chemically damaged nucleobases and preventing premature termination of DNA synthesis (Johnson et al., 2000). Bypass polymerases can operate in either an error-free or an error-prone manner, depending on lesion identity. For example, UV light–induced cis-syn cyclobutane pyrimidine dimers are readily bypassed by yeast DNA polymerase η in an error-free manner. In contrast, the (−)-trans-anti-N²-guanine adduct of benzo[a]pyrene (B[a]P) diolepoxide is correctly bypassed by polymerase κ by incorporating a C opposite the bulky adduct, but it is misread by polymerases η and χ, giving rise to G → T transversion mutations (Zhang et al., 2000). This process is at the core of mutagenesis in both prokaryotes and eukaryotes, including humans (Loeb and Loeb, 2000; Goodman, 2002; Christmann et al., 2003).

Since DNA is continually under attack by exogenous and endogenous damaging agents, proper functioning of the DNA repair system is crucial for the maintenance of genetic information. Most DNA repair systems take advantage of the double-stranded nature of the DNA molecule by removing a portion of the damaged strand and filling the gap using the complementary strand as a template. However, DNA

**FIGURE 2.2** O⁶-alkylguanine-induced mutation.
polymerases involved in repair generally have lower fidelity than the major replicative DNA polymerases (Friedberg et al., 2001). There are several classes of DNA-repair genes associated with signaling and regulation of DNA repair and with distinct repair mechanisms, including mismatch repair (MMR), base excision repair (BER), nucleotide excision repair (NER), direct damage reversal, and DNA double-strand break (DSB) repair. The mechanisms by which these DNA repair genes and their corresponding proteins are involved in human DNA repair and the specificities of the human error-prone DNA polymerases involved in bypass of DNA replication-blocking lesions are the subject of a comprehensive review by Christmann et al. (2003). The current status of knowledge supports the view that defects in DNA repair lead to accumulation of mutations in the genome and eventually to the development of cancer.

2.2.1.2 Protooncogenes and Tumor Suppressor Genes: Regulators of Cell Growth

The normal cellular functions of protooncogenes and tumor suppressor genes are related to cell growth control, including cell proliferation, differentiation, apoptosis, and genomic stability (Piris et al., 1996; Bertram, 2001; Kumar et al., 2003). In a normal cell, orderly progression through the cell cycle is ensured by periodic activation of cyclin-dependent kinase (CDK) complexes. While growth factors enable the cell to progress through the cell cycle “checkpoints,” growth inhibitory factors up-regulate CDK inhibitors, leading to cell cycle arrest. In carcinogenesis, including initiation and promotion, activation of protooncogenes through “change-of-function” mutations mobilizes signaling cascades and leads to uncontrolled cell division, whereas inactivation of tumor suppressor genes (antioncogenes) relieves the inhibition of the cell cycle. Mutant alleles of protooncogenes are called oncogenes and are considered dominant because they are capable of transforming cells in the presence of their normal counterpart. Tumor suppressor genes are considered recessive oncogenes because damage in both their normal alleles is required for transformation. Two additional types of genes involved in the carcinogenic process are genes regulating apoptosis and genes encoding proteins involved in cellular response to DNA damage (e.g., DNA repair proteins). Genes that regulate apoptosis may behave as dominant or recessive oncogenes. Mutations in DNA repair genes decrease or eliminate the ability of cells to repair nonlethal DNA damage before DNA replication takes place, leading to further mutations.

The following sections describe the functions and mutations of several growth control genes that are frequently altered in the carcinogenesis process.

2.2.2 Molecular and Cellular Events during Initiation and Promotion of Carcinogenesis

2.2.2.1 Activation of Oncogenes

Oncogenes are usually classified according to the function of their corresponding cellular protooncogenes in the signal transduction pathways, and include oncogenes related to growth factors, receptors, and signal transducers (both membrane-bound
and cytoplasmic), and nuclear transcription factors. Within the above classes, more than 100 oncogenes have been identified (Piris et al., 1996; Bertram, 2001).

The ras gene family [Harvey (H), Kirstein (K), and neuroblastoma (N)] consists of low-molecular-weight monomeric proteins with guanosine 5'-triphosphate (GTP)-binding activity (Barbacid, 1987). The ras proteins are found at the inner surface of the cell membrane. Following stimulation by growth factors or other mitogenic stimuli, they bind GTP and slowly hydrolyze it to guanosine 5'-diphosphate (GDP). The GTP-bound ras is a functionally active molecule capable of interacting with a large number of signal transduction molecules, including those stimulating cell proliferation. Oncogenic activation of ras usually results from point mutations that are centered on codons 12 and 13 (binding of GTP) and 61 (hydrolysis of GTP to GDP). Mutated ras lacks GTPase activity. At codon 12, the most frequent alteration is a G → T transversion causing a glycine → valine substitution. This mutation appears to be typically induced at an early stage of carcinogenic process. For example, ~39% of the hyperplastic lesions in the lung (vs. 42% of adenocarcinomas) had codon 12 mutations. The K-ras codon 12, 13, and 61 mutations are frequent in human tumors and have been detected in 30 to 50% of lung and colon carcinomas and 90 to 95% of pancreatic carcinomas (Bos, 1989).

The activation protein 1 (AP1) is a transcription factor capable of activation of several genes including ras protooncogenes (Curran and Franza, 1988). It is a heterodimer of the c-jun and c-fos proteins. The jun/fos heterodimer has a higher binding affinity for DNA (TGACTCA sequences) than jun homodimers do. The fos proteins are incapable of forming dimers and binding to DNA directly. AP1 activity is regulated at both the transcriptional level (expression of the fos and jun genes) and post-translational level (modification of the existing fos and jun proteins). Activation of protein kinase C (PKC) by growth factors increases the transcription of both c-fos and c-jun and also directly phosphorylates both proteins, resulting in increased transcriptional activity. The activities of fos and jun may also be influenced by the redox status of a cell in that the reduction of a cysteine residue in the DNA-binding domains by chemical reducing agents or a nuclear redox factor (ref-1) stimulates DNA-binding activity (Abate et al., 1990). The fos or jun mutations are rarely detectable in human cancers.

The myc gene family includes nuclear phosphoproteins c-myc, N-myc, and L-myc (Marcu et al., 1992). They bind to DNA through dimerization with other proteins, e.g., max (myc-associated X) protein. Both myc and max contain helix-loop-helix and leucine repeat motifs. The max/max homodimers and myc/max heterodimers bind to the same DNA sequence (CACGTG) present in the regulatory region of target genes. In contrast to unstable c-myc protein, which is rapidly synthesized in response to a variety of mitogenic stimuli, max is stable and present in nonproliferating cells in relatively constant amounts. Hence, myc/max formation is likely dependent on the rate of c-myc synthesis. Although binding of the heterodimers to DNA activates gene transcription, the max/max homodimers function as transcriptional repressors. Thus, overexpression of max reverts the oncogenic effect of excess of c-myc protein. In normal cells, c-myc is expressed only in S-phase of the cell cycle. In tumors, this controlled expression is lost, and c-myc is overexpressed throughout the cell cycle, driving the cells continuously towards proliferation. Over-
expression of the myc family genes have been detected in human tumors including breast carcinomas, small cell carcinomas of the lung, and neuroblastomas (Field and Spandidos, 1996). In addition, specific chromosomal translocations involving c-myc gene have been characterized in Burkitt’s lymphoma.

2.2.2.2 Inactivation of Tumor Suppressor Genes

The repression, inactivation, dysfunction, or loss of tumor suppressor genes may result in transformation of normal cells and the promotion of the neoplastic process in initiated cells. The retinoblastoma susceptibility gene (Rb) is the prototype of a tumor suppressor gene whose inactivation plays a key role in the development of several human tumors (Weinberg, 1991). Although numerous new putative tumor suppressor genes are being discovered, the functions of several genes in this class including p53, DCC (deleted in colorectal cancer), and APC (adenomatous polyposis coli) continue to be the subjects of intense investigations.

The p53 tumor suppressor gene is often referred to as “the guardian of the genome” since it plays an important role in gene expression relevant to cell cycle control, DNA repair, and apoptosis (Harris, 1996a; 1996b). The gene encodes a 53-kDa nuclear protein, which is a negative regulator of the G1/S phase transition in the cell cycle. The subcellular localization of p53 protein varies over the cell cycle in that it is present in the cytoplasm in the G1 phase and localizes to the nucleus at the beginning of the S phase. In normal cells, DNA damage inflicted by carcinogens, mutagens and/or ionizing radiation can trigger p53 activation. The p53 protein acts as a transcription factor, up-regulating the expression of several genes (Figure 2.3). p53-mediated up-regulation of CDK inhibitor (e.g., p21) leads to cell cycle arrest in the late G1 phase. This allows time for DNA repair by p53-mediated up-regulation of DNA repair genes, e.g., GADD45 (growth arrest and DNA damage). Successful DNA repair allows cells to proceed with the cell cycle. If the repair fails, p53-induced activation of the Bax gene promotes apoptosis of irreversibly damaged cells (Hall et al., 1993; Fritsche et al., 1993). In cells with loss of p53 function, DNA damage does not trigger up-regulation of p53-dependent genes, and hence, no cell cycle arrest or induction of apoptosis takes place (Figure 2.3).

The majority of the p53 base changes detected in human cancers are observed in exons 5, 7, and 8, with typical mutational “hot spots” at codons 245 (GGC→TGC), 248 (Cgg→CTG), 249 (AGG→ATG), and 273 (CGT→CTT) (Greenblatt et al., 1994; Hussain and Harris, 1999). Because p53 exons 5 to 8 correspond to a sequence-selective DNA binding domain of the p53 protein, mutations in this region inactivate the p53 protein by altering its ability to recognize promoter sequences. For example, p53 gene products containing base substitutions in codons 248 and 273 can no longer act as transcription factors for certain downstream genes, e.g., p21 and Bax (Walker et al., 1999). The loss of functional p53 leads to genomic destabilization, clonal expansion of the affected cells, and an increased likelihood of further genetic damage (Greenblatt et al., 1994). Genetically damaged cells continue to proliferate, acquiring additional mutations, potentially yielding malignant tumors.
The *p53* is the most commonly altered gene in a wide variety of human tumors including cancers of the lung, breast, colon, esophagus, head, neck, and skin (Hollstein et al., 1991). In the Li-Fraumeni syndrome, a syndrome of familial susceptibility to cancer at an early age, a single *p53* point mutation in the germ line is associated with a high risk of bone and soft-tissue sarcomas, breast cancer, and other neoplasia.

### 2.2.2.3 Alteration of Genes Regulating Apoptosis

In contrast to necrosis or the passive form of cell death, apoptosis is an active or “programmed” form of cell death (Gregory, 1996). The striking difference between these two forms of cell death is the efficiency with which apoptotic (but not necrotic) cells undergo phagocytosis by macrophages. Hence, apoptotic cells are seldom encountered *in vivo*. Apoptosis, like proliferation or differentiation, is regarded as a critical point of cellular control. Hence, modulation of apoptosis may influence the evolution or destruction of malignant cells. The release of mitochondrial cytochrome *c* is considered to be a key event in apoptosis, and it is regulated by *bcl-2* (B cell lymphoma/leukemia 2) family genes. Some members of this family, e.g., *bcl-2*, inhibit apoptosis by preventing the release of cytochrome *c*, whereas others, such as *Bax*, promote apoptosis by stimulating cytochrome *c* release. The interaction of *bcl-2* and *Bax* proteins determines their ratio and hence, cell survival or death following apoptotic stimulus. As stated in the preceding section, *p53* can induce apoptosis via transcriptional activation of *Bax* in cells that are unable to repair DNA damage (Figure 2.3).
2.2.3 Molecular and Cellular Events during Progression of Carcinogenesis

A number of oncogenes and tumor suppressor genes are genetically altered in cancer cells, and those alterations accumulate in a stepwise manner during tumor progression (Yokota, 2000). This is evidenced by a greater number of genetic alterations in late-stage than in early-stage tumors. Moreover, the frequencies of alterations in a particular set of genes may be higher in late-stage than in early-stage tumors, whereas the frequencies in another set of genes may be high in both early- and late-stage tumors. Genetic models of tumor progression have been developed for several types of human cancers. The adenoma→carcinoma sequence is particularly well understood in the evolution of colorectal cancers (Fearon and Vogelstein, 1990). The morphologically identifiable stages include colon epithelial hyperplasia followed by formation of early, intermediate, and late adenomas that progressively undergo malignant transformation to carcinomas. The associated genetic alterations involve inactivation of the APC tumor suppressor gene (loss or mutation of the APC locus on chromosome 5q), loss of DNA methylation, mutation of the ras gene on chromosome 12p, loss of a tumor suppressor gene on chromosome 18q, and ultimately loss of p53, and deletions of chromosomes 17p and 18q. Although malignant tumors are monoclonal in origin, by the time they are detectable, their cell population is heterogeneous. The heterogeneity associated with tumor progression most likely results from multiple mutations that accumulate independently in different cells generating subclones with different characteristics (Yokota, 2000).

Mutations in cancer-related genes are responsible for the development of six fundamental changes in cell physiology that collectively determine malignant tumor growth (Hanahan and Weinberg, 2000):

1. Self-sufficiency in growth signals
2. Insensitivity to growth-inhibitory signals
3. Evasion of apoptosis
4. Limitless replicative potential
5. Sustained angiogenesis
6. Ability to invade and metastasize

The order of acquisition of these capabilities varies across different cancers. In some tumors, a particular mutation may confer several capabilities simultaneously, while in other tumors two or more mutations may be needed to acquire a given trait.

During progression of tumorigenesis, a complex interplay among cytokines constituting a major class of intercellular signaling molecules along with hormones, neurotransmitters, and prostaglandins/leukotrienes also takes place (Naylor and Balkwill, 1996). Cytokines are polypeptides of low molecular weight (generally <80 kDa) that bind with high affinity to cell surface receptors and at picomolar concentrations may influence the synthesis of macromolecules and the mode of cell behavior. The production of cytokines in vivo is transient and rigidly controlled. Most mammalian cells secrete a range of cytokines including inflammatory mediators such as interferons, tumor necrosis factors, and interleukins, as well as classic growth factors.
Cytokines are pleiotropic and have multiple biological activities regulating cell proliferation, differentiation, and cell death. Any particular cytokine may have both tumor-promoting and tumor-suppressing effects, depending on the microenvironment of the tumor itself. Tumor-promoting effects include stimulation of neoplastic growth, inhibition of the host response to the tumor, degradation of the extracellular matrix, promotion of tumor stroma generation and neovascularization, and induction of resistance to cytotoxic factors. Tumor-suppressing effects include inhibition of neoplastic proliferation, induction of differentiation, activation of the host antitumor responses, induction of vascular necrosis, and direct cytotoxic effects on tumor cells.

2.3 Dietary Initiators and Promoters of Carcinogenesis in Humans

It is estimated that in the United States, ~30% of cancer death can be attributed to tobacco smoke and another 30% to dietary factors (Trichopoulos et al., 1996). Mechanisms by which certain food carcinogens or promoters may initiate or influence carcinogenesis are briefly discussed in the sections below. Food carcinogens derived from a variety of sources are comprehensively described in Chapters 4 through 8 of this volume.

2.3.1 Examples of Dietary Initiators

Tissue-specific tumorigenicities, DNA target sites, target genes, and mutations therein induced by selected human carcinogens are shown in Table 2.1.

Aflatoxins are produced by fungi, chiefly Aspergillus sp., which are ubiquitous in soils of all except the Arctic and Antarctic regions. The levels of contamination by aflatoxins of grains, seeds, nuts, and vegetables depend on temperature, humidity, and storage conditions and are much higher in hot and humid climates than in the cool and dry zones (Wogan, 1992). Aflatoxin-contaminated feed or purified aflatoxin B$_1$ (AFB$_1$) is carcinogenic for the liver of several animal species. In human populations, the relative risk of AFB$_1$-induced liver cancer (hepatocellular carcinoma) is synergistically increased in individuals exposed to hepatitis B virus (Qian et al., 1994; Henry et al., 1999). The mechanism of action of AFB$_1$ involves metabolic activation by the cytochrome P450 (CYP) 3A4 of human liver to form AFB$_1$ exo-8,9-epoxide, which binds covalently to the N7 position of guanine. The resulting 8,9-dihydro-8-(N7-guanyl)-9-hydroxy-AFB$_1$ adduct (Figure 2.4, structure 1) can be stabilized by imidazole ring opening to produce the corresponding formamidopyrimidine, i.e., the FAPY adduct (Essigmann et al., 1977; Smela et al., 2001). Epidemiological studies have revealed the presence of AFB1-DNA adducts in human urine in areas where the diet is contaminated with AFB$_1$ (Ross et al., 1992) and a characteristic mutation at p53 codon 249 (AGG→AGT, i.e., G→T transversion) in human liver tumors (Hsu et al., 1991). Positive dose-response correlations between estimated dietary exposure to AFB$_1$ and the levels of AFB$_1$-derived DNA adducts in urine and the frequency of codon 249 p53 mutations have been established (Balmain and Harris, 2000). Moreover, exposure of human liver cells to AFB$_1$ in
vitro produces the same \( p53 \) mutation leading to inhibition of apoptosis and enhancement of cell growth.

**Heterocyclic aromatic amines** (HAAs), e.g., 2-amino-3-methylimidazo[4,5-\( f \)]quinoline (IQ) and 2-amino-1-methyl-6-phenylimidazo[4,5-\( b \)]pyridine (PhIP), represent the respective IQ- and non-IQ-type compounds that are formed by heating of mixtures containing creatine, amino acids, and sugars or amino acids and proteins, respectively (Sugimura, 2000). High levels of HAAs, especially of PhIP, are found in grilled (exposed directly to flame) meat and fish or meats cooked for prolonged times (Felton et al., 1986). HAAs are mutagenic and genotoxic in bacterial and mammalian cell systems (Pfau et al., 1999), and carcinogenic in rodents and nonhuman primates (Schut and Snyderwine, 1999; Sugimura, 2000). Although the liver is the preferential tumor target site for the HAAs, PhIP induces mammary gland, colon, and prostate tumors in rats. Metabolic activation of HAAs proceeds via CYP1A2-catalyzed \( N \)-hydroxylation of the amino group, esterification of the \( N \)-hydroxy derivatives via a phase II enzyme(s), and dissociation of the esters to DNA-reactive nitrenium ions. These ions target primarily the C8 atom of guanine for covalent modification of DNA, as evidenced by the adduct formed with a PhIP-derived electrophile (Figure 2.4, structure 2). Its DNA repair product \( N^2 \)-(2'-deoxyguanosin-8-yl)PhIP has been determined in the urine of rats exposed to PhIP (20 mg/kg b. wt. for 6 days) but not in the urine of humans ingesting PhIP (~5 \( \mu \)g) in cooked meat (Fang et al., 2004). Tumors induced by HAAs in rodents contain

<table>
<thead>
<tr>
<th>Carcinogen</th>
<th>Tumor Site(s) ( ^a )</th>
<th>DNA Target Site(s)</th>
<th>Target Gene(s)</th>
<th>Mutation(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin ( B_1 )</td>
<td>Liver</td>
<td>N7-G</td>
<td>( p53 ) (Ser codon 249)</td>
<td>G→T</td>
</tr>
<tr>
<td>Heterocyclic aromatic amines</td>
<td>Liver</td>
<td>C8-G</td>
<td>H-( ras ) (codons 12, 13)</td>
<td>G→T</td>
</tr>
<tr>
<td>PhIP</td>
<td>Colon, mammary gland, prostate</td>
<td>( p53, APC, \beta)-catenin</td>
<td>A→T</td>
<td></td>
</tr>
<tr>
<td>Benzo( [a] )pyrene</td>
<td>Skin, lung, stomach</td>
<td>( N^2)-G, ( N^6)-A</td>
<td>( p53, H-( ras ) (codon 61)</td>
<td>G→G</td>
</tr>
<tr>
<td>( N )-nitrosamines</td>
<td>Compound-specific: liver, lung, esophagus, forestomach, pancreas, bladder</td>
<td>N7-G, ( O^6)-G, ( O^2)-T</td>
<td>K-( ras ) (codon 12)</td>
<td>G→A, T→C</td>
</tr>
<tr>
<td>Acrylamide</td>
<td>Multisite</td>
<td>N7-G, N3-A</td>
<td>Not determined</td>
<td>G→C, A→G</td>
</tr>
</tbody>
</table>

\( ^a \) Based on bioassays in rodents.

\( ^b \) Tumor target in humans and other species.

TABLE 2.1
DNA Target Sites and Genotoxic Effects *In Vivo* of Food-Derived Carcinogens
FIGURE 2.4 Structures of food-derived carcinogen–DNA adducts: 1) aflatoxin B$_1$-N7-2'-deoxyguanosine (AFB$_1$-N7-dG); 2) 1-methyl-2-amino-6-phenylimidazo[4,5-b]pyridine-C8-2'-deoxyguanosine (N$^6$-BPDE-dG); 3) N2-benzoflavone diolepoxide-2'-deoxyguanosine (N2-BPDE-dG); 4) O6-methyl-2'-deoxyguanosine (O6-Me-dG); 5) N7-(2-carbamoyl-2-hydroxyethyl)-2'-deoxyguanosine (N7-GA-dG); 6) xanthosine; 7) 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG); 8) 1,N$_2$-propeno-2'-deoxyguanosine (1,N$_2$-propeno-dG); dR = deoxyribose.
mutations in the cancer-related genes including H-ras, K-ras, p53, APC, and β-catenin (reviewed by Schut and Snyderwine, 1999). In PhIP-induced colon tumors in rats, a characteristic frameshift mutation in the APC gene consists of guanine (adjacent to adenine) deletion in the 5’-GGGA-3’ sequences (Kakiuchi et al., 1995; Nagao et al., 1997). It is considered that this mutation in the APC and p53 genes of human colorectal cancers may serve as a fingerprint of dietary exposures to PhIP and other HCAs (Nagao, 1999).

Polycyclic aromatic hydrocarbons (PAHs) are among the products of incomplete combustion of organic matter. The major sources of human exposure to PAHs consist of deposits of PAH-containing particles from polluted air in water and on plants and PAHs generated during food processing (e.g., smoking) and charcoal-broiling of meats and fish (Phillips, 1999). Median dietary intake of PAHs in nonsmokers is 3 μg per day, and cigarette smoke adds up to 15 μg per day for a heavy smoker. Urinary 1-hydroxypyrene is usually employed as a biomarker of human exposure to PAHs (Scherer et al., 2000). Among PAHs, B[a]P is a suspected human carcinogen (IARC, 1983). B[a]P is metabolically converted to the DNA-reactive (+)-anti-7,8-dihydroxy-c9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPDE), which yields primarily N2-guanine adducts (Osborne et al., 1976). Among the four possible stereoisomers of the adduct, the predominant one is (+)-trans-anti-7R,8S,9S,10S-tetrahydrobenzo[a]pyrene (N2-BPDE-dG) (Figure 2.4, structure 3). This adduct is a promutagenic lesion that accumulates in tumor target tissues of B[a]P-treated animals (Talaska et al., 1996) and in tissues of smokers and occupationally exposed humans (Randerath et al., 1989; Phillips, 1996; Kriek et al., 1998; Rojas et al., 2000).

N-nitrosamines exhibit tumor target selectivity depending on the structure of the nitrosamine and the species employed (Hecht, 1997). N-nitrosodimethylamine (NDMA) and N-nitrosopyrrolidine (NPYR) are especially prevalent in foods including cured meats, cheese and other dairy products, and beer. Because modifications of food processing in industrialized countries have decreased the levels of the nitrosamines, the average daily intake of NDMA and NPYR through the diet is now estimated at 1 μg per person (Bartsch and Spiegelhalder, 1996). However, the levels of these volatile N-nitrosamines present in cigarette smoke add significantly to human exposure (Hecht, 1997). N-nitrosamines may also be formed endogenously in the acid environment of stomach from sodium nitrite, used as a food additive, and nitrosatable amines, e.g., dimethylamine abundant in fish (Mirvish, 1995). The endogenous nitrosation poses an increased risk of gastric and esophageal cancers in humans. N-Nitrosamines are metabolically activated via a P450-catalyzed α-hydroxylation to unstable intermediates that decompose spontaneously to electrophilic DNA alkylating species. Although the major DNA adduct formed from NDMA is N7-methyl-2’-deoxyguanosine, O6-methyl-2’-deoxyguanosine (O6-Me-dG) (Figure 2.4, structure 4), produced initially in minor amounts, is a major mutagenic adduct causing GC→AT transition mutations (Loechler et al., 1984). This adduct, and also O4-ethyl-thymidine, formed from N-nitrosodiethylamine, appear relevant for the initiation of hepatocellular carcinoma (Swenberg et al., 1991).

Acrylamide (AA) is a ubiquitous food contaminant that reaches concentrations of up to 3.9 mg/kg in various heat-processed starchy foods (Tareke et al., 2002).
The formation of AA is a multistep process initiated by the Maillard reaction between asparagine side chains and sugars (Mottram et al., 2002; Stadler et al., 2002). Based on multiorgan carcinogenicity in rodents, AA has been classified as a possible human carcinogen (IARC, 1986). AA has also been found to be mutagenic in human cells (Besaratinia and Pfeifer, 2003). In both rodents and humans, AA is metabolically activated via a P450-catalyzed reaction to form glycinamide (GA), a DNA-reactive epoxide. After treatment of mice and rats with AA, N7-(2-carbamoyl-2-hydroxy-ethyl)-deoxyguanosine (N7-GA-dG) (Figure 2.4, structure 5), a depurinating GA-derived DNA adduct, rather than carboxyethyl adducts that would result from direct DNA alkylation by AA, was detected in tumor target tissues (Segerbäck et al., 1995). Likewise, after treatment of mice with AA or GA, N7-GA-dG was the major adduct in several tissues, and N3-(2-carbamoyl-2-hydroxy-ethyl)-deoxyadenosine (N3-GA-da), another depurinating adduct, was a minor one (Gamboa da Costa et al., 2003). The levels of these adducts were higher after treatment with GA than with AA, suggesting that GA is a genotoxic metabolite of AA.

2.3.2 Examples of Dietary Promoters

Halogenated aromatic hydrocarbons (HAHs), including polychlorinated biphenyls, dibenzo-\(p\)-dioxins and dibenzofurans, are recognized as nongenotoxic carcinogens that act as tumor promoters. Although tumor promoters usually have no carcinogenic activity of their own, they can potentiate the effects of genotoxic carcinogens by a number of different mechanisms that usually involve altered gene expression. A prototype compound of this class, 2,3,7,8-tetrachlorodibenzo-\(p\)-dioxin (TCDD), is designated as a “group 1 carcinogen” (i.e., an agent with sufficient evidence of carcinogenicity for humans) based on its multisite carcinogenicity in animal models and epidemiological evidence in occupationally exposed workers (IARC, 1997). The tumor-promoting activity of TCDD and other HAHs has been linked to their high-affinity binding to the aryl hydrocarbon receptor (AhR) (Poland and Knutson, 1982). The HAH-AhR complex acts as a transcription factor for a number of genes, increasing their expression (Safe, 1995). Among the genes up-regulated by TCDD is CYP1A1, which is involved in carcinogenic metabolism and activation. The promitogenic activity of AhR agonists may depend on TCDD-mediated activation of \(ras\)-dependent signaling cascades. Upon ligand binding, both AhR and Src kinase are released from the 90-kDa shock protein and translocated to the nucleus and cell membrane, respectively. Src kinase mediates phosphorylation of epidermal growth factor receptor that, in turn, provides an activation signal to \(ras\) and other downstream effectors (Schwarz et al., 2000). This leads to a wide range of species- and tissue-specific biological effects, including the loss of normal cell–cell communication and inhibition of apoptosis (De Haan et al., 1994), which contribute to tumor promotion. TCDD and other HAHs are ubiquitous environmental contaminants. Although their levels in the environment are generally low, their accumulation in animal tissues (due to lipophilicity and slow metabolism) may result in much higher concentrations of these compounds in foods. A recent study by Hites et al. (2004) has revealed relatively high levels of contamination with organochlorine compounds of farmed salmon.
from Europe and suggested potential health risks associated with contaminated fish consumption.

Epidemiological studies suggest that red meat intake is associated with an increased risk of colorectal cancer (Norat et al., 2002). Since *heme* content is ~10-fold higher in red than white meat, this iron-bearing prosthetic group of hemoproteins (e.g., myoglobin or hemoglobin) has been proposed to account for the increased cancer risk. The hemoproteins are digested in the upper gastrointestinal tract, delivering heme with ferrous iron oxide to the colon. Heme has been shown to generate lipid peroxyl radicals (LOO\(^\cdot\)) that are capable of DNA modification (Sawa et al., 1998). Supplementation of the diet with hemin (used in place of heme) increased epithelial cell proliferation in the colon of noninitiated rats (Sesink et al., 1999) and formation of aberrant crypt foci (ACF) (size and number) in the colon of rats initiated with azoxymethane, an experimental colon carcinogen (Pierre et al., 2003). The hemin-induced ACF promotion was counteracted by diets high in calcium or oxidation-resistant fats or antioxidants.

### 2.4 ENDOGENOUS DNA DAMAGE

In addition to DNA adducts induced by exogenous chemicals, normal human tissues contain a multitude of lesions produced as a result of spontaneous depurination, oxidation, and deamination. The levels of these endogenous DNA adducts (1 per 10\(^8\) to 1 per 10\(^6\) nucleotides) may be much higher than those induced by exogenous agents (Marnett and Burcham, 1993).

Hydrolytic deamination of the exocyclic amino groups of adenine, guanine, and cytosine produces hypoxanthine, xanthine, and uracil, respectively (Figure 2.4, structure 6 of xanthosine). Because these deaminated products have altered base-pairing properties, polymerase bypass of these lesions leads to transition mutations (Singer and Grunberger, 1983). Although spontaneous deamination in double-stranded DNA is relatively slow, *in vivo* deamination of DNA bases is dramatically accelerated by reactions with nitric oxide, the ubiquitous cellular mediator and cytotoxic product of inflammatory cells (Nguyen et al., 1992). Fortunately, deaminated lesions are rapidly repaired, primarily via the BER pathway. Deamination of the rare DNA base 5-methylcytosine results in thymine, which is a normal DNA base and cannot be repaired. This is thought to be the basis for frequent C\(\rightarrow\)T transition mutations at endogenously methylated CG sites (Rideout et al., 1990).

DNA oxidation by endogenous reactive oxygen species including hydrogen peroxide, superoxide, hydroxyl radical, singlet oxygen, and peroxynitrite, can take place in normal tissues as a result of aerobic metabolism, immune response, and inflammation (Halliwell et al., 1992). Because of their low redox potential, guanine bases within DNA are the primary targets of oxyradicals. Oxidation of guanine gives rise to a variety of products, including 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxo-dG) (Figure 2.4, structure 7). The extent of oxidative DNA damage increases with age, but this effect can be reduced in an animal model via dietary restriction (Hamilton et al., 2001). In humans, decreased caloric intake reduces the amounts of biomarkers of oxidative damage (e.g., thymine glycol and 8-oxo-dG) excreted in urine.
(Simic and Bergtold, 1991). Furthermore, diets rich in proteins, fats, and carbohydrates but lacking fruits and vegetables increase the level of oxidative damage.

Exocyclic DNA adducts, e.g., 1,N6-etheno-dA, N2,3-etheno-dG, 3,N4-ethenocytosine, 1,N2-propano-dG (Figure 2.4, structure 8), and 3-β-β-D-2¢-deoxyribofuranosylpyrimidino[1,2-α]purin-10(3H)-one can be produced in vivo as a result of lipid peroxidation (Nair et al., 1999; Marnett, 2000). The mechanism of their formation involves bifunctional DNA alkylation by lipid-derived α,β-unsaturated aldehydes (e.g., malondialdehyde, trans-4-hydroxy-2-nonenal) and base propanals or phosphoglycoaldehydes resulting from the free radical oxidation of deoxyribose in DNA. These adducts are relatively long-lived in DNA and are strongly mispairing because their formation leads to the blockage of two H-bonding positions of the parent base. Trans-4-hydroxy-2-nonenal, derived from radical oxidation of ω-3 and ω-6 polyunsaturated fatty acids, has been recently demonstrated to preferentially bind to codon 249 of the human p53 gene, a major mutational “hot spot” in many human cancers (Chung et al., 2003).

Abasic sites (AP sites) are formed via the cleavage of the glycosidic bond and a release of a free base. AP sites can be spontaneously produced from purines and form rapidly from N7-alkylated guanines (a half-life of 20 to 100 hours). In addition, an oxidized AP site can be produced as a result of oxidative damage at the ribose. The number of AP sites is 50,000 to 200,000 per mammalian cell, and it is the greatest in brain, followed by colon and heart, and liver, lung, and kidney (Nakamura and Swenberg, 1999).

The presence of endogenous DNA damage in normal tissues may explain the carcinogenic activity of nongenotoxic carcinogens. Many of the endogenously produced lesions have been demonstrated to have mispairing abilities similar to or greater than those of DNA adducts induced by exogenous chemicals. Increased cell proliferation in the presence of “background” endogenous DNA damage will inevitably induce mutations, potentially contributing to human cancer. However, it is important to recognize that the presence of a DNA adduct in a tissue does not necessarily indicate a carcinogenic risk, as other factors downstream of DNA adduct formation (e.g., DNA repair and cell proliferation) play an important role in determining the overall cancer risk.

### 2.5 CONCLUDING REMARKS

Genetic damage leading to uncontrolled cell growth is at the core of carcinogenesis. Change of function mutations in critical genes are induced primarily through damage to the structural integrity of DNA via covalent binding of metabolically activated carcinogens to DNA bases to yield carcinogen–DNA adducts. Over the past 25 years, progress in the development of sophisticated analytical technologies for the detection and quantitation of carcinogen–DNA adducts advanced the limits of detection to one adduct in 10⁷ to 10⁹ nucleotides, and in some cases to one adduct in 10¹² nucleotides (Poirier et al., 2000). Such sensitivities allow the assays of DNA adducts in tissues of humans exposed to occupational, environmental, tobacco smoke, chemotherapeutic, and dietary carcinogens (Hemminki, 1993; Bartsch 2000). Further-
more, these ultrasensitive methods allow delineation of specific DNA damage arising from oxidative stress and lipid peroxidation, which are believed to be driving forces in several human cancers. Although the positive correlations between the level of exposure and that of DNA adducts were reported for several carcinogens, significant interindividual variations in adduct levels have been shown in populations with comparable exposure to carcinogens. This observation and characterization of genetic polymorphism in xenobiotic-metabolizing enzymes (Graham et al., 1991) led to a recognition of “pharmacogenetic variants” of host susceptibility to carcinogenic insults. Thus, a combination of genotyping and measurements of macromolecular adducts may eventually provide an insight into an individual’s risk of developing cancer.

Although it is unequivocally accepted that carcinogenesis is a multistage process, a clear separation of the three major stages — initiation, promotion, and progression — is becoming obscure with discoveries of many new cancer-related genes. Heritable changes in these genes leading to changes or loss of their functions involved in regulation of cell proliferation, differentiation, apoptosis, and response to genetic damage during initiation and promotion of carcinogenesis are relatively well understood. However, the roles of these genes in the progression to a malignant phenotype and metastasis of cancer remain unclear (Yokota, 2000). Two different molecular approaches have been taken to advance our understanding of these late stages of carcinogenesis:

1. Identification of genes whose alterations accumulate during cancer progression
2. Identification of genes whose expression is responsible for the acquisition of metastatic potential

Suitable assay systems, e.g., reconstituted organ rather than cell culture systems, need to be developed for the functional analyses of gene products with respect to metastasis.

Further improvements in DNA sequence analysis are desired to allow identification of specific mutations caused by a specific agent and characteristic for a particular stage of carcinogenesis. Dietary factors, along with exposure to infectious agents, tobacco products, and environmental carcinogens, play a major role in chemical carcinogenesis. Cancer development requires a series of molecular alterations regulating cellular responses and events, each of which can be affected by carcinogenic and anticarcinogenic food components. Advancements in our knowledge of dietary carcinogens and their mechanisms of action can potentially lead to decreased cancer risk and incidence and improved outcomes of cancer treatment.

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3 Metabolism of Chemical Carcinogens

Zofia Mazerska

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3.1 INTRODUCTION

Organisms are exposed to a large number of xenobiotics (compounds that are foreign to the organism but can be metabolized by it) that penetrate through the skin and respiratory system. In addition, specific groups of foreign substances are delivered to the human organism by the gastrointestinal tract. These include drugs, natural food constituents, and chemical pollutants found in food and drinking water. Organisms deal with these substances, which are usually lipophilic, by means of enzymatic biotransformation systems that defend against the toxic action of xenobiotics. Excre-
tion of the xenobiotic is the simplest means of detoxification; however, this can be accomplished only in the case of relatively polar, hydrophilic compounds able to pass through the kidneys. Therefore, a lipophilic xenobiotic must undergo some kind of biotransformation to be converted into a relatively hydrophilic substance.

The systems of biotransformation enzymes comprise Phase I and Phase II enzymes. In Phase I, a xenobiotic undergoes transformation involving oxidation, reduction, or hydrolysis. Often a hydroxyl group is introduced into the lipophilic molecule, giving rise to more polar and more reactive product in comparison to the parent compound. In Phase II, a xenobiotic or its Phase I metabolite undergoes a conjugation with an endogenous molecule facilitating, e.g., excretion. Phase II can be divided into conjugations of electrophiles, catalyzed for example by glutathione-S-transferases, and conjugations of nucleophiles, catalyzed among others by glucuronyl transferases. Although Phase II usually leads to detoxification, some cases of bioactivation are also observed. Phase I transformations are frequently associated with bioactivation. It is becoming obvious that the transport of xenobiotics and their metabolites out of the cell can also be a crucial determinant of their final effect in a living organism. These transport processes are sometimes referred to as Phase III of metabolism and are considered as a supplementary line of the organism’s protection against the toxic action of xenobiotics (Van Iersel et al., 1999).

Metabolism is a crucial step in chemical carcinogenesis since most carcinogens require metabolic activation in order to exert their biological effects. Metabolism may also play a beneficial role, detoxifying the reactive metabolites. Pioneer works on metabolism of carcinogens demonstrating ring hydroxylation of 2-naphthylamine were performed between 1938 and 1941 (Miller, 1994). In 1947 James and Elisabeth Miller, who worked with the azo dye \(N,N\)-dimethyl-4-aminoazobenzene, showed the binding of some colored products to proteins (Miller and Miller, 1947). Moreover, the amount of protein-bound dye in rat liver was correlated with the carcinogenicity determined for a set of related dyes. Metabolism of \(N,N\)-dimethyl-4-aminoazobenzene was also studied in the first cell-free enzymatic system developed nicotinamide adenine dinucleotide phosphate (NADPH)-fortified rat liver microsomes (Mueller and Miller, 1948). Subsequent works in 1952 indicated the induction of rat liver enzymes by methylcholanthrene (Richardson et al., 1952) and human enzymes by barbiturates (Remmer, 1957). The development of more advanced experimental techniques enabled demonstration of the induction of aryl hydrocarbon metabolism by polycyclic aromatic hydrocarbons (PAHs) in cell culture (Nebert and Gelboin, 1968). Several years later, the existence of the “Ah” receptor, the function of which depends on xenobiotic ligands, was reported (Poland et al., 1976). In 1973, Kellerman and Show indicated that humans could be grouped into different phenotypes on the basis of the induction level of aryl hydrocarbon hydroxylase in human lymphocytes (Kellerman et al., 1973a). These authors also showed that the incidence of lung cancer in smokers is correlated with the hydroxylase level in their lymphocytes (Kellerman et al., 1973b). This was probably the first significant result in molecular epidemiology in the area of chemical carcinogenesis. Subsequent studies on carcinogen metabolism have been aimed at finding out the link between the level of expression of metabolizing enzymes and human susceptibility to cancer.
3.2 ENZYMATIC SYSTEMS INVOLVED IN METABOLISM OF CARCINOGENS

Studies on the enzymes catalyzing metabolic transformations of xenobiotics were initiated by Omura and Sato when the pigment enzyme in liver microsomes, termed cytochrome P450, was discovered (Omura and Sato, 1962). Cytochrome P450 was recognized as a terminal oxidase in the microsomal electron transport chain (Estabrook et al., 1963). The group of P450 proteins represents the enzymes of the Phase I xenobiotic transformations associated with activation of xenobiotics. In the 1970s, it was commonly believed that activated carcinogens were so reactive that they could not diffuse far from the site of reaction. It was even hypothesized that only nuclear enzymes could be involved in the activation of carcinogens preceding their DNA binding. In contrast to this view, several works showed that the distribution of metabolites, even those with a 30-sec half-life, could be widespread (Kapitulnik et al., 1978; Guengerich, 2000).

Phase II metabolism involves detoxification of xenobiotics or their reactive metabolites by conjugation with endogenous substrates. What has been appreciated in recent years is the variety of ways in which most of the conjugation enzymes activate procarcinogens. The typical mediators of Phase II detoxification of carcinogens are glucuronosyltransferases (UGT) and glutathione-S-transferases (GST). Epoxide hydrolases (EH), sulfotransferases (SULT), and N-acetyltransferases (NAT) are also among the conjugation enzymes giving rise to less toxic metabolites. However, the two latter enzymes are also strongly involved in the activation of carcinogens. The common types of reactions mediated by carcinogen-metabolizing enzymes are presented in Table 3.1.

### 3.2.1 CYTOCHROMES P450

The term cytochrome P450 refers to a family of heme proteins present in all types of mammalian cells except for mature red blood cells. Cytochrome P450–like

### TABLE 3.1
Reactions Catalyzed by the Enzymes Involved in Carcinogen Metabolism

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Reactions</th>
<th>Subcellular Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP</td>
<td>Oxidation at carbon, nitrogen, and sulfur atom</td>
<td>Microsomes</td>
</tr>
<tr>
<td>UGT</td>
<td>Conjugation of glucuronic acid with –OH, –NH</td>
<td>Microsomes</td>
</tr>
<tr>
<td>SULT</td>
<td>Conjugation of SO₃ with –OH, –NH</td>
<td>Cytosol</td>
</tr>
<tr>
<td>GST</td>
<td>Glutathione conjugation of electrophiles</td>
<td>Cytosol and microsomes</td>
</tr>
<tr>
<td>NAT</td>
<td>Acetyl transfer to –NH, –OH</td>
<td>Cytosol</td>
</tr>
<tr>
<td>EH</td>
<td>Hydration of epoxides (hydrolysis)</td>
<td>Cytosol and microsomes</td>
</tr>
</tbody>
</table>

enzymes also occur in plants and prokaryotes. Substrates for mammalian P450 enzymes include endogenously synthesized compounds such as steroids and lipids, such as prostaglandins and leukotrienes (eicosanoids), as well as exogenous compounds such as medicines, food additives, or environmental pollutants that enter the body through food sources, inhalation from the air, or absorption through the skin. The contradictory points regarding the function of P450 have been addressed (Guengerich, 2001). Some researchers claim that these enzymes have physiological substrates and that the reactions performed on xenobiotics constitute a protective mechanism only accidentally. The other view is that these enzymes are not particularly critical to life and are part of a general, nonspecific system of environmental stress management. Guengerich supported the latter view by demonstrating that some people lack certain metabolic enzymes. These individuals generally fare well unless they are exposed to particular drugs, the action of which is seriously dependent on a single missing enzyme. Experiments with knock-out mice also demonstrated that a major evolutionary role of hepatic P450 is to protect mammals from environmental insult (Peters et al., 1999).

The structures of bacterial and mammalian cytochromes P450 are similar. The cytochromes P450 of eukaryotic organisms are all bound to membranes of endoplasmic reticulum (microsomes) or mitochondria, whereas most bacterial P450s are water soluble. All microsomal P450s have a highly hydrophobic sequence at their amino terminus, which targets and anchors the enzyme molecules to the reticulum membrane.

The action of P450 consists of monooxygenation of various substrates. Such reactions require molecular oxygen and a supply of reducing equivalents from NADPH or nicotine adenine dinucleotide (NADH). The electron transport does not occur directly from the electron donor to the P450 heme. The microsomal P450 system, embedded in the endoplasmic reticulum membrane, includes two components: cytochrome P450 and NADPH–cytochrome P450 reductase (Figure 3.1). Two electrons needed for the catalytic cycle of cytochrome P450 are donated from NADPH and are transported one by one from flavine adenine dinucleotide (FADH) and flavine mononucleotide (FMNH). The first electron mediates oxygen binding to the heme iron; the second cleaves the oxygen molecule to generate active oxygen species able to bind to the substrate (Guengerich et al., 1997).

Cytochrome P450 enzymes catalyze a wide variety of oxidation reactions including aromatic and aliphatic hydroxylations, N- and S-oxidation, and oxidative dealkylation. Many isoforms of cytochrome P450 exist; they have overlapping but distinct substrate specificities to foreign chemical compounds. The genomes of mammalian species include about 50 to 80 P450 genes; some plants contain even larger numbers of P450 genes, whereas the unicellular eukaryote Saccharomyces cerevisiae has only three (Omura, 1999).

The increasing number of cytochrome P450 isoforms prompted a systematic classification of all P450s into “families” and “subfamilies,” with serial numbers based on the degree of similarity in their amino acid sequence. All members of a particular family, e.g., CYP1, display at least 40% similarity to other members of that family, whereas members of a subfamily, e.g., CYP1A, have at least 50% similarity. Several hundred P450 genes from different organisms have been
sequenced, and the number of families of cytochrome P450 has now reached nearly 200, including about 40 families of animal P450s, 25 of fungal P450s, 50 of plant P450s, and 80 of bacterial P450s (Omura, 1999).

The activation of carcinogens in humans appears to be carried out only by a small number of P450 isoforms. The CYP1A family consists of two members, which are about 70% identical in their sequences. CYP1A1 is essentially an extrahepatic enzyme; human CYP1A2, in contrast, is expressed only in the liver. Some principal carcinogens that are substrates for the specific human P450 isoforms are presented in Table 3.2.

Although the total level of liver microsomal P450s does not vary much among humans, the levels of many of the particular isoforms of P450 may vary by as much as one to three orders of magnitude; this variation is related to several factors. The first is genetic polymorphism, where heritable DNA changes lead to the lack of production of some P450s. The next is the lack of inducibility of a given isoform or synthesis of a P450 form with altered catalytic activity. Polymorphism is usually defined as a genetically determined difference affecting at least 2% of a population. Different races often display similar patterns of P450 expression. At the same time, polymorphisms that show strong racial links also exist.

The knowledge about interindividual variations in human P450 isoforms and their relation to the prediction of cancer risk has met much optimism. Such a relation between CYP1A1 polymorphism and lung cancer risk induced by smoking was first pointed out in 1990 and is still under intensive study (Kawajiri et al., 1990).

FIGURE 3.1 The microsomal P450 enzyme system and electron transport.
# TABLE 3.2
Human P450 Enzymes Involved in the Activation of Some Carcinogens

<table>
<thead>
<tr>
<th>CYP1A1</th>
<th>CYP1A2</th>
<th>CYP2A6</th>
<th>CYP2E1</th>
<th>CYP3A4</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Benzo[a]pyrene" /></td>
<td><img src="image2.png" alt="PhIP" /></td>
<td><img src="image3.png" alt="N,N-Dimethylnitrosamine" /></td>
<td><img src="image4.png" alt="Benzene" /></td>
<td><img src="image5.png" alt="7,8-Dihydroxy-7,8-dihydrobenzo[a]pyrene" /></td>
</tr>
<tr>
<td><img src="image6.png" alt="2-Amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine (PhIP)" /></td>
<td><img src="image7.png" alt="2-Amino-3,5-dimethylimidazo-[4,5-f]quinoline (MeIQ)" /></td>
<td><img src="image8.png" alt="4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)" /></td>
<td><img src="image9.png" alt="Styrene" /></td>
<td><img src="image10.png" alt="Acrylonitrile" /></td>
</tr>
<tr>
<td><img src="image11.png" alt="4-Aminobiphenyl" /></td>
<td><img src="image12.png" alt="2-Naphthylamine" /></td>
<td><img src="image13.png" alt="Vinyl chloride" /></td>
<td><img src="image14.png" alt="Vinyl chloride" /></td>
<td><img src="image15.png" alt="Vinyl chloride" /></td>
</tr>
<tr>
<td><img src="image16.png" alt="2-Naphtylamine" /></td>
<td></td>
<td></td>
<td><img src="image17.png" alt="Carbon tetrachloride" /></td>
<td><img src="image18.png" alt="17β-Estradiol" /></td>
</tr>
</tbody>
</table>

Another reason for variation in levels of P450 enzymes is their induction and inhibition by exogenous factors. Many P450s are known to be induced by drugs, foods, alcoholic beverages, tobacco smoke, or environmental pollutants. The mechanism of induction has been studied most thoroughly for CYP1A1. In the case of this isoform, the induction occurs through AhR (Aryl hydrocarbon Receptor). AhR is a ligand-dependent transcription factor that regulates the expression of a battery of genes in a wide range of species and tissues (Denison and Nagy, 2003). The model of AhR action is presented in Figure 3.2. An inducer, e.g., PAH, enters the cell and binds to the cytosolic AhR, which exists as a multiprotein complex. This complex is translocated to the nucleus, where the ligand–AhR is released. Afterwards, the ligand–AhR dimerizes with the nuclear protein Arnt. Such a conversion leads to a ligand–AhR–Arnt complex, which expresses high affinity to DNA. Binding to the specific DNA recognition site, the DRE site, stimulates the transcription of the CYP1A1 gene. Binding to other recognition sites can stimulate other genes. The presence of AhR transduction pathways was shown in various species, tissues, and cell types. Therefore, many of the toxic and biological effects of xenobiotics, including food components, might result from their binding to AhR. It has also been suggested that endogenous AhR–ligands should exist in the human organism (Denison and Nagy, 2003; Handschin and Meyer, 2003).

Dioxins and food carcinogens, such as PAHs, represent the most widely characterized class of AhR ligands. However, the ability of many dietary plant compounds, such as indole-3-carbinol (I3C), dibenzoylmethanes, curcumin, and carotenoids, to bind to AhR and/or to induce AhR-dependent gene expression has also been reported (Jeuken et al., 2003).
3.2.2 OTHER CARCINOGEN-METABOLIZING ENZYMES

3.2.2.1 UDP-Glucuronosyl Transferases (UDPGT; EC 2.4.1.17)

Glucuronidation, the reaction of Phase II metabolic transformation, involves the transfer of the glucuronic acid group from uridine diphosphate (UDP)-glucuronic acid to an acceptor group of a substrate. UDP-glucuronosyl transferases, UGTs, catalyze this reaction (Formula 1). A significant number of functional groups, e.g., –OH, –COOH, –NH₂, –SH, and C–C, are conjugated in this way. The UDP conjugation is considered a fundamental natural mechanism for detoxification and elimination of lipophilic waste chemicals from the body. In general, glucuronides are less biologically or chemically reactive than their corresponding parent aglycones are, and they exhibit higher polarity and excretability. However, a number of exceptions to this rule are now known. These include two classes of glucuronides with electrophilic properties, N-O-glucuronides of hydroxamic acids (N-hydroxy-N-acetyl-arylamines) and acyl glucuronides of carboxylic acids (Formula 2). Furthermore, several other glucuronides are known to express higher pharmacological or toxicological activities compared with their parent aglycones, for example, glucuronides of morphine, all-trans-retinol, and all-trans-retinoic acid, as well as natural and synthetic estrogens (Ritter, 2000).

UGTs are membrane-bound enzymes (Figure 3.3A) associated with smooth endoplasmic reticulum (ER) membrane. The protein molecule is anchored in the membrane by one peptide fragment. The localization of the active site of UGT inside the lumen of the ER requires a specific carrier of the UDP-glucuronic acid to cross the membrane and reach its binding site on UGT (Figure 3.3B). There are strong

R-O-Gluc
Ar-O-Gluc
R-NH-Gluc
Ar-NH-Gluc
R-COO-Gluc
Ar-COO-Gluc

Formula 1

N-O-glucuronides
Acyl glucuronides

Formula 2
suggestions that the active enzyme is a dimer consisting of two UGT polypeptide chains. Thus, the substrate could diffuse through the bilayer membrane by the channel formed within two monomers and bind to the active site. After the conjugation, the glucuronide product is exported into the cytosol via the same channel (Radominska-Pandya et al., 1999).

Two large families of UGTs have been found. The UGT1 family includes nine known isoforms in humans (UGT1A1 and UGT1A3 through UGT1A10) and seven in the rat. The UGT2 family is divided into three subfamilies: 2A, 2B, and 2C. Although the liver is the main site of glucuronidation, UGTs have been expressed in several extrahepatic tissues, as exemplified by 2B7, which is expressed not only in the liver but also in the gastrointestinal tract, kidney, and brain. The major

endogenous substrates for the human UGT1A family are bilirubin, steroid hormones, bile acids, retinoids, and thyroid hormones. The substrate specificity of the UGT2B family resembles that of UGT1A; however, UGT1A isoforms glucuronidate a significantly larger number of xenobiotic substrates than do the UGT2B enzymes.

The key classes of xenobiotics that are UGT substrates include phenols, anthraquinones, flavones, and many drugs. Foodborne carcinogens also undergo biotransformation by glucuronidation. For instance, the heterocyclic aromatic amine MeIQx, metabolized by primary human and rat hepatocytes, was converted by UGT to three different glucuronides (Langouët et al., 2001) (Formula 3).

3.2.2.2 Sulfotransferases (SULT; EC 2.8.2)

Sulfotransferase enzymes transfer the sulfonate group from 3'-phosphoadenosine-5'-phosphosulfate, PAPS, to a nucleophilic group of their substrates (Formula 4). Like glucuronidation, it is a conjugation reaction of Phase II. In mammals, two classes of sulfotransferases can be distinguished. One class metabolizes macromolecular endogenous structures and comprises mainly membrane-bound forms localized in the Golgi apparatus. The other class consists of cytosolic proteins that metabolize xenobiotics and small endogenous compounds (such as hormones and neurotransmitters). All cytosolic sulfotransferases studied are members of the superfamily termed SULT.
SULTs are beneficial in the metabolism of numerous xenobiotics. Many sulfates and sulfamates formed by SULTs are stable. This, together with water solubility, is favorable for their excretion. However, sulfotransferases rarely inactivate ultimate carcinogens and mutagens because these are normally electrophiles, whereas SULTs transfer the sulfonate group to nucleophilic functional groups. Nevertheless, SULTs may sequester procarcinogens by competing with activation pathways. For example, sulfo-conjugation of aromatic amines leading to the formation of sulfamates may compete with N-hydroxylation and the subsequent activation by N-O-acetylation or N-O-glucuronidation. Acetylamino fluorene-N-sulfate (Formula 5) was the first electrophilic metabolite of a carcinogen to be discovered (DeBaun et al., 1968). However, it was demonstrated subsequently that sulfation is an important step in the activation of many rodent hepatocarcinogens, such as 4-aminobenzene, safrole, 2′,3′-estrargole, hydroxymethylbenzo[a]pyrene, and many others. The sulfate group can act as a leaving group and lead to the formation of strongly electrophilic cations. Such a heterolytic cleavage of the sulfate group is facilitated if the resulting cation is stabilized by inductive effects or by mesomerism. Otherwise sulfuric esters are rather stable (Formula 6). Food carcinogens such as benzylic and allylic alcohols, as well
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as aromatic hydroxylamines and hydroxamic acids, are frequently esterified by SULTs, giving rise to the above-described reactive sulfates.

In humans, 11 SULT forms have been detected: three in the SULT1A family, SULT1B1, two in the SULT1C family, SULT1E1, SULT2A1, two SULT2B1 forms, and SULT4A1. SULT1A1 levels are very high in the liver; this form is present at lower levels in many other tissues. Low levels of SULT1A2 have been detected in the liver; however, this form has not been found in any extrahepatic tissues. On the other hand, the level of SULT1A3 is high in the gut, detectable in many other extrahepatic tissues, and negligible in the liver (Glatt, 2000).

Nearly 100 compounds were mutagenic in SULT-expressing cells but inactive or much less active in the corresponding SULT-deficient control cells. The activated compounds were members of the following classes: benzylic alcohols, allylic alcohols, aromatic hydroxylamines, aromatic hydroxamic acids, or secondary nitroalkanes. Benzyllic aldehydes and ketones, represented by 1-formylpyrene or acetylpyrene, showed SULT-dependent mutagenic effects under conditions that led to their reduction to the corresponding alcohols. Aromatic amines and amides, including food-derived carcinogens, showed SULT-dependent carcinogenicity only in the presence of N-hydroxylation activity, provided usually by CYP1A2. It was shown that the liver carcinogen acetylaminofluorene (AAF) is strongly mutagenic only in cells coexpressing rat CYP1A2 and rat SULT1C1 (Glatt et al., 1998).

### 3.2.2.3 Glutathione S-Transferases (GST; EC 2.5.1.18)

GSTs are dimeric enzymes that catalyze the conjugation of glutathione, GSH, to electrophilic xenobiotics in order to inactivate them and facilitate their excretion from the body. Glutathione conjugation is considered to be a crucial Phase II pathway
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of detoxification of carcinogenic metabolites. Several carcinogens, such as the reactive benzo\[a\]pyrene diolepoxide, aflatoxin-2,3-epoxide, and reactive sulfates, are substrates for GSTs. Thus, GSTs have an essential protective function in chemical carcinogenesis. On the other hand, the conjugating agent, GSH itself, is an important factor in the detoxification of xenobiotics due to the nucleophilicity of the sulfur atom and antioxidant properties. In a limited number of cases, however, carcinogens are activated through GST-catalyzed conjugation.

GST isoforms described in humans, rats, and mice are divided into at least seven classes, five of which are cytosolic and two of which are membrane-bound proteins in microsomes. The cytosolic isoforms are designated A, M, P, T, S, and K (for alpha, mu, pi, theta, sigma and kappa classes). For example, the human alpha class GST is designated as hGSTA (Van Iersel et al., 1999).

As with P450 isoforms, the substrate selectivities of GST isoforms are broad and overlapping. However, some differences between the various classes exist. Thus, class alpha isoforms exhibit peroxidase activity, and class mu isoforms perform the GSH-conjugation of polycyclic aromatic hydrocarbon epoxides and diolepoxides, whereas class theta has a notable catalytic activity towards methylene chloride and several related halogenated alkanes and alkenes. The conversion of aromatic hydrocarbon epoxides is a well-known detoxification pathway, whereas GST-dependent metabolism of dihaloalkanes leads to a reactive intermediate, episulfonium ion, forming DNA adducts. A “reactivation” of the detoxified hydroxyethylene conjugate has also been observed (Guengerich, 1992) (Formula 7). In contrast, polychlorinated alkenes are metabolized in the presence of liver GSTs by an addition–elimination reaction to give S-(haloalkenyl)glutathione conjugates followed by cleavage to cysteine S-conjugates (Formula 8). The latter are urinary metabolites detoxified into mercapturic acids, which, in turn, are activated by cysteine conjugate β-lyase to yield reactive electrophiles (Dekant, 2003).

In humans, a clear polymorphism has been observed for classes mu, pi, and theta isoforms. The glutathione S-transferase gene, GSTM1, is expressed in only 50% of the samples from populations analyzed. In contrast to GSTM1, the occurrence of GSTT1 null genotype frequency is observed in only about 15% of the European population. This deleted genotype is related to ethnic background. The
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GSTM1 null genotype or GSTM1 deficiency has been associated with increased levels of DNA damage and with higher risks of some forms of cancer. The GSTM3 isoform seems to play an important role in determining the risk of lung cancer. The GSTM1 genetic polymorphism has been extensively studied with respect to metabolic activation and detoxification of tobacco carcinogens within the framework of the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens (GSEC). Analyses of the data from 9500 subjects surprisingly revealed no evidence of increased risk of lung cancer among carriers of the GSTM1 null genotype and no evidence of interaction between GSTM1 genotype and either smoking status or cumulative tobacco consumption (Benhamou et al., 2003). Moreover, no protection by GSTM3 was observed in heavy smokers, whereas in smokers with tobacco consumption of less than one pack per day, GSTM3 was protective (Piipari et al., 2003). Therefore, the elucidation of GST-related cancer risk factors still needs intensive study.

Glutathione S-transferases are inducible enzymes; however, the relative rates of induction are low compared to those observed for P450s. The activity of GSTs can be induced by the essential oils eugenol and anise, as well as by some components of cruciferous vegetables.

Sometimes, the conjugate of the detoxified compound with glutathione is a strong inhibitor of GSTs. Many naturally occurring phenols such as plant phenols, ellagic acid, caffeic acid, and ferrulic acid or quercetin have been shown to effectively inhibit GSTs in a competitive manner (Formula 9). On the other hand, irreversible GST inhibition is displayed by several quinones including natural agents juglone and vitamin K as well as synthetic quinones, e.g., tetrachloro-1,2- and 1,4-benzoquinones.

The regulation of GST level in the cell was shown to depend on antioxidant-responsive elements (ARE) and xenobiotic-responsive elements (XRE) that recognize specific DNA sequences (Van Iersel, 1999). Also, several protein binding sites are involved in the complex mechanism leading to the induction of GSTs genes.
3.2.2.4 **N-acetyltransferases (NAT; EC 2.3.1.5)**

*N*-Acetyltransferases (NATs) are Phase II metabolizing enzymes that catalyze the transfer of an acetyl moiety from acetyl-CoA to the nitrogen or oxygen atom of primary arylamines, hydrazines, and their N-hydroxylated metabolites. NATs therefore play an important role in the detoxification and/or activation of substrates including constituents of cigarette smoke, numerous occupational carcinogens, and heterocyclic amines in cooked meat. In general, N-acetylation produces less active metabolites, while O-acetylation generates more dangerous products because of easy displacement of the leaving group formed.

NAT enzymes have been identified in several species. In humans, two functional isoforms of *N*-acetyltransferase, NAT1 and NAT2, have been described. Interindividual genetic variations have been shown to cause differences in NAT1 and NAT2 protein levels and activity. This acetylation polymorphism in the human population, with its ubiquitous exposure to food-derived aromatic and heterocyclic amines, suggests that NAT1 and NAT2 acetylator genotypes are important modifiers of human cancer susceptibility. NAT2 slow-acetylator phenotypes are at higher risk for urinary bladder cancers induced by amines, for which N-acetylation is a detoxification step. In contrast, for carcinogens where activation consists of O-acetylation, NAT2 rapid-acetylator phenotypes are at higher risk for colon cancer. Several studies have also found an association between the NAT1 genotype and various cancers; however, these findings are less consistent (Hein, 2002). Although NAT1 and NAT2 have different substrate specificities, some substrates, e.g., 2-aminofluorene, are metabolized by both NAT1 and NAT2. NAT1 can even catalyze the metabolism of compounds that are primarily metabolized by NAT2 in slow acetylators (Wormhoudt et al., 1999) (Formula 10).

3.2.2.5 **Microsomal Epoxide Hydrolase (mEH; EC 3.3.2.3)**

Epoxides are often generated from olefins or PAH by P450s. Some of these epoxides (oxiranes) are among the most common electrophilic metabolites responsible for carcinogenic action. An example of a simple olefin epoxide is styrene epoxide. Also, vinyl chloride undergoes epoxidation and then rearranges rapidly to carcinogenic
2-chloroacetaldehyde (Formula 11). These reactive intermediates can be stabilized in the presence of microsomal epoxide hydrolase (mEH) or alcohol dehydrogenase (Guengerich, 1992). The hydrolytic cleavage of the epoxide, a Phase II metabolic transformation, is a general task of microsomal epoxide hydrolase and is a beneficial process for the cell. However, some actions of epoxide hydrolase may create an intermediate that undergoes repeated activation with P450. This occurs in mEH-mediated hydrolysis of 7,8-dihydro-7,8-epoxy-benz(a)pyrene. The formed dihydriodiol is a good substrate for CYP1A1, and the product obtained, 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-BP, is a particularly strong genotoxin (Wormhoudt et al., 1999).

Broad interindividual variability in the enzymatic activity of microsomal EH is considered to reflect the genetic polymorphisms, which consist of two point mutations, one resulting in a 40% decrease and the other in a 30% increase in the activity of mEH in vitro. Our knowledge about the contribution of these variant alleles to cancer susceptibility is rather limited. However, several reports suggest that low mEH activity may be a risk factor for colon cancer. Low mEH activity is also suspected to be associated with hepatocellular and ovarian cancers (Clapper, 2000).
3.2.3 The Role of Enzymes of the Intestinal Microflora in Bioactivation of Foodborne Carcinogens

Numerous findings have shown that intestinal microorganisms and lactic bacilli present in dairy products play a role in the activation and detoxification of food-derived carcinogens including tryptophan derivatives, nitrosamines, aflatoxins, aromatic and heterocyclic amines, and polycyclic aromatic hydrocarbons. What is more, the intestinal metabolism of carcinogens is influenced by the exposure of mammals to certain substances, e.g., crude oils (George et al., 2001) or biphenyls (Chung and Adris, 2003). Several types of enzymes have shown to be involved in the actions of microflora strains: beta-lyase, azo- and nitro-reductases, sulfatase, and beta-glucuronidases.

However, the results obtained are still not unequivocal. The question remains whether the intestinal microflora plays an important role in the bioactivation or detoxification of foodborne carcinogens and whether it plays any role at all in carcinogen metabolism (Kadlubar, 1994).

It was shown, for instance, in the case of carcinogenic tryptophan derivatives TRP-P-1 and TRP-P-2, that these compounds were not metabolized in the gut of animals but formed the “bound fecal residues.” Other heterocyclic amines formed upon cooking, IQ, MeIQ, MeIQx, and DiMeIQx, were also demonstrated to have a binding capacity to various bacterial strains. Such binding was found to diminish their mutagenic effect (Knasmüller, 2001). These carcinogens are probably sequestered by the carbohydrate moieties of bacterial cell walls.

On the other hand, food-derived carcinogenic amines were converted by representatives of the human microflora to active mutagens. For instance, the incubation of IQ with human fecal microflora resulted in the formation of a stable hydroxy metabolite, 7-OHIQ, a very potent direct-acting mutagen in Salmonella strain TA98 (Formula 12). However, the data from experiments with 7-OHIQ and mammalian cells, as well as rodents, did not indicate any mutagenic effect. In contrast, IQ itself delivered intrarectally caused the induction of colon tumors. The conclusion was that the presence of 7-OHIQ in the intestinal tract of humans is unlikely to result in increased colon cancer risk in individuals consuming IQ and related amines (Knasmüller, 2001).

Other processes involving the gut microflora might lead to the activation of foodborne amines. The bacterial beta-glucuronidase is able to split glucuronide conjugates of carcinogenic amines (Formula 13). The cleavage products can be
activated to DNA-reactive metabolites in the colon or other organs. The intestinal bacteria may also affect the activity of liver enzymes involved in carcinogen activation (Kassie et al., 2001). The enzyme activity in conventional human flora–associated and germ-free rats was compared (Onoue et al., 1997). The results indicated that the selected intestinal bacterial strains behave as promoters; however, in the majority of cases they are inhibitors of carcinogen-activating enzymes.

Summing up, the most important mechanism by which intestinal bacteria may influence carcinogenic risk involves activation of heterocyclic aromatic amines (HAAs) through cleavage of glucuronide conjugates of HAAs by bacterial enzymes. On the other hand, the essential detoxification mechanism of intestinal microflora appears to rely on direct binding of carcinogens to the bacterial cell wall. It is also possible that protective effects in vivo are due to the enzymatic activities of the microflora. Finally, it cannot be excluded that intestinal bacteria may induce or inhibit the action of metabolizing enzymes in the liver, e.g., isoforms of cytochrome P450 or the conjugation enzymes.

3.2.4 CONCLUDING REMARKS

Several new concepts about the role of metabolic transformations in carcinogenesis have been developed recently. The broad knowledge gathered for cytochromes P450 paved the way for studies on the mechanisms that govern the action of other enzymes. At present, xenobiotic metabolizing enzymes are known to play both beneficial and detrimental roles. They are induced by various exogenous agents, but endogenous compounds are suspected as well. The observed differences in enzyme levels can explain the variable cancer susceptibility of experimental animals to carcinogens. There is also some evidence for enzymatic polymorphism as a risk factor for lung cancer in humans. However, interindividual variations in the enzymes involved in carcinogen metabolism in humans are still under extensive study. The isoform divergence, polymorphism, and low specificity of the regulation of gene expression make it difficult to predict the metabolic pathway as well as the biological action of the obtained metabolites. However, it seems that such a divergence is aimed at the best adaptation of the organism to different circumstances where defense against the toxic action of xenobiotics is the crucial task of metabolic enzymes.

Considering all of the above, the association between dietary constituents and the metabolism of procarcinogens appears to be complex. On the one hand, some
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food components express procarcinogenic properties and may be activated by metabolizing enzymes to ultimate carcinogens. On the other hand, the carcinogenic metabolite of the food component may be detoxified by enzymatic systems. Furthermore, many nutrients are able to induce or inhibit carcinogen-metabolizing enzymes. Therefore, the elements of the diet may influence the carcinogenic action of non–food-derived procarcinogens.

3.3 METABOLIC PATHWAYS OF SELECTED FOODBORNE CARCINOGENS

In recent years, substantial new evidence on the metabolic pathways of carcinogens has been reported. Findings related to well-known dietary carcinogens are presented below.

3.3.1 POLYCYCLIC AROMATIC HYDROCARBONS (PAHS)

The best-known metabolic pathway of benzo[a]pyrene (BP) in human tissues involves CYP1A1 and EH to form 7,8-dihydro-7,8-diol and then CYP1A1 or CYP3A4 to catalyze the subsequent epoxidation. GSTM1 plays an important role in the detoxification of the arene oxides (Formula 14). In cultured human bladder cells, benzo[a]pyrene (BP) is metabolized by CYP1A1 and mEH to form BP-7,8-diol. BP-7,8-diol is then metabolized by CYP1A1 or CYP3A4 to form BP-7,8-diol-9,10-epoxide. GSTM1 plays an important role in the detoxification of the arene oxides.

**Formula 14**
systems as well as in human lung and larynx, the formation of 9-hydroxy-BP and its subsequent conversion to 4,5-epoxide was also observed. CYP1A1, CYP2C9, and CYP3A4 have been each shown to catalyze these oxidations (Kadlubar and Badawi, 1995).

Benzanthracene, BA, is predominantly metabolized to 5,6-, 8,9-, or 10,11-dihydriodiols by several P450s (Formula 3.2). Benzanthracene-3,4-dihydiol (BA-3,4-dihydrodiol) is a minor metabolite, but it is 10- to 20-fold more carcinogenic than the parent BA or any other BA-dihydriodiols. Under oxidative conditions, BA-3,4-dihydiol can undergo oxidation, which is followed by radical hydroxylation leading to BA-3,4-dihydro-2,3,4-triol. In another pathway, BA-3,4-dihydrodiol is oxidized by dihydrodiol dehydrogenase to form \( \alpha \)-catechol. Such a pathway suppresses the formation of carcinogenic dihydiol epoxides. However, the catechols may be oxidized to \( \alpha \)-quinones, which are reactive towards macromolecules either directly or through generation of reactive oxygen species (Seike et al., 2003).

\[ \text{Formula 15} \]
The methyl-substituted PAHs are carcinogenic owing to their further metabolism to proximate hydroxymethyl metabolites. This type of benzylic alcohol (7-HMBA in Formula 16) is less carcinogenic than are methyl derivatives. However, they may be activated to reactive esters and/or halides to form highly carcinogenic electrophilic metabolites capable of covalent binding to cellular nucleophiles. It has just been shown that formyl hydrocarbon metabolites (7-FMBA in Formula 16) are reduced with rat liver microsomes to hydroxymethyl derivatives. Therefore, hydroxymethyl metabolites may be an important common intermediate in the activation of methyl-substituted PAHs (Horn et al., 2003).

3.3.2 AFLATOXIN B1 (AFB₁)

AFB₁ is the most carcinogenic mycotoxin and shows considerable variability in carcinogenicity in different animal species. The characterization of a guanyl AFB₁ DNA adduct allowed researchers to postulate that the epoxide is the reactive electrophilic product involved in the reaction with macromolecules. The epoxide was synthesized, and the \textit{exo} isomer was shown to be $10^3$ times more genotoxic than the \textit{endo} form. Despite the short lifetime of the reactive epoxide, it is still
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stable enough to migrate into the cell nucleus in cells of target tissues and modify DNA therein (Guengerich, 2001) (Formula 17). The studies on metabolic pathways indicated that human CYP3A4 was considerably involved in the exo epoxidation of AFB₁, whereas CYP1A2 also produced equal amounts of the endo isomer. Both CYPs 1A2 and 3A4 and their mRNAs have been detected in human lung and liver tissues. Nonetheless, CYP1A2 has a more important role than CYP3A4 does in the bioactivation of low levels of AFB₁ (Van Vleet et al., 2002). The detoxification of epoxides runs by GSH conjugation, and this reaction probably contributes to the differences in sensitivity of various animal species and human individuals to AFB₁. Epoxide hydrolase does not participate in the hydrolysis of epoxide, as the rapid nonenzymatic reaction is observed. The subsequent transformation of the hydroxylated metabolite consists of ring opening, which gives rise to dialdehyde. The latter is not a genotoxic agent and is generated from both the exo and endo epoxides. Nevertheless, dialdehyde may contribute to the acute toxicity of AFB₁. It is also postulated that dialdehyde might be rapidly reduced back by the aldehyde reductase.

The link between AFB₁ metabolism and human cancer risk is still not clear. Higher levels of CYP3A4 observed in human liver tissue are consistent with a role of this enzyme in hepatocellular cancer. The most involved in conjugation of exo-8,9-epoxide of AFB₁ in humans is an M1 form of GSH transferase. However, the results pertaining to the role of GSTM1 polymorphism obtained from epidemiological studies failed to provide a clear-cut answer (McGlynn et al., 1995).

Formula 17
3.3.3 Heterocyclic Aromatic Amines (HAAs)

The structures of food-derived HAAs consist of a heterocyclic aromatic ring and an amine group attached to this ring. Such derivatives undergo metabolic transformations like those observed for simple aromatic amines (Formula 18). The heterocyclic nitrogen atom usually does not take part in enzymatic activation responsible for carcinogenesis.

The general pathway of aromatic amine metabolism involves conversion to N-hydroxyarylamines catalyzed by cytochrome P450 enzymes. Under acidic conditions the elements of water are lost to generate reactive nitrenium ion, which can react with DNA (Formula 19).

The reactive nitrenium ion is also formed at neutral pH in reactions with a good leaving group. The latter reaction usually involves NAT- or SULT-mediated esterification and is observed in some experimental animal systems (Kadlubar and Hammons, 1987).

The major enzymes involved in the activation of arylamines in human liver are CYP1A1 and CYP1A2 (Table 3.2). Some arylamines are oxidized to N-hydroxy derivatives by peroxidases and the microsomal flavin monooxygenase.

HAAs, derived from thermally processed meat, are activated mainly according to the pathway presented above. Taking that pathway into account, individuals whose intake of meat is high and who are of a “rapid” CYP1A2 and/or N-acetyltransferase or sulfotransferase genotype or phenotype may be at increased risk of developing colon cancer.
On the other hand, several pathways of detoxification of HAAs are also known. These include N-glucuronidation, N-sulfation, and N-acylation of the parent amines and also NADH-dependent reduction of N-hydroxy metabolites of HAAs. In the case of N-hydroxy-PhIP, the formation of N₂-glucuronide has also been demonstrated. However, HAAs have not been found to be substrates for the glutathione S-transferases. A notable exception is the activated PhIP derivative, N-acetoxy-PhIP, which is a substrate of the human GSTA1-1 isoform (Coles et al., 2001).

A specific subject of interest in the metabolic activation of carcinogens is the elucidation of the relations between human and animal metabolism of these compounds. In the case of HAAs, this problem has been studied extensively but the results are still equivocal. For instance, studies with microsomes from recombinant yeast strains expressing human and rat CYP1A1 genes demonstrated that metabolic activation of a dozen HAAs led to similar levels of mutagenicity (Kanazawa et al., 1999). Nevertheless, many works reported differences in the metabolic products generated with human and rat liver microsomes, hepatocytes, and cytochromes CYP1A2 (Formula 20) (Languøët et al., 2001; Turesky et al., 2001). There are two groups of hydroxylated metabolites: 2-hydroxyamino (MeIQ-HONH) and 8-hydroxymethyl (8-CH₂OH IQx) derivatives. Both have been found in humans and rats; however, rat liver microsomes were 10- to 15-fold less active in this reaction than were their human counterparts. What is more, the carboxylic derivative (8-COOH-IQx) was observed only in humans, whereas rat microsomes and CYP1A2, but not human ones, extensively catalyzed ring oxidation at the C-5 position of MeIQx (5-HO-MeIQx). Identical relations resulted from comparison of human and rat hepatocyte cells.

Like MeIQx, another carcinogenic amine, PhIP, gave the reactive hydroxyamino metabolite (HONH-PhIP), also with an efficiency much greater with human than with rat enzymes. Similarly to MeIQx, PhIP undergoes glucuronidation at the N² and N3 positions to stable conjugates. Glucuronidation occurs at the N²-hydroxy group or directly at the N3 amino group (Formula 21).

In conclusion, the differences in the HAA metabolic pathways between animal and human microsomes, cytochromes, and hepatocytes indicate that carcinogenicity data from the rat may underestimate the human health risk associated with these carcinogens.

### 3.3.4 N-nitrosamines

Carcinogenic N-nitrosoamines originate mainly from protein foods and are also formed from nitrate precursors, which are abundant in leafy and root vegetables. Under the acidic conditions of the stomach, the amines present in food are activated by reaction with nitrate, leading to N-nitrosamines (Bartoszek, 2002). P450-mediated hydroxylation at position α to the nitroso group is a key activation step of nitrosamines (Formula 22). The subsequent demethylation and dehydration give rise to the next reactive intermediate, diazomethane, which is a strong methylating agent. CYP2A3 and CYP2A6 are the main P450s involved in the activation metabolism of N-nitrosamines.
Metabolism of Chemical Carcinogens

$N$-nitroso piperidine (NPIP) and its homologue $N$-nitrosopyrrolidine (NPYR) may be generated from endogenous nitrosation of dietary piperidine and pyrrolidine, respectively. These secondary amines, like other amines, are easily nitrosated in the acidic environment in the stomach. NPIP is activated by P450s to hydroxypiperidine, and the unstable $\alpha$-hydroxy-NPIP is decomposed to electrophilic reactive intermediates that can react with DNA to yield adducts (Formula 23). The products of hydrolysis observed in vitro rapidly cyclize to noncarcinogenic tetrahydrofurane (THF) and tetrahydropyran (THP) derivatives (Wong et al., 2003). Hence, in the

**Formula 20**

$N$-nitrosopiperidine (NPIP) and its homologue $N$-nitrosopyrrolidine (NPYR) may be generated from endogenous nitrosation of dietary piperidine and pyrrolidine, respectively. These secondary amines, like other amines, are easily nitrosated in the acidic environment in the stomach. NPIP is activated by P450s to hydroxypiperidine, and the unstable $\alpha$-hydroxy-NPIP is decomposed to electrophilic reactive intermediates that can react with DNA to yield adducts (Formula 23). The products of hydrolysis observed in vitro rapidly cyclize to noncarcinogenic tetrahydrofurane (THF) and tetrahydropyran (THP) derivatives (Wong et al., 2003). Hence, in the
case of N-nitrosamines, P450s are involved in both activation and detoxification, and the final carcinogenic effect will be determined by the balance between these two metabolic pathways.

3.4 CONCLUDING REMARKS

Food carcinogens delivered to the human organism undergo various enzymatic transformations, and the obtained metabolites participate in numerous biochemical
interactions. On the one hand, they can be metabolized to secondary metabolites that are less or more toxic. On the other hand, they can influence the metabolic pathways of other xenobiotics, including carcinogens, and not only those derived from foods. Noncarcinogenic elements of the diet may also influence metabolic transformations of carcinogens. A variety of enzymatic systems are involved in metabolic reactions of procarcinogens. They are frequently divided into Phase I and Phase II transformations, and recently Phase III has been proposed. The transformations of each phase may lead to more reactive intermediates, resulting in carcinogenic products, or may be involved in detoxification of reactive metabolites. Therefore, the final result of delivering food carcinogens into the body depends on the interactions between enzymatic pathways. What is more, interindividual variations in enzyme levels complicate the outcome of metabolic transformations. However, the polymorphism of enzyme expression may be a marker of individual susceptibility of humans to various forms of cancer (Luch, 2005). Considering the ability of nutrients and nonnutrients to influence the levels of metabolic enzymes, nutrigenomics proposes to create a complex pattern of specific genes and proteins, the expression of which is influenced by dietary components. Such patterns might
be applied in the future as a common marker of the human cancer risk resulting from dietary habits (Müller and Kersten, 2003).

REFERENCES


Carcinogenic and Anticarcinogenic Food Components


4 Genotoxic Food Components

Agnieszka Bartoszek

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4.1 INTRODUCTION

In the developed countries, chronic diseases account for the majority of premature deaths. The still-increasing human exposure to pollutants with mutagenic and carcinogenic activity or other environmental factors with various adverse effects, including the potential to facilitate tumorigenesis, in households, workplaces, and large industrialized areas, was the first demonstrated anthropogenic hazard to human health. More recently, the local lifestyle, including tobacco habits, excessive alcohol use, still later the lack of regular exercise, and above all dietary traditions, have also become implicated in the occurrence of heart disease, stroke, adult diabetes, and diverse types of cancer. Among the main risk factors for cancer, diet is particularly important because of daily, lifetime exposure and the presence of both caloric and other nutritional components. The current consensus view is that some 30% of cancers (though estimates as high as 90% can be found in literature) may be attributable to diet, either through ingestion of compounds that initiate or promote cancer or through the lack of protective substances (e.g., Manson and Benford, 1999; Weisburger, 1999).
Carcinogenic food components can be classified in different ways. One of the classifications relies on the distinction between genotoxic and epigenetic carcinogens. Hermann Druckrey, during a 1973 conference on evaluation of genetic risks of environmental chemicals held in Sweden, suggested the following definition: “In order to describe the components of chemical interaction with genetic material, the term genotoxic is proposed as a general expression to cover toxic, lethal, and heritable effects to karyotic and extrakaryotic genetic material in germinal and somatic cells” (Ramel, 1973). Table 4.1 presents the most important genotoxic effects and methodologies to detect them used in experimental research as well as in institutional risk assessment. Theoretically, even a single contact of an organism with a genotoxin can result in DNA damage, which if “translated” upon replication into mutation may lead to an “initiated” cell.

Epigenetic factors operate mainly as promoters of cancer and usually require high and sustained exposures. For example, dietary fat, constituting about 40% of ingested calories, can act as an enhancer of cancer induction (Weisburger and Williams, 2000). Another classification takes into account the fact that dietary tumor-inducing factors can be delivered to organism exogenously with food or can be produced endogenously, e.g., reactive oxygen species (ROS) with genotoxic potential are generated during normal metabolism of nutrients. However, this distinction is not always clear-cut since numerous xenobiotics including food carcinogens are capable of inducing oxidative stress with the result of endogenous generation of ROS.

The source of mutagenic and carcinogenic foodborne substances is the basis of the classification that is probably most important from consumers’ point of view. The first group includes natural substances contaminating food products, such as mycotoxins. The second group, which is largest and presumably poses the greatest risk, comprises mutagens and carcinogens formed during food processing, especially thermal processing. Subsequent parts of this chapter will discuss these two groups of compounds in some detail. The third group, proposed here as a separate category, consists of carcinogenic and mutagenic substances that are found in food and drinking water as a result of environmental pollution (e.g., heavy metals) or agricultural practices (e.g., pesticides). Environmental carcinogens (discussed elsewhere in this volume) were long thought to represent a negligible risk factor as regards food (Ames et al., 1990); however, new data suggest that they deserve more careful consideration. The fourth group is food additives. The latter food components, in contrast to the previous three groups, are introduced into food products purposefully as stabilizers, colorants, flavoring agents, nutritional supplements, etc. It seems that their role as cancer risk factors might require rethinking.

4.2 GENOTOXICITY IN RISK ASSESSMENT OF DIET-RELATED CARCINOGENIC FACTORS

Genotoxicity assessment has been proposed by several official agencies in the United States and Europe alike (Table 4.2) to evaluate environmental risk, also as a support of carcinogenicity (Dearfield et al., 2002; Madle et al., 2002). There are convincing reasons for that. Analysis of the properties of 10,000 chemicals suggests that those that have genotoxic potential are more likely to be human carcinogens. In experimental
## TABLE 4.1
Major Assays for Determination of Genotoxic Effects of Compounds Found in Food

<table>
<thead>
<tr>
<th>Model</th>
<th>Genotoxic Effect</th>
<th>Assay Rationale and Related Mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td>Mutagenicity:</td>
<td>Mutated histidine-dependent strain reverses to histidine-independent phenotype upon specific mutation (an array of strains with different mutations is available)</td>
</tr>
<tr>
<td></td>
<td><em>Ames Salmonella typhimurium</em> test</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em> LacZ test</td>
<td>Upon mutation bacteria regain functional beta-galactosidase, which releases blue galactose derivative coloring colonies of revertants</td>
</tr>
<tr>
<td></td>
<td>Induction of SOS repair</td>
<td>Chromotest revealing bacteria with the induced SOS error-prone repair in response to DNA damage</td>
</tr>
<tr>
<td><strong>Eukaryotic cells and organisms</strong></td>
<td>Mutagenicity in cultured cell lines</td>
<td>Mutations at the <em>hprt</em> locus; transgenic Chinese hamster fibroblast V79 cells expressing human activating or detoxifying enzymes additionally discern metabolic routes leading to formation of mutagenic metabolites</td>
</tr>
<tr>
<td>(H, including humans)</td>
<td><strong>Unscheduled DNA synthesis (UDS)</strong></td>
<td>The induction of DNA repair synthesis in response to xenobiotic exposure in the absence of scheduled replicative DNA synthesis</td>
</tr>
<tr>
<td></td>
<td>Total DNA adducts (<strong>32P</strong>-postlabeling)**</td>
<td>TLC or HPLC resolution of <strong>32P</strong>-labeled DNA adducts with autoradiographic or online radioactivity detection, respectively; applicable mainly to bulky aromatic DNA-binding compounds</td>
</tr>
<tr>
<td></td>
<td>DNA adducts (specific types including oxidative DNA damage)**</td>
<td>Chromatographic (HPLC or GC) analysis of modified DNA components with the use either of synthetic adducts as standards or radiolabeled compounds; immunological methods employing antibodies raised against particular modified nucleobases including commercially available ELISA tests</td>
</tr>
<tr>
<td></td>
<td>DNA breaks and alkali labile DNA damage (comet assay)**</td>
<td>Genotoxic insult determined at the level of a single cell after DNA unwinding under alkaline conditions and electrophoresis; more fragmented nuclear DNA travels farther in agarose gel forming a “comet tail” whose length reflects the extent of DNA damage</td>
</tr>
<tr>
<td></td>
<td>Sister chromatid exchanges (SCE)**</td>
<td>Sister chromatids differentially stained to visualize exchanges of reciprocal fragments in mitotic cells blocked at metaphase</td>
</tr>
<tr>
<td></td>
<td>Chromosomal aberrations (FISH)**</td>
<td>Oligonucleotide probes attached to different Fluorochromes for individual chromosomes In Situ Hybridized with metaphase chromosomes and visualization of translocations under fluorescence microscope</td>
</tr>
<tr>
<td></td>
<td>Aneuploidy**</td>
<td>Abnormalities in chromosome number or appearance in metaphase cells</td>
</tr>
<tr>
<td></td>
<td>Micronuclei**</td>
<td>Formation of small nuclei-like DNA-containing bodies in response to xenobiotic exposure</td>
</tr>
</tbody>
</table>
models, DNA damage, and in particular DNA adducts formed upon chemical reaction between activated form of the genotoxin and DNA, is related to tumor formation (Rosenkranz, 2003; Baird and Mahadevan, 2004). Not surprisingly then, in practice, risk assessment of genotoxic substances is closely related to the evaluation of carcinogenicity. Many food components have genotoxic potential and more are produced endogeneously during digestion; hence, the above reasoning also applies to food.

In the case of molecular epidemiology for cancer prediction in humans, the biomarkers of DNA damage in response to exposure to carcinogenic factors prevail as well. Although these markers include blood and urinary levels of a chemical or its metabolites, much greater emphasis is placed on urinary metabolite-bound nucleobases, DNA adducts, gene mutations, or other DNA alterations. These are even suggested by some researchers to represent the determinants of cancer occurrence and distribution in human population. It has been proposed that the measurement of DNA adduct levels in target tissue has the potential to be not only an exposure marker but an individual cancer risk bioindicator, hence a predictive measure of disease outcome in response to environmental exposure (Ottender and Lutz, 1999; Preston, 2003). Therefore, such measurements in humans exposed to food carcinogens at doses corresponding to those normally consumed would be of great value for the risk assessment of diet-related cancer. The insufficient sensitivity of methods hampered such studies until the introduction of ultrasensitive accelerator mass spectrometry (AMS). The latter technique enabled the detection of DNA adduct formation in humans administered low, dietary-equivalent doses of radioactively labeled typical food carcinogens. Patients soon to have colon surgery were included in these studies. The level of aflatoxin B₁ DNA adducts in human colon (nontarget tissue) approached the AMS level of detection and thus could not be quantified. However, albumin adduct levels showed a linear dose-response relationship down to 0.16 ng/kg b.w. The formation of DNA adducts by MeIQx (see further sections) seemed to follow a linear dose-response relationship in humans (the range from 3.5 down to 0.35 µg/kg was analyzed), suggesting the absence of a threshold dose (Manson and Benford, 1999). Also, the formation of 168 adducts per 10^{12} nucleotides by PhIP in

<table>
<thead>
<tr>
<th>Model</th>
<th>Genotoxic Effect</th>
<th>Assay Rationale and Related Mechanisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transgenic animals</td>
<td>Mutagenicity</td>
<td>Mice (or rats) with introduced bacterial reporter lacI (BlueMouse®) or lacZ (MutaMouse®) genes, mutations in which are determined in vitro following in vivo exposure to xenobiotics</td>
</tr>
</tbody>
</table>

\[a\] Thin-layer chromatography.  
\[b\] High-performance liquid chromatography.  
\[c\] Gas chromatography.  
\[d\] Enzyme-linked emmunosorbent assay.

<table>
<thead>
<tr>
<th>TABLE 4.1 (Continued) Major Assays for Determination of Genotoxic Effects of Compounds Found in Food</th>
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<tbody>
<tr>
<td><strong>Model</strong></td>
</tr>
<tr>
<td>Transgenic animals</td>
</tr>
</tbody>
</table>

\[a\] Thin-layer chromatography.  
\[b\] High-performance liquid chromatography.  
\[c\] Gas chromatography.  
\[d\] Enzyme-linked emmunosorbent assay.
human colon DNA was demonstrated after the administration of 20 µg of this compound in 14C-labelled form 4 to 6 h prior to surgery (Cupid et al., 1998). At the moment, AMS technique is far from routine, but perhaps in the future such measurements will become one of the daily tools of molecular epidemiology and cancer risk assessment.

### 4.3 GENOTOXIC FOOD COMPONENTS

The following sections will concentrate only on genotoxic food carcinogens. This subject was covered in two recent reviews published in the *Chemical and Functional*
Properties of Food Components series (Bartoszek, 2001; 2002). Therefore, the current review is designed to include relevant information omitted in previous articles and to update developments in the field of diet-related genotoxic factors. Most importantly, the reversal of genotoxic effects of food carcinogens has been used recently in experimental studies on potential dietary chemopreventive agents. The most promising findings are being verified in human intervention studies and even in a few case-control clinical studies (Salama and Au, 2003).

4.3.1 Mycotoxins

Mycotoxins are secondary fungal metabolites produced by molds in the genera Aspergillus, Penicillium, and Fusarium. Many of these compounds are highly toxic and display mutagenic, teratogenic, and estrogenic activity as well as having been found to be highly carcinogenic in many animal species including fish, rodents, and nonhuman primates (Bushby and Wogan, 1984). Because of considerable resistance to thermal processing, mycotoxins are widespread contaminants of foods and feeds.

Looking back on human history, it seems that mycotoxins troubled agricultural production since its earliest days. Poisoning by ergot is mentioned in the Bible. It has been hypothesized that Fusarium toxins and zearalenone were the reason for the eradication of the Etruscans and pestilence in Athens in the fifth century B.C. Later, Fusarium toxins were implicated in human and livestock diseases from the medieval period in Europe to colonial times in America. There have been also speculations that mysterious sudden deaths of archeologists entering Egyptian tombs were caused by inhalation of mycotoxins, ochratoxin A in particular (Formula 1).

Aflatoxins are the most important mycotoxins found in contaminated foods such as corn, grains, peanuts, soy sauce, and dried fruits and feeds such as cottonseed meal, particularly in subtropical regions where the climate favors the growth of fungi. These compounds were structurally identified in the 1960s, and since then their toxicology has been the subject of extensive research. Aflatoxin B$_1$ (AFB$_1$) produced by Aspergillus flavus is one of the most powerful foodborne carcinogens known and one of the first compounds classified by the International Agency for Research on Cancer (IARC) as a known human carcinogen (IARC, 1993). In the 1990s, case-control studies conducted in Shanghai confirmed a significant link between dietary aflatoxin exposure and hepatocarcinoma incidence and showed dramatic 60-fold increase in the risk of liver cancer when exposure was coupled with chronic hepatitis B infection, suggesting striking chemical–viral interaction (reviewed by Wild and Turner, 2002). In Europe, the currently most threatening

![Aflatoxin B1](attachment://formula1_1.png)  
![Aflatoxin G1](attachment://formula1_2.png)
route of AFB1 contamination is from its 10-hydroxy metabolite AFM1 found in bovine milk. According to European Union (EU) regulations from 1998, the maximum allowable level of aflatoxins B1, B2, G1, G2, and M1 in food is set at 5 to 10 ppb (Creppy, 2002).

AFB1 is activated by cytochrome P450 to an 8,9-epoxide, which binds covalently to DNA. The adducts formed convert to a stable ring-opened formamidopyrimidine derivative. Repair of these lesions leads to mutations with a predominance of G-to-C transversions. Such specific mutation was observed in codon 249 of the p53 tumor suppressor gene in more than 50% of AFB1-related tumors (Aguilar et al., 1993). Hydroxylated metabolites and other naturally occurring aflatoxins are poorer substrates for epoxidation and consequently are less mutagenic and carcinogenic. Induction of mitotic recombination is another genotoxic effect exerted by AFB1 (Stettler and Sengstag, 2001). Also, stimulation of the formation of ROS and secondary free radicals by AFB1 and AFM1 was reported (Towner et al., 2003).

While vaccination against hepatitis B virus and the reduction of aflatoxin food contamination is one approach to reducing liver cancers, total elimination of these factors may not be possible. Therefore, chemopreventive food components have been proposed as an alternative strategy. For example, it was demonstrated that dietary cabbage increased the activity of detoxifying enzymes in rat liver, which paralleled an over 80% decrease in the binding of AFB1 to hepatic DNA (Whitty and Bjeldanes, 1987). In contrast, black tea theafulvins, which inhibited some detoxifying enzymes, potentiated the mutagenicity of AFB1 in the Ames test, probably due to the decreased deactivation of the ultimate mutagenic metabolite (Catterall et al., 2003). The most promising clinical studies carried out to date involved chlorophyllin, a water-soluble form of chlorophyll. This compound is a potent antimutagen capable of forming tight molecular complexes with AFB1, thereby impeding its absorption. The studies were carried out in a rural region of China (Daxin Township, Qidong), where an extremely high incidence of hepatocellular carcinoma associated with dietary exposure to aflatoxins had been noted. Administration of chlorophyllin to study participants led to a 50% reduction in the median level of urinary excretion of aflatoxin-N7-guanine, a biomarker of increased risk of liver cancer development (Egner et al., 2003).

Ochratoxin A, zearalenone, and sterigmatocystin (Formula 2) are other abundant foodborne mycotoxins and hence may be important human health risk factors. Zearalenone was shown to cause reproductive problems in animals. Sterigmatocystin forms DNA adducts and induces liver cancers in vivo (Jonker et al., 1999). The carcinogenicity of the latter two compounds in humans has not been established to date. Many more studies have been devoted to ochratoxin A (OTA).
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In contrast to aflatoxins, OTA is a major contaminant of improperly stored food in northern climates of Europe and North America. Its major sources are cereals and cereal products, resulting in chronic human exposure. OTA has been also found in several other food products such as wine, coffee, pork, poultry, and bovine milk.

It seems that both genotoxic and nongenotoxic mechanisms may contribute to carcinogenic properties of this compound. OTA was demonstrated to induce SCE and SOS (sister chromatid exchange) DNA repair in vitro as well as DNA strand breaks in the liver and kidney of rats. However, mutagenicity testing in the Ames test mostly showed negative results (Zepnik et al., 2001). OTA also facilitates OH• formation in vivo and copper-promoted oxidative damage of plasmid DNA (Manderville et al., 2003), as well as guanine-specific DNA adduct formation (Pfohl-Leszковicz et al., 1991). Though some studies questioned the previously proposed role of oxidative metabolism in the genotoxicity of OTA (Zepnik et al., 2001), recent reports provided definitive evidence that OTA phenoxyl radical is an intermediate in the reaction with DNA (Obrecht-Pflumio and Dirheimer, 2001; Dai et al., 2003).

OTA has been detected in human plasma samples in several European countries (average ranges 0.02 to 2.3 ng/ml). It reached levels as high as 100 ng/ml in the Balkan peninsula (Jimenez et al., 1998), a region where this toxin is implicated in a fatal kidney disease called Balkan endemic nephropathy in which patients suffer from high incidences of kidney, pelvic, ureter, and urinary bladder carcinomas (Castegnaro et al., 1987). Daily human intake of OTA may range from 0.7 to 4.7 mg/kg b.w.; a provisional tolerable weekly intake of 100 ng/kg b.w. has been allocated by the World Health Organization (WHO) (2001).

Certain fungi distributed worldwide produce the mycotoxins patulin and citrinin (Formula 3). The former is a frequent contaminant of apples and apple products. Many other fruits, including grapes, pears, peaches, and berries, have also been shown to contain patulin. Citrinin is found mostly in cereals: maize, wheat, barley, and rice (CAST, 2003). In animal experimental models, both the genotoxicity and the carcinogenicity of these two mycotoxins were demonstrated. Recent studies carried out in human embryonic kidney cells and lymphocytes revealed that patulin and citrinin are genotoxic and incur oxidative DNA damage in human-derived cells; patulin is the much more potent genotoxin (Liu et al., 2003). Though human data are lacking, current knowledge suggests that both these compounds may be yet another public health concern.

Fumonisins are ubiquitous contaminants of corn and other grain products produced by several Fusarium species. There are 14 fumonisins known, of which fumonisin B₁ (FB₁) is the most frequent (Formula 4). The level of these compounds
in corn ranges from undetectable to 150 ppm (Shephard et al., 1996). Because of its widespread presence, the U.S. Food and Drug Administration (FDA) evaluated FB₁ in chronic bioassays in rats and mice, which indicated that it induced liver tumors in male rats and kidney tumors in female mice. A limited number of studies examining the genotoxic potential of fumonisins have been reported. Their results revealed that these mycotoxins (including FB₁) are nongenotoxic and non–DNA reactive compounds. An early effect of FB₁ exposure in target organs is apoptosis. FB₁ may be the first example of an apparently nongenotoxic agent producing tumors through a mode of action involving apoptic necrosis, atrophy, and consequent abnormal regeneration (Dragan et al., 2001).

To summarize, mycotoxins pose a substantial foodborne carcinogenic risk that is very difficult to eradicate, is resistant to processing, and displays different mechanisms of action. The most effective strategy to combat the negative health impact of these toxins should involve monitoring of food products and chemopreventive dietary measures, especially in high-risk regions. Much to inventors’ surprise, corn genetically modified for insect resistance, so called Bt corn, owing to restricted feeding of pests on kernels turned out to be less liable to infection by fungi; consequently, the kernels were less contaminated with aflatoxins and fumonisins. These results indicate that under some conditions, genetic engineering of corn for insect resistance may enhance its safety for animal and human consumption (Munkvold and Hellmich, 1999).

4.3.2 \textit{N-Nitroso Compounds}

\textit{N}-nitroso compounds (NOC) include \textit{N}-nitrosamines and \textit{N}-nitrosamides. They are produced in the reaction of nitrite or nitrogen oxides with secondary amines and \textit{N}-alkylamides. Environmental human exposure to NOC originates from the use of tobacco products, pollution generated by industry (e.g., the rubber industry), and diet (Formula 5). The structures of four NOC most commonly found in foods are given below.

Alkylation of DNA following activation of NOC by cytochrome P450 is generally assumed to represent the primary event in the carcinogenicity of these compounds. However, several other genotoxic activities were demonstrated for NOC including mutagenicity, induction of strand breaks, unscheduled DNA synthesis (UDS), and micronuclei formation (Wolf et al., 2003).
Dietary human exposure may result either from ingestion of preformed NOC or from their endogenous formation from precursors found in food: nitrates (III) and (V) and amines. Table 4.3 compares the contents of major NOC in some food products with those in cigarette smoke, which is regarded as an abundant source of nitrosamines.

According to Cassens (1995), foods contaminated with preformed NOC can be divided into six groups:

1. Nitrite-cured meats
2. Smoked foods (fish or meat), which upon processing are exposed to nitrosating nitrogen oxides present in the smoke

<table>
<thead>
<tr>
<th>Food Product</th>
<th>DMN</th>
<th>DEN</th>
<th>NPYR</th>
<th>NPIP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meat:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacon, uncooked</td>
<td>1 to 9.5</td>
<td>0 to 2</td>
<td>&lt;17</td>
<td>0</td>
</tr>
<tr>
<td>Cured meats</td>
<td>1 to 80</td>
<td>&lt;40</td>
<td>10 to 105</td>
<td>&lt;60</td>
</tr>
<tr>
<td><strong>Fish:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh or frozen</td>
<td>3 to 18</td>
<td>&lt;147</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Smoked, baked, or processed</td>
<td>6 to 177</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Vegetables:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetables (range for 16 tested)</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>0 to 20</td>
<td>0 to 0.4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Beverages:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>0.8 to 3.3</td>
<td>0.1 to 1.83</td>
<td>0.09 to 0.2</td>
<td>0.03</td>
</tr>
<tr>
<td>Alcoholic beverages</td>
<td>&lt;10</td>
<td>&lt;0.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><strong>Cigarette mainstream smoke (ng/cigarette)</strong></td>
<td>0.1 to 180</td>
<td>0 to 25</td>
<td>30 to 60</td>
<td>0 to 9</td>
</tr>
</tbody>
</table>

3. Products exposed to nitrogen oxides as a result of drying by combustion gases, e.g., the malt used in beer and whiskey production

4. Pickled and salt preserves, especially pickled vegetables, in which microbial reduction of naturally occurring nitrate (V) to nitrate (III) takes place

5. Foods stored under humid conditions favoring growth of fungi involved in generation of nitrosoamines

6. Products contaminated with NOC as a result of migration and formation of these compounds from food contact material (e.g., rubber baby bottle nipples)

The first mentioned group, nitrate (III)-cured meats, contain by definition a relatively high concentration of a nitrosating agent (about 150 mg NaNO$_3$ per kg meat); they also contain considerable amounts of NOC (Table 4.3). It has been shown that in mice fed a semipurified diet enriched with 18% of pork hot dogs, a mean daily fecal NOC output increased 3.7 to 5.0 times compared to control animals (Mirvish et al., 2003).

Endogenous synthesis of NOC from precursors, natural secondary amines and residual nitrate (III), also provides an important portion of these carcinogens to the human organism. Surprisingly, the major source of nitrate (III) involved in endogenous NOC formation is the enterosalivary circulation of dietary nitrate (V), mostly (over 85%) of plant origin (Iijima et al., 2003). Nitrate (V) is absorbed from the small intestine, and 25% of this is taken up by the salivary gland and resecreted to the mouth. Here it is reduced to nitrate (III) by bacteria inhabiting the tongue. The salivary nitrate (III) concentration may reach up to 200 $\mu$M after ingesting a typical salad portion (Mowat et al., 1999). The acidification of ingested nitrate (III) by acidic gastric juice may convert it into nitrosating species such as N$_2$O$_3$, NO$^+$, or NOSCN, which form N-nitrosamines in reaction with dietary amines. Ascorbic acid effectively competes for these nitrosating agents, thereby diminishing NOC formation. However, this protection has its advantages and disadvantages since one of the products of the reaction with ascorbic acid is nitric oxide, which diffuses to surrounding epithelium exerting substantial nitrosative stress there (Iijima et al., 2003).

Since 1956, when dimethylnitrosamine (NDMA) was recognized as a rat liver carcinogen (Magee and Barnes, 1956), NOC have belonged to the most investigated groups of carcinogens. The majority induce tumors in all species examined so far, in a highly organ-specific manner (Hecht, 1997). There is also a growing body of evidence that diet-derived NOC are involved in the etiology of human cancer of the stomach, esophagus, nasal cavity, and brain and leukemia. In the two latter cases, early exposure is particularly detrimental because of the young organism’s poor capacity of detoxification and rapid cell divisions converting DNA lesions into mutations. Nine case-control studies have linked the consumption of cured meat by pregnant women with brain cancer in their children; also, hot dog consumption by children (12 per month) was linked with childhood leukemia (Mirvish et al., 2002).

As is clear from above-described modes of NOC exposure, their elimination as human risk factors seems not only impossible but would even be undesirable, taking into account specific protection by nitrate (III) against foodborne pathogens, including Clostridium botulinum. Therefore, a number of measures regulating the use of
nitrate (III) have been adopted, and several studies on chemoprevention of NOC carcinogenesis have been undertaken in order to diminish risk without losing benefits (Cassens, 1995).

4.3.3 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) were identified as carcinogenic constituents of coal tar in the early twentieth century (Phillips, 1983). They are produced by the incomplete combustion of organic matter and consequently are widely distributed in the human environment. The mechanism of formation involves degradation of complex molecules to yield free radicals, which recombine into PAHs. Alternatively, aromatization of ring structures such as phytosterols to, for instance, chrysene may occur (Smith et al., 2001). IARC has classified many of these compounds, including benzo[a]pyrene (B[a]P), which is viewed as a prototype of this group of compounds, as Group 2A or 2B carcinogens (IARC, 1983) (Formula 6).

Human exposure to PAHs can be attributable to occupational, environmental, and dietary sources. The general population is exposed to these carcinogens through polluted air, drinking water, and predominantly, food. Around 70 different PAHs have been identified in foodstuffs; the most abundant are B[a]P and B[a]A (benzo[a]anthracene). Table 4.4 presents the contents of two latter compounds in some popular food products.

PAHs enter food as a result of environmental pollution, e.g., fish living in polluted waters, or due to heat processing; hence, they are found in broiled or barbecued meat and fish in considerable amounts, reaching levels of B[a]P as high as 50 μg/kg (Larsson et al., 1983). The major precursors of foodborne PAHs are phytosterols, sugars, amino acids, and lipids. Another nonnegligible risk related to dietary PAHs (along with heterocyclic aromatic amines [HAA]) comes from cooking oil fumes, formed especially during so-called stir-frying. It has been shown that the concentration of PAHs in such fumes is high enough to form detectable levels of DNA adducts in human lung cancer cells. Moreover, this type of exposure seems associated with lung cancer in nonsmokers and is the most common cause of cancer among women in Taiwan (Yang et al., 2000).

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PAHs are procarcinogens that undergo metabolic activation prior to exerting mutagenicity and carcinogenicity. Diol epoxides are metabolites capable of covalent binding to DNA, which is the most widely accepted mechanism of induction of carcinogenic processes by PAHs. Recent epidemiological studies have suggested that apart from DNA adducts, exposure to PAHs can cause oxidation of nucleobases. These genotoxic effects are brought about by other metabolites, namely o-quinones,
which induce nonenzymatically oxidative DNA damage via redox cycling of the quinone moiety (Seike et al., 2003).

B[\textit{a}]P has been also used as a model food and environmental carcinogen in numerous studies on chemopreventive properties of nonnutritive components of edible plants. This research demonstrates that anticarcinogenic phytochemicals can inhibit B[\textit{a}]P-induced carcinogenesis at various stages. For example, isothiocyanates inhibited lung tumor multiplicity (Hecht et al., 2000), and tea polyphenols decreased DNA damage (Yen et al., 2004), while coumarins inhibited human cytochrome 450, thereby blocking DNA adduct formation (Kleiner et al., 2003). Even this limited selection of studies suggests that cancer risk associated with exposure to PAHs might be modulated by proper composition of diet.

### 4.3.4 HETEROCYCLIC AROMATIC AMINES

Heterocyclic aromatic amines (HAA) are mutagenic compounds produced upon thermal processing of protein-rich foods (Table 4.4). Of more than 20 compounds that have been identified in this family, the most abundant are MeIQx and PhIP, whose daily consumption may amount to several µg per person (Wakabayashi et al., 1992; Layton et al., 1995).

HAA are formed from natural components of muscle foods. Factors reported to affect their formation include pH, precursor concentration, and processing time and

<table>
<thead>
<tr>
<th>Food Product</th>
<th>B[\textit{a}]P</th>
<th>B[\textit{a}]A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh vegetables</td>
<td>2.8 to 24.5</td>
<td>0.3 to 43.6</td>
</tr>
<tr>
<td>Vegetable oils</td>
<td>0.4 to 1.4</td>
<td>0.8 to 1.1</td>
</tr>
<tr>
<td>Coconut oils</td>
<td>43.7</td>
<td>98.0</td>
</tr>
<tr>
<td>Tea</td>
<td>3.9</td>
<td>2.9 to 4.6</td>
</tr>
<tr>
<td>Oysters and mussels</td>
<td>1.5 to 9.0</td>
<td>—</td>
</tr>
<tr>
<td>Fish, smoked</td>
<td>0.83</td>
<td>1.9</td>
</tr>
<tr>
<td>Mackerel, broiled</td>
<td>0.9</td>
<td>2.9</td>
</tr>
<tr>
<td>Ham, smoked</td>
<td>3.2</td>
<td>2.8</td>
</tr>
<tr>
<td>Sausage, cooked</td>
<td>12.5 to 18.8</td>
<td>17.5 to 26.2</td>
</tr>
<tr>
<td>Steak, charcoal-broiled</td>
<td>8.0</td>
<td>4.5</td>
</tr>
<tr>
<td>Beef, barbecued</td>
<td>3.3</td>
<td>13.2</td>
</tr>
<tr>
<td>Cigarette mainstream smoke</td>
<td>20 to 25</td>
<td>20 to 35</td>
</tr>
</tbody>
</table>

Carcinogenic and Anticarcinogenic Food Components

In general, longer cooking time increases the quantity, while temperature has decisive impact on the kind of HAA produced (Robbana-Barnat et al., 1996). At temperatures exceeding 300°C, as on surface layers of grilled meat, the products of amino acid and protein pyrolysis dominate (Formula 7).

Normal cooking temperatures (150 to 200°C), like those in an oven during baking or in the oil during frying meat, facilitate the reactions of creatine and creatinine with amino acids and sugars, generating derivatives of quinoline, quinoxaline, and pyridine (Formula 8).

Dietary HAA (Table 4.5) are highly mutagenic, as has been demonstrated in bacteria, cultured cells, and animals. They form DNA adducts in vitro, in rodents administered these compounds with diet, and in suckling pups exposed to them via mother’s milk (Schut and Snyderwine, 1999). HAA exert also other genotoxic effects such as DNA strand breaks and micronuclei formation and are able to transform mouse fibroblasts in vitro. Interestingly, these activities do not appear to be directly related to mutagenic potency (Pfau et al., 1999). HAA are carcinogenic in rodent bioassays, inducing tumors in a variety of tissues; IQ was shown to induce carcinomas also in nonhuman primates (Adamson et al., 1994).

Despite over three decades of research, connecting HAA to human cancers is elusive due to the low dose in the human diet and difficulty in determining accurate intakes over a lifetime. It is known that dietary-relevant doses of PhIP or MeIQx are sufficient to induce DNA adducts in the human colon (Manson and Benford, 1999). Nonetheless, a comprehensive case-control study, designed to estimate HAA risk on the background of common polymorphisms in genes implicated in metabo-
lism of these compounds, suggested that they do not play an important role in the etiology of colorectal cancer in humans (Sachse et al., 2002). To complicate further the interpretation of epidemiological data, a number of investigations with the use of PhIP and MeIQx as model food carcinogens showed that virtually all studied dietary chemopreventive components prevent at least some of the genotoxic effects of HAA. The latter was also observed in nutritionally relevant situations. For instance, addition of onion to meat decreased HAA formation in fried patties (Kato et al., 1998), white cabbage juice lowered by over 80% the level of mutations induced by PhIP or MeIQx in the Ames test (Borowska et al., 2004), and lactic bacteria prevented DNA damage by a HAA mixture in rats (Zsivkovits et al., 2003). Thus, it might be conceivable that although HAA are highly genotoxic substances, their undesirable effects are relatively easy to eradicate.

### 4.3.5 Acrylamide

Until 2000, acrylamide (AA) was regarded solely as a high-production-volume chemical whose polymers are widely used in a variety of industries. Based on animal studies, it was classified by IARC as probable human carcinogen (IARC, 1994). Not surprisingly then, in humans occupationally exposed to AA, its hemoglobin adducts were observed. However, an unexpectedly high background level of these adducts was also found in unexposed persons (Bergmark, 1997). This observation prompted further research, which revealed a previously unrecognized source of acrylamide — heat-processed starchy foods (Tareke et al., 2000) such as potato chips and bread; coffee was later found to contain this substance as well (Table 4.6). It was documented that AA is formed during the Maillard reaction between asparagine and
### TABLE 4.5
Chemical Names, Abbreviations, and Sources of Foodborne HAA

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Abbreviation</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Amino-1,4-dimethyl-5(H)-pyrido[4,3-(b)]indole</td>
<td>Trp-P-1</td>
<td>Tryptophan pyrosylate, meat drippings, broiled fish</td>
</tr>
<tr>
<td>3-Amino-1-methyl-5(H)-pyrido[4,3-(b)]indole</td>
<td>Trp-P-2</td>
<td>Tryptophan pyrosylate, broiled fish</td>
</tr>
<tr>
<td>2-Amino-6-methyl-dipyrido[1,2-(a):3,2-(d)]indole</td>
<td>Glu-P-1</td>
<td>Glutamic acid pyrosylate</td>
</tr>
<tr>
<td>2-Amino-6-methyl-dipyrido[1,2-(a):3,2-(d)]indole</td>
<td>Glu-P-2</td>
<td>Glutamic acid pyrosylate</td>
</tr>
<tr>
<td>2-Amino-5-phenylpyridine</td>
<td>Phe-P-1</td>
<td>Phenylalanine pyrosylate</td>
</tr>
<tr>
<td>4-Amino-6-methyl-1(H)-2,5,10(b)-tetraazafluoranthene</td>
<td>Orn-P-1</td>
<td>Ornithine pyrosylate</td>
</tr>
<tr>
<td>2-Amino-9(H)-pyrido[2,3-(b)]indole</td>
<td>AceC</td>
<td>Soybean globulin pyrosylate, grilled meat, fried fish</td>
</tr>
<tr>
<td>2-Amino-3-methyl-9(H)-pyrido[2,3-(b)]indole</td>
<td>MeAceC</td>
<td>Soybean globulin pyrosylate, meat drippings</td>
</tr>
<tr>
<td>1-Methyl-9(H)-pyrido[2,3-(b)]indole</td>
<td>Harman</td>
<td>Protein pyrosylate, fried bacon</td>
</tr>
<tr>
<td>2-Amino-3-methyl-imidazo[4,5-(f)]quinoxaline</td>
<td>IQ</td>
<td>Broiled sun-dried fish, meat drippings, fried fish, broiled meat</td>
</tr>
<tr>
<td>2-Amino-3,4-dimethyl-imidazo[4,5-(f)]quinoxaline</td>
<td>MeIQ</td>
<td>Broiled sun-dried fish, meat drippings, fried fish, charbroiled hamburger</td>
</tr>
<tr>
<td>2-Amino-3-methyl-imidazo[4,5-(f)]quinoxaline</td>
<td>IQx</td>
<td>Fried meat, meat drippings</td>
</tr>
<tr>
<td>2-Amino-3,8-dimethyl-imidazo[4,5-(f)]quinoxaline</td>
<td>MeIQx</td>
<td>Fried meat, dry-heated meat, cooking fumes, commercial pet foods</td>
</tr>
<tr>
<td>2-Amino-3,4,8-trimethyl-imidazo[4,5-(f)]quinoxaline</td>
<td>4,8-DiMeIQx</td>
<td>Fried meat, dry-heated meat, meat drippings, charbroiled hamburger</td>
</tr>
<tr>
<td>2-Amino-3,7,8-trimethyl-imidazo[4,5-(f)]quinoxaline</td>
<td>7,8-DiMeIQx</td>
<td>Heated mixture of creatinine, glycine, and glucose</td>
</tr>
<tr>
<td>2-Amino-1-methyl-6-phenyl-imidazo[4,5-(b)]pyridine</td>
<td>PhIP</td>
<td>Fried meat (especially chicken), dry-heated meat, charbroiled hamburger</td>
</tr>
<tr>
<td>2-Amino-1,6-dimethyl-imidazo[4,5-(b)]pyridine</td>
<td>DMIP</td>
<td>Dry-heated chicken, pork, and fish; meat drippings</td>
</tr>
</tbody>
</table>

*Note:* The three major dietary HAA are given in bold.

reducing sugars (Mottram et al., 2002; Stadler et al., 2002); hence, it is generated parallel, from the same precursors, with flavors and browning.

Studies on different potato cultivars showed that glucose and fructose were determinants of AA formation, while agricultural practice (conventional/organic) had no influence (Amrein et al., 2003). Although heat processing is prerequisite to AA generation, model studies on homogenized potatoes showed that higher temperatures (200°C) combined with prolonged heating reduced its levels due to degradation/elimination processes. The effects of addition of antioxidants were small or nonexistent (Rydberg et al., 2003).

It is accepted that the carcinogenicity of AA is associated with its genotoxic potential. The primary event involves oxidative metabolism producing a mutagenic DNA-reactive metabolite, glycidamide (Formula 9). Glycidamide was demonstrated to form several different DNA adducts both in vitro and in vivo (Gamboa da Costa et al., 2003).

The discovery that carbohydrate-rich foodstuffs, nutritionally important and thus consequently consumed in large quantities, contain high levels (up to mg per kg) of a probable human carcinogen was alarming, and the assessment of the impact of AA on cancer risk became an obvious task. Recent reports have concluded that the carcinogenic risk associated with dietary exposure to AA might not be negligible (Konings et al., 2003), and six out of 10,000 individuals may develop cancer due to such exposure (Dybing and Sanner, 2003).

---

**TABLE 4.6**

Acrylamide Levels in Some Food Products and Estimated Intakes in Scandinavian Countries

<table>
<thead>
<tr>
<th>Food Product</th>
<th>Variability of Acrylamide Level (µg/kg)</th>
<th>Daily Intake (µg/day) Sweden</th>
<th>Daily Intake (µg/day) Norway</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread</td>
<td>0 to 360</td>
<td>3.5</td>
<td>3.3</td>
</tr>
<tr>
<td>Cookies/biscuits</td>
<td>36 to 430</td>
<td>3.3</td>
<td>2.3</td>
</tr>
<tr>
<td>French fries</td>
<td>120 to 1030</td>
<td>4.9</td>
<td>2.4</td>
</tr>
<tr>
<td>Potato chips</td>
<td>120 to 2760</td>
<td>2.8</td>
<td>5.9</td>
</tr>
<tr>
<td>Coffee</td>
<td>25 to 370</td>
<td>12.0</td>
<td>9.5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>31.0</td>
<td>33.5</td>
</tr>
</tbody>
</table>


---

**Formula 9**

\[
\begin{align*}
\text{CH}_2\text{NH}_2\xrightarrow{\text{CYP 2E1}}\text{O} \quad \text{CYP 2E1} \\
\text{O} \quad \text{NH}_2 \\
\end{align*}
\]

Acrylamide \quad Glycidamide
4.3.6 MISCELLANEOUS FOODBORNE GENOTOXIC AGENTS

Modern processing introduces many natural and artificial compounds — food additives — into food products in order to improve their functional properties. It is an obvious assumption that such chemicals are reasonably free of risk to human health. Recent evaluations were therefore somewhat surprising; more than 40% of food additives tested turned out to be carcinogenic in one or more rodent groups. If this percentage is extrapolated to all substances added to food in the United States, it would imply that more than 1000 of them are potential rodent carcinogens (Johnson, 2002). Also, commercially available liquid smoke flavorings seem not to be risk free. Some of these complex mixtures formed upon wood pyrolysis were shown to be more cytotoxic than is cigarette smoke condensate. In addition, they displayed mutagenic potential in a bacterial test specifically detecting base-pair substitutions (Putnam et al., 1999).

In contrast to food additives, ethanol, though not itself carcinogenic, has been long known to be a risk factor for several cancers, in particular those of the upper gastrointestinal tract, liver, and colon. In conjunction with smoking, risk may increase up to tenfold (Kune and Vitetta, 1992). Several mechanisms for this increase due to ethanol have been proposed. The most important are associated with the enhancement of the genotoxic effect of N-nitrosoamines, present both in cigarette smoke and in a number of foodstuffs. Ethanol, by inducing CYP 2E1, facilitates mutagenic activation of nitrosoamines (Mori et al., 2002). In nursing rats, ethanol administered prior to nitrosoamines caused a tenfold rise in O\textsuperscript{6}-methylguanine in the mammary gland. In the sucklings, this coexposure markedly increased DNA methyl adducts in the lung and kidney (Chhabra et al., 2002). In addition, production of potentially genotoxic ethanol metabolites such as acetaldehyde as well as 1-hydroxyethyl and hydroxyl radicals near DNA by the liver nuclear metabolizing system was proposed (Díaz Gómez et al., 1999). The above data suggest that ethanol may provoke a genotoxic insult in many ways.

Mutagenic products of pyrolysis including methylglyoxal (Formula 10) and the less active glyoxal and diacetyl are commonly found in coffee (roasted, instant, caffeine-free), roasted tea, soy sauce, and brandy-type alcoholic beverages. Instant coffee powder may contain between 20 and 100 μg/g of each of these compounds. Methylglyoxal, which is also a spontaneous product of glucose metabolism and forms during transformation of some xenobiotics, displays the strongest mutagenic activity and is capable of acylation of guanines in DNA. The copresence of hydrogen peroxide increases its mutagenicity by 30-fold (Nagao et al., 1986; Kalapos, 1999). Another risk factor associated with coffee consumption is the presence of caffeine.

\[
\text{Glyoxal} \quad \text{Methylglyoxal} \quad \text{Diacetyl}
\]

**Formula 10**
This alkaloid has been long known to inhibit DNA repair and more recently to induce Phase I enzymes (CYP 1A2). These two properties may explain the enhancement of HAA carcinogenicity by caffeine (Tsuda et al., 1999).

Despite apparent cancer-promoting activities, both caffeine and methylglyoxal, depending on the situation, were shown either to promote or to inhibit carcinogenesis. Such a dichotomous effect is not exceptional among natural food components. Research blooming since 1980 emphasized the possibility that reduction of cancer risk may be linked to diet rich in fruits and vegetables, owing mainly to nonnutritive phytochemicals produced by plants. Many of these compounds in purified form, however, were shown previously to be mutagenic (Ames et al., 1990) and more recently to exert other genotoxic and tumorigenic effects (Lee and Park, 2003; Paollini et al., 2004). Therefore, recommending regular consumption of isolated chemopreventive foodborne compounds as a means of cancer prevention seems very premature.

4.4 PRODUCTS OF OXIDATIVE PROCESSES

Obesity is one of the cancer risk factors that are generally agreed upon. In contrast, since the beginning of the twentieth century, several investigations have demonstrated inhibition of tumorigenesis in underfed mice. Also, numerous recent studies confirm that caloric and protein restriction inhibits experimental carcinogenesis (reviewed by Kritchevsky, 1999). These findings point to the importance of caloric intake to cancer development. One explanation is that normal metabolic processes taking place in the human organism generate ROS. They display high oxidative potential, thereby contributing to deleterious reactions with cellular components. A small but significant flux of ROS leaks away from mitochondria and endoplasmic reticulum. The leakage, it may be presumed, increases with the rate of metabolism and thus with the amount of nutrients consumed, and it exerts so-called internal oxidative stress. Targets of ROS include DNA, proteins, RNA, and lipids. Resultant mutations in cancer-related genes and oxidative modification of proteins and reactive aldehydes (byproducts of lipid peroxidation) are some key events that can increase cancer risk (Behrend et al., 2003; Hussain et al., 2003). Caloric restriction, apart from slowing down metabolism and thereby limiting internal oxidative stress, increases activity of antioxidant enzymes; it also leads to enhanced DNA repair. Probably, these events result in the observed modulation of oxidative damage and inhibition of carcinogenesis (Kritchevsky, 1999).

4.4.1 Reactive Oxygen Species with DNA-Damaging Potential

The function of ROS in pathological mechanisms is taking a central role in various fields of biomedical research, cancer research included. ROS are formed during normal metabolic conversion of nutrients. Exposures to environmental pollutants and food carcinogens are examples of diet-related external oxidative stress inducers. It is now well documented that oxidation reactions mediated by one-electron oxidants together with ROS (OH•, H₂O₂, ′O₂, O₃, HOCl) give rise to a wide variety of
modifications of cellular components. DNA is the most biologically significant target of oxidative attack. Estimates suggest an average of a few hundred “genotoxic hits” per day on the DNA of each of the cells in the human body (Dizdaroglu, 1997; Cadet et al., 2003). Oxidative DNA damage caused by ROS includes a multiplicity of different base oxidations; those resulting from hydroxylations are given below (Formula 11).

A recent review by Bjelland and Seeberg (2003) presents the structures of all currently known oxidized DNA base lesions and summarizes the knowledge on their mutagenic and toxic effects, as well as repair. ROS also induce other genotoxic insults such as DNA strand breaks, lesions to deoxyribose, abasic sites, and DNA-protein and DNA-DNA crosslinks.

In addition, proteins, such as those involved in DNA repair or signal transduction, apoptotic modulators, p53 protein, or DNA polymerases, can be modified both structurally and functionally when exposed to ROS. Thus, in addition to DNA damage, ROS promote cancer development by several epigenetic mechanisms, some of which are triggered due to oxidative modification of proteins leading indirectly to an increased rate of mutations (Hussain et al., 2003).

The assumption that DNA damage by ROS contributes significantly to cancer development implies that agents decreasing their formation should decrease cancer risk. Indeed, several human intervention studies confirmed that diets rich in foods with a high content of antioxidants decrease oxidative DNA and protein damage. However, supplementation with purified dietary antioxidants (e.g., beta-carotene) was not always protective.

### 4.4.2 Genotoxic Products of Lipid Peroxidation

Polyunsaturated fatty acid (PUFA) residues, either in food oils and fats or in cellular phospholipids, are extremely sensitive to oxidation, which takes place during storage and cooking of foods or in the body. Monounsaturated fatty acids (constituting over
70% in olive oil) are far less susceptible than PUFAs (prevailing in sunflower oil and fish oils, among others) to oxidative damage. Particularly susceptible are animal fats because they are devoid of protective antioxidants found in plant oils. The oxidative degeneration of food-derived PUFAs is enhanced upon thermal processing, including frying (Bartsch et al., 2002; Seppanen and Csallany, 2002).

The chain of nonenzymatic peroxidative reactions (induced, for example, by ROS) known as lipid peroxidation yields lipid hydroperoxides as the major initial reaction products. These are relatively short-lived and decompose to epoxides and aldehydes. The major enal breakdown products include malondialdehyde (MDA), acrolein, crotonaldehyde, and 4-hydroxynonenal (HNE) (Formula 12).

Compared to radicals and hydroperoxides, aldehydes are stable and can diffuse within or even escape from cells and attack targets (DNA, proteins) that are far from the site of the original event. Moreover, in contrast to hydroperoxides, which are not absorbed, aldehydes are readily absorbed from the diet. Therefore, in humans the toxicity of thermally processed PUFAs appears to come mainly from low-molecular-weight secondary oxidation products. In the organism, their concentration is influenced by endogenous oxidative stress and possibly the amounts consumed with foods (Seppanen and Csallany, 2002; De Bont and van Larebeke, 2004).

All four of the aldehydes mentioned above are genotoxic compounds. Upon reaction with nucleobases, they generate a variety of DNA adducts that are promutagenic and can cause genomic instability. The reactivity of enals towards guanine decreases with increasing chain length, MDA > acrolein > crotonaldehyde > HNE, in accord with the greatest mutagenicity for MDA and lowest for HNE. Characteristic for these compounds is formation of exocyclic adducts (though other agents induce such lesions as well). As aldehydes, they give rise to propano adducts. Following conversion to epoxyaldehydes by different oxidative processes, they yield etheno adducts (De Bont and van Larebeke, 2004). Examples are given below (Formula 13).

Since accurate methods of their determination have been developed, etheno and other exocyclic DNA adducts appear to be promising biomarkers in the assessment...
of risk incurred by oxidative stress. Moreover, measurements of these adducts may aid studies on efficacy of interventions by dietary antioxidants. In the case of nutrition, this could help to identify food components and other diet-related factors promoting or preventing oxidative DNA damage, hence the carcinogenic process (Diplock et al., 1998; Halliwell, 2000; Bartsch et al., 2002).

4.5 CONCLUSIONS

The purpose of this chapter has been to catalogue the major known and suspected genotoxic carcinogens found in the human diet as well as to discuss, whenever data allow, their impact on human cancer risk. Some of these carcinogens enter the food matrix before consumption (e.g., mycotoxins, environmental pollutants, compounds formed upon processing); others are produced endogenously during digestion. Probably no ready-to-eat food item devoid entirely of genotoxic activity can be found in the Western diet. Though carcinogenic activity of the substances described above has been demonstrated unequivocally in a broad array of experimental systems and suggested by a number of epidemiological data, their individual impact on diet-related cancer risk is still a subject of controversy.

In the literature, one can frequently encounter claims that the concentration of environmental pollutants in food is too low to pose risk of cancer. The same is suggested in the case of carcinogenic food components formed as a result of thermal processing. Especially, the previously accepted concept that no threshold exists in the potential of genotoxic carcinogens to exert a carcinogenic effect has been challenged by recent animal studies on MeIQx (Fukushima et al., 2003) and \( N\)-nitroso-diethylamine (NNDA) (Waddell, 2003). Results of both studies suggest sharp threshold doses necessary to induce cancers in rats by these carcinogens. Also, genetic factors are considered to underlie no more than 5% of tumor incidence. Thus, the question arises as to the reason for such high estimates (30% or more) proposed for diet-related cancers. The most feasible answer would be the combination of all these factors. The biological importance of major nutrients is fairly well understood; little is known, however, about minor components of the diet, including food carcinogens and anticarcinogens. Most of the studies cited here have been carried out with purified compounds in isolation from the food matrix, while the interaction between components in food, including nutrients, requires a more holistic approach. Moreover, cancer has both genetic and environmental components; the interplay between genes and the environment varies not only between individuals but also at different points in an individual’s lifetime. This notion is endorsed by studies in cohorts of twins showing a significant causation of cancer resulting from gene–environment interactions (Lichtenstein et al., 2000). Therefore, a key hope for the next years is that the sequencing of human genome will deliver, in the so-called postgenomic era, substantial benefits in terms of elucidation of carcinogenic mechanisms and possibilities of their prevention by dietary measures, e.g., functional foods. At the moment, the most reasonable approach to diminish cancer risk is to control and restrict human exposure to carcinogens, foodborne carcinogens in particular, and to advocate a lifestyle (quitting smoking, healthy diets) promoting chemopreventive behaviors.
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5 Impact of Food Preservation, Processing, and Cooking on Cancer Risk

Amanda J. Cross and Rashmi Sinha

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5.1 INTRODUCTION

Much of the international variation in cancer incidence has been attributed to dietary differences since large variation exists in rates of specific cancers, coupled with changes in incidence among migrant populations. Throughout the world certain foods, in particular meats, are preserved, processed, and cooked using different methods.

Thus far, the most convincing dietary associations are those found for meat consumption and the risk of several cancers. Over the years there have been several reviews, including two large consensus reports from the World Cancer Research Fund (WCRF) (WCRF, 1997) and the Committee on Medical Aspects of Food and Nutrition Policy (Department of Health, U.K., 1998). The recommendation from these reports was to prevent the average level of red meat and processed meat consumption
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from rising. Those consuming high levels (>140 g/day) were advised to reduce their intake. However, both panels agreed that the epidemiology was not consistent.

The WCRF report concluded that an association between meat and colorectal cancer was “probable.” In addition, this report concluded that there was a “possible” association between meat and cancers of the pancreas, prostate, breast, and kidney (WCRF, 1997).

5.2 PRESERVATION AND PROCESSING

Curing foods has been used for years as a method of preservation, particularly for foods such as meats. Curing generally involves adding salt, nitrate (V), or nitrate (III) to the food. The majority of the research regarding meat preservation methods has focused on cancers of the gastrointestinal tract; these studies were summarized by the WCRF report, which concluded that there was “possible” evidence for an association between cured meats and colorectal cancer (WCRF, 1997). There have been two recent reviews of studies on meat and colorectal cancer. The first concluded “a significant 49% increased risk was found for a daily increase of 25 g of processed meat” (Sandhu et al., 2001). The following year a review concluded that processed meat was associated with a significant 1.3-fold increased risk of colorectal cancer (Norat et al., 2002). Processed meat consumption has also been associated with risk of stomach cancer in several case-control (Correa et al., 1985; Risch et al., 1985; Buiatti et al., 1989; Ward and Lopez-Wu-Williams et al., 1990; Boeing et al., 1991; Gonzalez et al., 1991; Hoshiyama and Sasaba, 1992; Ward et al., 1997; Carrillo, 1999) and cohort studies (Chyou et al., 1990; Nomura et al., 1990; Kneller et al., 1991; van den Brandt et al., 2003).

Other cancers that have been associated with processed meat consumption include childhood leukemia, and cancers of the brain, oral cavity, pharynx, larynx, prostate, esophagus, and pancreas (Preston-Martin et al., 1982; Norell et al., 1986; Sarasua and Savitz et al., 1994; Peters et al., 1994; Michaud et al., 2001; Ngoan et al., 2002; Rajkumar et al., 2003; Levi et al., 2004; Risch, 2003).

5.2.1 SALTED FOODS

Salt is used throughout the world as a flavor enhancer and as a preservative. The WCRF concluded that salt was a “convincing” risk factor for nasopharyngeal cancer and a “probable” risk factor for stomach cancer (WCRF, 1997).

The majority of the research into salted foods and cancer risk has focused on stomach cancer. The rates of stomach cancer are higher in countries such as Japan and China, where the consumption of foods preserved with salt, such as Chinese-style salted fish, is high. Some case-control studies have shown a positive association between total salt intake and stomach cancer risk (La Vecchia et al., 1987; Graham et al., 1990; Nazario et al., 1993; Ramon et al., 1993), whereas others have not found an association (Hu et al., 1988; Jedrychowski et al., 1992; Harrison et al., 1997), and neither have the majority of cohort studies (Ikeda et al., 1983; Chyou et al., 1990; Nomura et al., 1990; Kneller et al., 1991; Kato et al., 1992; Galanis et al., 1998; van den Brandt et al., 2003). However, studies that were able to look specif-
ically at foods preserved with salt have found positive associations with stomach cancer risk. Case-control studies have found a positive association between stomach cancer risk and consumption of salted meat and fish (Haenszel et al., 1972; Kono et al., 1988; Buiatti et al., 1989; Demirer et al., 1990; Ward and Boeing et al., 1991; Palli et al., 1992; Ramon et al., 1993; Lee et al., 1995; Lopez-Carrillo, 1999). In addition, a cohort study found a twofold increased risk of stomach cancer with salted fish consumption (Kneller et al., 1991).

Other cancer sites that have been associated with salted food consumption include the nasopharynx (Armstrong et al., 1983; Yu et al., 1986; Yu et al., 1989; Ning et al., 1990; Zheng et al., 1994; Sriamporn et al., 1992; Yu et al., 1988), oral cavity (Zheng et al., 1992), esophagus (Gao et al., 1994), and colorectum (Knekt et al., 1999).

Salted meats and fish are also sources of nitrate (III) and \(N\)-nitroso compounds (NOCs). The effect of salted foods on cancer risk may therefore be entangled with the effects of exposure to NOC. Some foods, such as salted fish, are preserved using nitrate (III) salts and are thus a source of both salt and exogenous NOC from the reaction between the nitrate (III) and the secondary amines present in the fish. It is known that Chinese salted fish, for example, contains high levels of NOCs.

5.2.2 Nitrates (V)/(III) and \(N\)-Nitroso Compounds (NOCs)

Nitrate (III) is added to processed meat as an antibacterial agent against Clostridium botulinum and as a cosmetic agent to react with myoglobin to produce the characteristic red-pink color of cured meats. Nitrate (V) and nitrate (III) are known precursors for NOC formation and therefore, nitrate (III) added to meat can form NOC in the meat. Therefore, it is hypothesized that increasing exposure to nitrate (V) and nitrate (III) increases exposure to NOCs. However, dietary sources of nitrate (V) and nitrate (III) have not shown consistent associations with cancer risk. Some studies have found a positive association between foods high in nitrate (III), such as bacon and hot dogs, and esophageal cancer (Rogers et al., 1995), nasopharyngeal cancer (Ward et al., 2000), noncardia gastric cancer (Mayne et al., 2001), pancreatic cancer (Coss et al., 2004), and childhood leukemia and brain cancer (Kuijten et al., 1990; Peters et al., 1994; Sarasua and Savitz, 1994). However, several cohort and case-control studies have not shown an association with colorectal (Knekt et al., 1999) or gastric cancer risk (Chyou et al., 1990; Van Loon et al., 1997). A possible explanation for the lack of association between nitrate (V) and cancer risk is that one of the major sources of dietary nitrate (V) is vegetables. Vegetables are also a source of vitamin C, which is a known \(N\)-nitrosation inhibitor.

NOCs are among the most powerful chemical carcinogens; therefore, even small amounts in the human body could be influential in carcinogenesis. The carcinogenicity of NOCs has been tested in 39 different animal species including 6 species of primate, and tumors have been induced in all species so far examined at a variety of anatomical sites.

Humans are exposed to NOCs from exogenous sources and by endogenous formation within the body. Exogenous exposure to NOCs occurs mainly from tobacco and the consumption of preserved or heat-treated foods. NOCs are found
in foods processed by smoking or direct fire-drying, which uses sufficient heat to oxidize molecular nitrogen to nitrogen oxides, which are able to nitrosate amines present in foods such as meat.

When nitrate (III) is in acidic conditions, such as those found in food processing operations, the nitrosating agents dinitrogen-, tri-, and tetra-oxide can form. To reduce the formation of NOCs under these conditions, ascorbic acid may be added to such processing procedures to scavenge nitrosating agents and can therefore reduce N-nitrosation reactions. With such improvements in food processing technology, the concentration of NOCs in nitrate (III)-cured meats has decreased, but traditional methods of curing are still used in many countries. Daily NOC intake from dietary sources has been estimated to be 36 to 140 μg, with bacon and beer being the main sources (Gangolli et al., 1994).

The association between exogenous NOC exposure and cancer was investigated in 73 cases of colorectal cancer in a Finnish cohort of 9985 individuals (Knekt et al., 1999). This study investigated whether intake of N-nitroso-dimethylamine (NDMA) or foods rich in N-nitrosamines is predictive for colorectal cancer. NDMA intake came from smoked and salted fish (52%) as well as cured meats and sausages (48%). This study found a significant twofold increased risk of colorectal cancer in those with a high intake of NDMA.

Other gastrointestinal tract cancers have been associated with NOC exposure. The stomach is known to be a potential site for endogenous N-nitrosation. Some studies have estimated overall NOC intake and have found significant positive associations with stomach cancer risk (Gonzalez et al., 1994; La Vecchia et al., 1995; Pobel et al., 1995), while other studies have found a positive association between the consumption of individual foods known to contain NOC, such as cured sausages, and stomach cancer risk (Correa et al., 1985). A study of esophageal cancer conducted in two different areas of China, a low- and a high-risk area, showed that NOC levels in the diet and daily excretion of NOC were significantly higher in the area at high risk for this cancer (Lin et al., 2002).

Consumption of foods high in NOCs has also been associated with an increased risk of upper aerodigestive tract cancers (Rogers et al., 1995), and childhood exposure to NOCs has been specifically associated with nasopharyngeal cancer (Ward et al., 2000). In addition, NOC exposure has been investigated as a risk factor for brain cancer. A meta-analysis found a positive association with cured meat consumption and brain-cancer risk (Huncharek and Kupelnick, 2004). However, this cancer is rare and difficult to study prospectively.

5.2.3 Smoked Foods — Polycyclic Aromatic Hydrocarbons (PAHs)

Smoke-curing foods is also used as a method of preservation. Foods may be preserved by using burning wood directly, which results in smoke engulfing the food and giving it a smoked flavor. Alternatively, foods may have a smoked flavor from liquid smoke, which is made by condensing smoke from burning wood. Both of these methods expose the food to potentially carcinogenic compounds. Smoke is known to be an abundant source of NOCs; therefore, the consumption of smoked
foods increases an individual’s exposure to these compounds. Moreover, there is an additional risk associated with smoked foods: the incomplete combustion of organic matter results in the formation of polycyclic aromatic hydrocarbons (PAHs), which can be metabolized into mutagenic compounds. For further discussion of these compounds see Section 5.3.2.

5.3 COOKING METHODS

According to the WCRF report, cooking methods are “possibly” contributing to the risk of both stomach and colorectal cancers (WCRF, 1997). Of seven case-control studies that have investigated the effect of cooking method on colorectal cancer risk (Lyon and Mahoney, 1988; Young and Wolf, 1988; Peters et al., 1989; Gerhardsson de Verdier et al., 1991; Kampman et al., 1999; Butler et al., 2003; Wohlleb et al., 1990), three showed an increased risk for frying (Peters et al., 1989; Gerhardsson de Verdier et al., 1991; Butler et al., 2003) and two found an increased risk for grilling/barbecuing (Gerhardsson de Verdier et al., 1991; Wohlleb et al., 1990).

Frying/grilling has also been associated with an increased risk of pancreatic cancer (Norell et al., 1986) and lung cancer (Sinha et al., 1998a).

The degree to which the meat is cooked is also thought to affect risk of some cancers. Of seven studies investigating the role of meat doneness on colorectal adenoma or cancer, five found a significant increased risk with well-done meat (Gerhardsson de Verdier et al., 1991; Lang et al., 1994; Sinha et al., 1999; Nowell et al., 2002; Butler et al., 2003). In addition, well-done meat has been associated with an increased risk of lung cancer (Sinha et al., 1998a) and breast cancer (Zheng et al., 1998).

5.3.1 HETEROCYCLIC AROMATIC AMINES (HAAs)

Cooking methods and meat doneness have been used as surrogate measures of individual exposure to mutagens formed when meat is cooked by high-temperature methods such as pan-frying or grilling. The mutagens formed by such high-temperature cooking methods are heterocyclic amines (HAAs). HAAs are formed from the reaction between creatine or creatinine (found in muscle meats), amino acids, and sugars. HAA formation increases with the temperature and duration of cooking and depends on the type of meat and the cooking method. Early work analyzed cooked meat for HAAs and found that high levels of exposure are possible with optimal cooking time and temperature. Table 5.1 shows the effect of different cooking methods and doneness levels on HAA concentration in meat, using beef steak as an example. This table clearly shows that HAA concentration is highest in very well-done meat cooked by the methods that expose the meat to the highest temperatures (pan-frying and grilling/barbecuing). As the formation of HAAs increases with cooking time and temperature, this may explain some of the associations found for meats cooked well-done by high-temperature cooking techniques such as frying or grilling.

In 1993, the International Agency for Research on Cancer found that there was sufficient evidence from experimental animal studies to conclude that the HAAs IQ (2-amino-3-methylimidazo(4,5-f)quinoline), MeIQ (2-amino-3,4-dimethylimidazo(4,5-f)quinoline), MeIQx (2-amino-3,8-dimethylimidazo(4,5-f)quinoxaline),
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and PhIP (2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine) were carcinogenic (IARC, 1993). The most abundant HAAs in cooked meat are PhIP and MeIQx; after a cooked meat meal, they are also the two major absorbed HAAs. Over 20 individual HAAs have been identified, most of which are potent bacterial mutagens and at least 10 of which have been found to induce tumors in laboratory animals. HAAs produce tumors in a variety of organs such as the liver, lung, forestomach, colon, prostate, and mammary gland, as well as lymphomas in rodent and nonhuman primate models. PhIP, specifically, has been associated with an increased risk of intestinal and mammary adenocarcinomas in rodents (Ghoshal et al., 1994; Ito et al., 1997), as well as prostate tumors in rats (Shirai et al., 1999). MeIQx can induce tumors at multiple sites in rodents, such as the liver and lung, as well as lymphomas and leukemias (Ohgaki et al., 1987; Kato et al., 1988).

In human studies, questionnaires with detailed cooking and doneness information linked to an HAA database are used to estimate individual HAA intake. The HAA database was created by measuring levels of HAAs in a variety of meats, cooked by different methods to a range of doneness levels (rare, medium, well-done and very well-done). Estimates of HAA intake, using a questionnaire linked to this database, suggest that the mean daily intake of PhIP is between 43 and 110 ng/day, and the intake of MeIQx is between 14 and 47 ng/day.

In case-control studies, using meat doneness as a surrogate of HAA exposure, elevated risks have been shown for colorectal adenoma (Probst-Hensch et al., 1997; Sinha et al., 1999), as well as cancers of the colorectum (Gerhardsson de Verdier et al., 1991; Lang et al., 1994; Kampman et al., 1999), stomach (Ward et al., 1997),

<table>
<thead>
<tr>
<th>TABLE 5.1</th>
<th>HAAs and B[a]P Concentrations in Beef Steak Cooked by Different Methods to Varying Degrees of Doneness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooking Method</td>
<td>Doneness Level</td>
</tr>
<tr>
<td>MeIQx (ng/g)a</td>
<td>Oven-broiled</td>
</tr>
<tr>
<td></td>
<td>Pan-fried</td>
</tr>
<tr>
<td></td>
<td>Grilled/barbecued</td>
</tr>
<tr>
<td>PhIP (ng/g)a</td>
<td>Oven-broiled</td>
</tr>
<tr>
<td></td>
<td>Pan-fried</td>
</tr>
<tr>
<td></td>
<td>Grilled/barbecued</td>
</tr>
<tr>
<td>B[a]P (ng/g)b</td>
<td>Oven-broiled</td>
</tr>
<tr>
<td></td>
<td>Pan-fried</td>
</tr>
<tr>
<td></td>
<td>Grilled/barbecued</td>
</tr>
</tbody>
</table>

breast (Zheng et al., 1998), lung (Sinha et al., 2000), and prostate (Norrish et al., 1999). However, other case-control studies have not found an association (Muscat and Wynder, 1994; Augustsson et al., 1999). Very few studies have specifically used detailed questionnaires to capture meat cooking methods and doneness level to determine individual HAA intake. Three initial case-control studies have taken this approach. Two of these studies found a significant increased risk associated with HAAs; one found a 1.8-fold increased risk of colon cancer with DiMeIQx intake (Butler et al., 2003), the other found 2.1- to 2.5-fold increased risks for colorectal adenoma with DiMeIQx, MeIQx, and PhIP intake (Sinha et al., 2001). However, the third study found no association between estimated heterocyclic amine intake and cancer of the colon, rectum, bladder, or kidney (Augustsson et al., 1999).

The amount of HAAs formed during meat cooking can be reduced by reducing the heat and the length of the cooking time. In addition, preheating meat in the microwave removes the creatine and consequently reduces the amount of HAA formed.

### 5.3.2 Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs are mutagenic compounds formed in foods processed by smoking, such as meat, as well as in meat cooked by grilling/barbecuing. Meat cooked over a flame results in fat/meat juices dripping onto the hot fire, which yields flames containing a number of PAHs. These PAHs adhere to the surface of the food. Benzo[a]pyrene (B[a]P) is one of the most potent PAH carcinogens in animal studies and can induce leukaemia as well as gastric, pulmonary, forestomach, esophageal, and tongue tumors in rodents (Culp et al., 1998). Grilled and well-done steak, hamburgers, and chicken contain the highest levels of B[a]P, containing up to 4 nanograms of B[a]P per gram of cooked meat. Depending on individual factors, the total PAH intake may vary between 25 and 300 μg/day. The effect of cooking methods and doneness level on B[a]P concentration is shown in Table 5.1; this table demonstrates that appreciable levels of B[a]P are only present in meat cooked by barbecuing/grilling and that the concentration increases with increasing meat doneness level.

However, only one epidemiologic study has directly investigated the association between dietary intake of PAHs and colon cancer, and this population-based case-control study did not find an association (Butler et al., 2003).

### 5.3.3 Acrylamide

Acrylamide exposure has recently caused concern as a potential risk for a variety of cancers. Acrylamide is listed by the World Health Organization as a probable human carcinogen, and IARC concluded that it is “probably carcinogenic to humans” (IARC, 1994). It has long been known that humans can be exposed to acrylamide from certain occupational exposures, but it has only recently been recognized that exposure to acrylamide can occur from dietary sources. This, therefore, is an emerging area of research.

Acrylamide is formed at high temperatures from the reaction between certain amino acids, such as asparagine, and certain sugars. Studies have shown that acrylamide levels are high in heat-processed commercial foods and in foods cooked at
high temperatures. Acrylamide formation increases with increasing temperatures. These substances are formed in heated protein-rich foods, but the highest levels are formed in carbohydrate-rich foods, such as potato chips (Table 5.2).

Acrylamide has been shown to be mutagenic in a mouse cell line (Besaratinia and Pfeifer, 2003) but studies have not yet been conducted in human cell lines. In carcinogenicity studies in rodents, acrylamide administered via different routes increased the risk of cancers of the lung, skin, reproductive tract, thyroid, mammary gland, brain, central nervous system, and oral cavity (Bull et al., 1984; Johnson et al., 1986; Dearfield et al., 1995; Friedman et al., 1995). In addition, animals given acrylamide orally (Paulsson et al., 2002), or fed a diet high in fried foods (Tareke et al., 2000), had increased levels of haemoglobin DNA adducts.

Epidemiological studies have mainly focused on occupational exposure to acrylamide and have not found an elevated risk of cancer (Marsh et al., 1999; Granath et al., 2001). Very few epidemiological studies have addressed the issue of dietary exposure to acrylamide, mainly because it has only very recently been recognized as a potential hazard but also due to difficulties in exposure assessment.

The first epidemiological study to address the hypothesis that acrylamide may contribute to the risk of human cancer was carried out in Sweden. This was a population-based case-control study of several cancers including colorectal (n = 591), bladder (n = 263), kidney (n = 133), and 538 controls (Mucci et al., 2003). This study did not find an increased risk for any of these cancers with estimated acrylamide intake. However, this study was not able to consider the acrylamide content of all foods and therefore exposure may have been underestimated. A second study looked at fried foods as a risk factor in a case-control study of 527 individuals with laryngeal cancer (Bosetti et al., 2002). This study found significant elevated risks for fried beef/veal (1.6-fold risk), fried fish/shellfish (3-fold risk), fried eggs (1.9-fold risk), and fried potatoes (1.9-fold risk).

### TABLE 5.2
Levels of Acrylamide in Foods That Contribute the Most to Acrylamide Exposure

<table>
<thead>
<tr>
<th>Food</th>
<th>Acrylamide (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread</td>
<td>Not detectable to 59</td>
</tr>
<tr>
<td>Cereals</td>
<td>54 to 241</td>
</tr>
<tr>
<td>Cookies</td>
<td>81 to 168</td>
</tr>
<tr>
<td>Potato — boiled</td>
<td>Not detectable</td>
</tr>
<tr>
<td>— chips</td>
<td>264 to 536</td>
</tr>
<tr>
<td>— French fries</td>
<td>381 to 450</td>
</tr>
</tbody>
</table>

Source: Data from the U.S. Food and Drug Administration Web site: http://www.cfsan.fda.gov/~dms/acrydat2.html.
Fried and baked potatoes have been shown to be among the major sources of dietary acrylamide. A recent study used data from several hospital-based case-control studies conducted in Italy and Switzerland to consider whether fried and baked potatoes increased the risk of a variety of cancers (Pelucchi et al., 2003). The results showed no association between fried/baked potatoes and cancers of the oral cavity/pharynx, esophagus, larynx, colorectum, breast, and ovary.

Any potential associations between dietary sources of acrylamide and risk of human cancer are only just beginning to be explored. Therefore, this area needs further studies with improved exposure assessment and biomarkers of acrylamide intake.

5.4 CONCLUSIONS

Studies have shown that methods of food processing, preservation, and cooking can influence the risk of several cancers. Table 5.3 summarizes the associations between food preservation, processing, and cooking and cancer risk. This table shows whether a positive association has been found at a particular cancer site in human or animal studies; it does not, however, show the number of studies that found a positive association or the number of studies that found a null result for any particular exposure and cancer site.

Some measures have been taken to reduce the risk of exposure to certain potential carcinogens; for example, ascorbic acid is added to processed meat to reduce the formation of NOCs. However, much of the exposure arises from personal dietary choices, ranging from the consumption of foods that have been processed to the method of cooking the food.
### TABLE 5.3
A Summary of Cancers Positively Associated with Processed Foods and Mutagens Formed from Cooking Procedures

<table>
<thead>
<tr>
<th>Cancer Site</th>
<th>Salted Foods</th>
<th>Nitrate (V)/Nitrate (III)</th>
<th>NOCs</th>
<th>Processing Procedures</th>
<th>Cooking Method</th>
<th>Mutagens Formed from Cooking</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>High-Temperature Cooking Methods</td>
<td>Foods Cooked Well-Done</td>
<td>HAAs</td>
</tr>
<tr>
<td>Lung</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Colorectal</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Breast</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Prostate</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Bladder</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esophagus</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral cavity</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasopharyngeal</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper aerodigestive tract</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukemia</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central nervous system</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reproductive tract</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphomas</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: *, Positive associations found in human studies; †, Positive associations found in animal studies only.
REFERENCES


Carcinogenic and Anticarcinogenic Food Components


6 Environmental Contamination of Food

Marek Biziuk and Agnieszka Bartoszek

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6.1 INTRODUCTION

Intensive industrialization of the world has resulted in an increased input of toxic inorganic and organic compounds to the environment, drastically deteriorating the quality of surface and ground waters as well as agricultural land. Anthropogenic pollution of drinking water supplies and resources indispensable for food production has become a fact of life. The number of known organic compounds is now estimated to be about 16 million, 2 million of which are synthetic compounds. Every year, approximately 250,000 new compounds are synthesized, and about 1,000 of them are manufactured on an industrial scale. Presently, ca. 70,000 organic compounds are commercially available, with an annual global production of 100 to 200 million tons. About one-third of all organic compounds produced end up in the environment, including water (Biziuk and Przyjazny, 1996; Biziuk et al., 2001).

The vast majority of anthropogenic environmental pollutants are toxic compounds, dangerous not only to humans but also to animals and plants. For instance, around 700 chemical compounds, including more than 600 organic compounds, many of which are biologically active, have been detected in drinking water samples
Carcinogenic and Anticarcinogenic Food Components

(Moore and Ramamoorthy, 1984; Cotruvo, 1985; Sontheimer et al., 1985; Toft, 1985; de Leer, 1987). Moreover, numerous food and potable water pollutants are potentially mutagenic and carcinogenic; hence, at certain levels they may pose a serious health hazard (Ferguson, 1999). Consequently, there is a need for continuous monitoring of the degree of pollution of food and potable and surface waters by anthropogenic inorganic and organic compounds. Excessive concentrations of pollutants, above permitted maximum contaminant levels, not only may pose hazards to consumers but also signal the existence of uncontrolled discharge of wastes, improperly operating treatment plants, lack of enforcement of legislation dealing with food production and water management, or other violations of environmental laws.

Food and water pollutants can be divided into physical, biological, chemical, radioactive, inorganic, and organic. This chapter discusses only chemical organic and inorganic pollution. The main groups of organic pollutants found in food and drinking water comprise pesticides, polychlorinated biphenyls (PCB), polychlorinated dibenzo-p-dioxins (PCDD) and benzofurans (PCDF), volatile organohalogen compounds (VOX) including trihalomethanes (THM), volatile hydrocarbons, and polycyclic aromatic hydrocarbons (PAH). The most dangerous inorganic contaminants are heavy metals, asbestos, and nitrates (III) and (V).

Most environmental mutagens and carcinogens are introduced into organisms with food (Ferguson, 1999). It is assumed that concentrations of environmental carcinogens in food and tap water are very low, and the risk of cancer associated with their intake seems negligible relative to overall cancer risk rates. The tendency of several carcinogenic pollutants to accumulate in the human organism may, however, increase these estimates over a lifetime.

Sources of human exposure to some potent environmental carcinogens are shown in Table 6.1. Pesticides and benzo[a]pyrene are mainly ingested with food. This far-from-complete list leads to an obvious conclusion: obeying the Delaney clause* nowadays would cause famine in the majority of industrialized countries. Regarding mutagenic and carcinogenic substances in the human environment, including food, new measures have been adopted. Projections of risks are approached by two principal means: retrospective epidemiologic studies of exposed populations and surrogate toxicology studies using experimental animals, which enable estimation of acceptable daily intake, maximum admissible level, etc. Such studies have become the basis of classification of compounds in relation to their carcinogenic impact on human health. The International Agency for Research on Cancer (IARC), based in Europe (Lyon, France), and the U.S. Environmental Protection Agency (USEPA) have formulated the most frequently used classifications (Table 6.2).

The environmental pollutants found in food and drinking water belong mainly to Groups 2A and 2B of the IARC classification or Groups B1, B2, and C of the USEPA classification. Ironically, despite clear demonstration of their carcinogenicity in numerous experimental models, according to current risk assessment code of

---

* The Delaney clause in section 409(c) of the Pure Food and Drugs Act (U.S. Code, 1982a): Provided that no additive shall be deemed to be safe if it is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal.
### TABLE 6.1
Percent Distribution of Selected Environmental Contaminants in Various Sources of Human Exposure

<table>
<thead>
<tr>
<th>Compound</th>
<th>Drinking Water</th>
<th>Food</th>
<th>Air</th>
<th>Smoking</th>
<th>Daily Intake (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzo[a]pyrene</td>
<td>0.9</td>
<td>87</td>
<td>4</td>
<td>8</td>
<td>1.1</td>
</tr>
<tr>
<td>DDT</td>
<td>0.01</td>
<td>~100</td>
<td>0.01</td>
<td>—</td>
<td>10</td>
</tr>
<tr>
<td>Chlordane</td>
<td>0.01</td>
<td>~100</td>
<td>0.02</td>
<td>—</td>
<td>10</td>
</tr>
<tr>
<td>Lindane</td>
<td>0.1</td>
<td>~100</td>
<td>0.1</td>
<td>—</td>
<td>10</td>
</tr>
<tr>
<td>Vinyl chloride</td>
<td>&lt;1</td>
<td>5</td>
<td>95</td>
<td>—</td>
<td>210</td>
</tr>
<tr>
<td>Benzene</td>
<td>0.04</td>
<td>56</td>
<td>44</td>
<td>—</td>
<td>450</td>
</tr>
<tr>
<td>Trichloromethane</td>
<td>15</td>
<td>77</td>
<td>8</td>
<td>—</td>
<td>130</td>
</tr>
<tr>
<td>Trichloroethene</td>
<td>1</td>
<td>5</td>
<td>94</td>
<td>—</td>
<td>106</td>
</tr>
<tr>
<td>Tetrachloromethane</td>
<td>0.4</td>
<td>19</td>
<td>77</td>
<td>—</td>
<td>26</td>
</tr>
<tr>
<td>Tetrachloroethylene</td>
<td>0.3</td>
<td>4.9</td>
<td>95</td>
<td>—</td>
<td>105</td>
</tr>
</tbody>
</table>


### TABLE 6.2
Classification of Carcinogenic Substances According to IARC and USEPA Criteria

<table>
<thead>
<tr>
<th>Criteria</th>
<th>IARC</th>
<th>USEPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical is carcinogenic to humans (sufficient evidence from epidemiological studies)</td>
<td>Group 1</td>
<td>Group A</td>
</tr>
<tr>
<td>Chemical is probably carcinogenic to humans</td>
<td>Group 2</td>
<td>Group B</td>
</tr>
<tr>
<td>At least limited evidence of carcinogenicity to humans</td>
<td>Group 2A</td>
<td>Group B1</td>
</tr>
<tr>
<td>Usually a combination of sufficient evidence in animals and inadequate data for humans</td>
<td>Group 2B</td>
<td>Group B2</td>
</tr>
<tr>
<td>Possible human carcinogen (limited evidence of carcinogenicity in animals in the absence of human data)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not classified (inadequate animal evidence of carcinogenicity)</td>
<td>Group 3</td>
<td></td>
</tr>
<tr>
<td>IARC: no evidence of carcinogenicity to humans</td>
<td></td>
<td></td>
</tr>
<tr>
<td>USEPA: no evidence for carcinogenicity in at least two adequate animal tests in different species or in both epidemiological and animal studies</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

practice, most of the pollutants occurring in food and drinking water fall into the class of compounds whose carcinogenic effects are controversial.

### 6.2 BIOACCUMULATION AND BIOMAGNIFICATION

Some of the most dangerous compounds (pesticides, PAHs) are persistent in the environment, tend to bioaccumulate in plants and animal organisms, and are even subject to biomagnification in the food chain (Connell, 1990). People are at the top of the food chain and thus may ingest food with already enriched concentrations of toxicants.

*Bioaccumulation* is the capability of a substance to accumulate in an organism, or particular tissues of an organism, and can be described by a bioconcentration factor, for instance, the equilibrium constant of the concentration of a particular substance in aqueous organisms to its concentration in the water. Bioaccumulation strongly depends on the distribution coefficient octanol/water, specific for each compound.

Biomagnification is the process whereby a chemical, as it is passed through a food chain by trophic transfer, reaches increasingly higher concentrations in the tissues of animals at each higher trophic level. The biomagnification of environmental pollutants has been described mainly for closed water ecosystems where the follow up of food chains is possible. If an arbitrary concentration of unity is assigned to the trace of insecticides in water, then their concentration will rise in the direction plankton < crustaceans < small fish < carnivorous fish to reach in fish-eating birds a value of 125,000 (Figure 6.1). Another example, showing actual values, illustrates dramatic biomagnification of the most persistent organic pollutant, DDT, in the water environment of Lake Michigan (Eichler, 1982). The concentration of DDT in wet bottom silt was 0.014 mg/kg; in crustaceans, 0.41 mg/kg; in various fish (eelpout, ide), 3 to 6 mg/kg; and in fish-eating sea gulls, 2400 mg/kg.

![FIGURE 6.1 Bioaccumulation of xenobiotics at subsequent levels of the trophic pyramid in a water ecosystem.](image)
The most recent global assessment of organic contaminants in farmed salmon also points to the importance of biomagnification to the final concentration of carcinogens in food (Hites et al., 2004). This study revealed much higher levels of 14 organic contaminants (among them dioxin, PCBs, lindane, and DDT), additionally depending on the source of farmed fish, than those detected in wild salmon, driving the authors to the conclusion that consumption of farmed Atlantic salmon may pose a substantial health hazard including an increased risk of getting cancer. The large differences between the farmed and wild salmon contaminant levels were ascribed to concentrated feed obtained primarily from small pelagic fishes. The reported data were undermined and criticized for the following reasons: sample material was already two years old, permitted levels were not exceeded, we are all subject to a growing number of different substances, and the researchers mixed up units of their calculations (Eurofish, 2004). Causing a serious uproar in the fish industry, this study demonstrated yet another difficulty in environmental risk assessment — conflict with food producers.

6.3 ENVIRONMENTAL POLLUTANTS IN FOOD

6.3.1 Pesticides

Pesticides are a particularly important group of organic compounds hazardous to human health because of common use, persistence in the environment, and toxicity (Biziuk et al., 1996). They increase crop yields by reducing the amount of a crop that is lost to pests and control diseases transmitted by insects. By definition, pesticides are toxic compounds, as they are used in agriculture, industry, and households to kill weeds (herbicides), insects (insecticides), fungi (fungicides), rodents (rodenticides), mollusks (molluscicides), mites (acaricides), roundworms (nematicides), aphids (aphicides), and eggs (ovicides). They also serve as fumigants, attractants, or repellants. The chemical structures of pesticides are very diversified, including organohalogen compounds, organophosphates, carbamates, triazines, and phenol derivatives, among others. In nature, pesticides are transferred among different parts of the environment undergoing a number of transformations, such as hydrolysis, biodegradation, oxidation, photolysis, biotransformation, or metabolic reactions in living organisms. The present global annual production of pesticides is estimated to reach several hundred thousand tons. As a consequence of large production and high stability, pesticides are present in all parts of the environment, including air, water, and soil. The examples given in Table 6.3 demonstrate that waters contain pesticides even in remote uninhabited places such as Antarctica or the Arctic. As the knowledge of adverse effects on human health grew, some nonbiodegradable and particularly persistent pesticides were abandoned, while the most dangerous were banned in most countries (DDT and other organochlorine pesticides).

Pesticides in soil and surface water can enter food and drinking water. For humans, the major route of exposure to these pollutants is through the gastrointestinal system, via intake of food (because of bioaccumulation and biomagnification in the food chain) and water. Pesticides are readily accumulated in organisms in adipose tissue, liver, kidneys, brain, and heart. Some contents of pesticides in aqueous
organisms in various places of the world are listed in Table 6.4; similar levels of these substances can be found in commercial seafood products.

Humans eat considerable amounts of food in which pesticides are enriched, e.g., milk and dairy products, marine and freshwater fish, crustaceans, vegetables, poultry, and meat. Pesticides very slowly undergo metabolic transformation and are excreted with urine, feces, sweat, or milk, both in animals and humans. Table 6.5 shows that despite discontinued use of pesticides such as DDT in most developed countries, human lipid tissue and human breast milk in numerous places of the world still contain this compound. There is a correlation between the concentration of pesticides in human breast milk and adipose tissue and the intensity of application of particular pesticides in a given region. This effect is apparent in the case of DDT in lipid tissue of people from Zaire as well as in human breast milk in Hong Kong, the two countries in which this compound was still in use at the time of measurements.

*Organochlorine pesticides* are regarded as the group of pesticides most dangerous to human health, displaying carcinogenic, mutagenic, and teratogenic activity as well as causing cardiovascular and neurological diseases. Chlordane, DDT, DDE, tox-
Organochlorine pesticides cause DNA damage and exhibit cancer-promoting properties. Moreover, they are endocrine-disrupting chemicals, i.e., xenoestrogens, and this potential to interact with the endocrine system at various levels (interfering with hormone synthesis, transport, and clearance and with receptor recognition and binding) is believed to be most important for carcinogenicity of these pollutants (Choi et al., 2004, Poli et al., 2003, Zeljezic and Garaj-Vrhovac, 2001). Although the endocrine-disrupting potential of chemicals showing estrogen-modulating effect was closely related to their mutagenicity and carcinogenicity, estrogenic effects were the most predominant in pesticides (Choi et al., 2004).

Since organochlorines accumulate and persist in human adipose tissue and are present in human breast milk, they were suggested as a risk factor in breast cancer. It has also been suggested that such estrogenic compounds may affect testicular cancer. Indeed, the incidence of malignant tumors in terminally ill patients from the general population was significantly correlated with the concentration of DDE (main metabolite of DDT) in adipose tissue (Moore and Ramamoorthy, 1984). Also, statistical analysis of data on women in New York suggested a significant risk of breast cancer in those with higher DDE levels in adipose tissue (Wolff et al., 1993).

### TABLE 6.4
The Concentration (ng/g Lipid or ng/g Dry Tissue) of Selected Pesticides in Marine Organisms from Various World Locations in the Period 1992 to 1999

<table>
<thead>
<tr>
<th>Place (Country)</th>
<th>Source</th>
<th>Year</th>
<th>Lindane</th>
<th>DDT</th>
<th>HCB&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greenland</td>
<td>Fish&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1992</td>
<td>1.5 to 6.2</td>
<td>70 to 1446</td>
<td>3.6 to 84</td>
</tr>
<tr>
<td>Atlantic, North Sea</td>
<td>Cod-liver oil&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92/98</td>
<td>n.d. to 73</td>
<td>30 to 1605</td>
<td>n.d. to 130</td>
</tr>
<tr>
<td>Red River (Vietnam)</td>
<td>Mollusks, shrimp, fish&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95/96</td>
<td>0.62 to 1.76</td>
<td>4.9 to 180</td>
<td>0.05 to 0.15</td>
</tr>
<tr>
<td>Alaska</td>
<td>Milk of female seals</td>
<td>1996</td>
<td>—</td>
<td>844</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Blood of female seals</td>
<td></td>
<td>—</td>
<td>1.4</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Blood of young seals</td>
<td></td>
<td>—</td>
<td>8.0</td>
<td>—</td>
</tr>
<tr>
<td>Pomerania Bay (Poland)</td>
<td>Fish muscles&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1996</td>
<td>1.5 to 2.0</td>
<td>3.5 to 15.3</td>
<td>6.9 to 8.7</td>
</tr>
<tr>
<td></td>
<td>Fish liver&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>3.2 to 4.9</td>
<td>24.4 to 58</td>
<td>4.1 to 8.1</td>
</tr>
<tr>
<td>Michigan (U.S.)</td>
<td>Green frog tissues&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1998</td>
<td>0.06</td>
<td>1.24</td>
<td>—</td>
</tr>
<tr>
<td>Pacific (Sweeper Cove)</td>
<td>Cod liver&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1999</td>
<td>5.1</td>
<td>1094</td>
<td>43.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> ng/g lipid.

<sup>b</sup> ng/g dry tissue.

<sup>c</sup> Hexachlorobenzene.

*Note: n.d., not detected.*

### TABLE 6.5
The Concentration (ng/g Lipid) of Selected Pesticides in Human Breast Milk and Adipose Tissue in Various Countries during the Period 1979 to 1990

<table>
<thead>
<tr>
<th>Source</th>
<th>Country</th>
<th>Year</th>
<th>HCH</th>
<th>DDT</th>
<th>HCB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human breast milk</td>
<td>Sweden</td>
<td>1979</td>
<td>0.17</td>
<td>1.90</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Germany</td>
<td>79/80</td>
<td>0.44</td>
<td>1.51</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>Great Britain</td>
<td>79/80</td>
<td>0.22</td>
<td>1.71</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Denmark</td>
<td>1982</td>
<td>0.08</td>
<td>1.15</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>Hong Kong</td>
<td>1985</td>
<td>16.0</td>
<td>13.80</td>
<td>0.05</td>
</tr>
<tr>
<td>Human adipose tissue</td>
<td>Japan</td>
<td>1981</td>
<td>3.6</td>
<td>3.8</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>India</td>
<td>1982</td>
<td>10</td>
<td>15</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Zaire</td>
<td>1983</td>
<td>4.2</td>
<td>63</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>U.S.</td>
<td>1984</td>
<td>0.62</td>
<td>4.1</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Canada</td>
<td>1984</td>
<td>0.08</td>
<td>3.4</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Italy</td>
<td>1984</td>
<td>0.13</td>
<td>8.2</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>The Netherlands</td>
<td>1986</td>
<td>0.28</td>
<td>2.5</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Poland</td>
<td>77/90</td>
<td>0.33</td>
<td>15.19</td>
<td>0.28</td>
</tr>
</tbody>
</table>


Formula 1
However, a subsequent prospective study carried out in a cohort of 57,040 women did not support this hypothesis (Krieger et al., 1994).

Another line of evidence indicated an association between occupational exposure to pesticides and increased risk of a variety of cancers (Choi et al., 2004, Poli et al., 2003). An association between prostate cancer among farmers and exposure to organochlorine insecticides and acaricides was shown (Alavanja et al., 2003; Settini et al., 2003). Chlordane can cause anemia and different forms of leukemia, while exposure to hexachlorobenzene can induce cancer of the liver, kidney, and thyroid (Nollet, 2000). In addition, a casual relationship between exposure to chlorinated pesticides in the house and garden, an increase of these pesticide contents in blood plasma, and childhood leukemia, lymphoma, and brain tumors has been reported (Heudorf et al., 2003).

Organophosphates and carbamates are two other groups of pesticides with documented adverse effects on human health (Formula 2). Organophosphate and carbamate pesticides are extensively used due to their high insecticidal activity and relatively low persistence. The toxic effects of organophosphorus compounds are due to the inactivation of acetylcholinesterase at muscarinic receptors for acetylcholine, causing symptoms such as cold sweating, salivation, nausea, bronchoconstriction, and a decrease in blood pressure (Nollet, 2000). Since the target enzyme of these pesticides, acetylcholinesterase (AChE), is common to neural transmission both in insects and in mammals, including humans, they represent a potential hazard for human health but are probably not related to neoplastic diseases.

Herbicides, i.e., triazine herbicides (simazine, atrazine), currently represent the most heavily used of all agricultural pesticides (Formula 3). The most common of these chemicals is atrazine, to which nearly 60% of the population of the United States is currently exposed daily. Atrazine, like organochlorine pesticides, is an endocrine disruptor (Birnbaum and Fenton, 2003). However, the results of five extensive oncogenicity studies carried out in rats and mice have led to the conclusion that the carcinogenic effects of high doses of atrazine observed in animals were strain, sex, and tissue specific and consequently have no biological relevance to humans (Stevens et al., 1999).

Genetically modified crops represent a new group of plantborne foods surrounded by much controversy regarding their environmental and dietary safety. The
most abundantly cultivated are edible plants, which are glyphosate tolerant (e.g., Roundup Ready soybeans) or produce insecticidal protein (Bt toxin) due to the incorporation of gene(s) from the soil bacterium *Bacillus thuringiensis* (e.g., Bt corn). A carcinogenic risk assessment has been conducted only in the case of glyphosate-tolerant plants, assuming that Bt proteins, whose maximum consumption at this moment of cultivation is estimated to be in the range of 1 to 10 mg/day compared to 100 to 300 g/day of total protein intake, are safe in this regard (Chassy, 2002) (Formula 4).

Glyphosate is the active ingredient of Roundup®, marketed as a nonselective, broad spectrum, postemergence herbicide. This herbicide is used to control weeds in plantations mainly of glyphosate-resistant genetically modified (GM) crops. To evaluate its carcinogenicity, two chronic administration studies were conducted in rats (in 1979 to 1981 and 1988 to 1990), in which the animals received much higher doses of glyphosate that can be anticipated in humans. In the first study, a significant increase in the incidence of interstitial cell tumors was observed in rat testes; however, the absence of such an effect with the higher doses used in the second study was the basis to exclude glyphosate from the carcinogenic category (WHO, 1994). On the other hand, reports on the metabolic fate of glyphosate in edible parts of GM plants are lacking; thus, no evaluation of the human health hazard of potential metabolites could be performed.

### 6.3.2 Polychlorinated Biphenyls (PCBs), Dibenzo-p-Dioxins (PCDDs), and Dibenzofurans (PCDFs)

*Polychlorinated biphenyls* are a group of 209 related compounds (referred to as congeners) that differ only in the number and sites of chlorine atoms attached to the parent biphenyl molecule (Formula 5). Since these compounds display high dielectric
constant and excellent heat absorption properties, they were used as hydraulic and heat transfer fluids, dielectric fluids, plasticizers, etc. Although the use of PCBs (similar to the situation with chlorinated pesticides) was restricted many years ago, they still remain widely distributed in the environment. They are very stable chemically and thermally and consequently are among the most persistent environmental pollutants entering different food chains to be eventually found in numerous food products consumed by people. A few examples of contents of PCBs in food products are given in Table 6.6 (Falandysz, 1999). PCBs are highly soluble in lipids; thus, they readily bioaccumulate in fatty and lipid-rich tissues and organs.

The toxicity of PCBs is associated with the presence of nonortho and monoortho substituted coplanar congeners. Biological assays based on PCB interaction with the aryl hydrocarbon (Ah) receptor (as ligands) demonstrated that the most toxic congeners are PCB15, PCB37, PCB77, PCB81, and PCB126. Intermediates produced during metabolism may be more toxic than parent compounds are (McFarland and Clarke, 1989). The most common sublethal effect of PCBs in all organisms is the increased activity of the hepatic microsomal mixed-function oxidase system. PCBs may also trigger the expression of an array of genes that are responsible for a variety of metabolic processes.

### TABLE 6.6

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pork</td>
<td>0.3</td>
<td>0.66</td>
<td>6.1</td>
<td>2.3</td>
</tr>
<tr>
<td>Beef</td>
<td>0.5</td>
<td>0.66</td>
<td>4.8</td>
<td>—</td>
</tr>
<tr>
<td>Chicken</td>
<td>0.8</td>
<td>0.88</td>
<td>17</td>
<td>—</td>
</tr>
<tr>
<td>Eggs</td>
<td>0.7</td>
<td>0.88</td>
<td>9.3</td>
<td>—</td>
</tr>
<tr>
<td>Cow milk</td>
<td>0.2</td>
<td>0.24</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Butter and cheese</td>
<td>0.1 to 3.5</td>
<td>1.3 to 2.9</td>
<td>130 to 160</td>
<td>32</td>
</tr>
</tbody>
</table>

of adverse effects including chloracne, thymic atrophy, liver damage, birth defects, immunotoxicity, and cancer (Smith and Gangolli, 2002). A correlation between increased concentrations of PCBs and some chlorinated pesticides (hexachlorobenzene [HCB], chlordanes) in the blood of mothers and an increasing incidence of testicular cancer of children has been reported. According to current hypothesis, testicular cancer is initiated during the fetal period, and exposure to endocrine disruptors, to which PCBs belong, has been of concern (Hardell et al., 2003). In addition, the ability to form DNA adducts by PCBs was demonstrated by the 32P-postlabeling method (McLean et al., 1996); DNA adducts with similar chromatographic properties were detected in the placentas of women environmentally exposed to organochlorines (Lagueux et al., 1999). Chemical DNA modification could thus be another mechanism underlying carcinogenic properties of this group of environmental pollutants.

Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) are formed as undesirable byproducts in industrial processes involving chlorine, during thermal or combustion processes connected with waste incineration, but also naturally, for example during forest fires. There are 75 congeners of PCDDs and 135 congeners of PCDFs (Formula 6). All these compounds are very persistent in the environment and can accumulate in the food chain, mainly in fat and fatty matrices (Travis and Nixon, 1996). Daily PCDD and PCDF intakes in the United States associated with the ingestion of different food items (Table 6.7) indicate that these compounds occur in particularly high concentration in fish oil. Rappe (1992) demonstrated the correlation between fish consumption and contents of PCDD/Fs in human blood (Table 6.8).

PCDD/Fs with the 2,3,7,8-chlorosubstitution are endocrine disrupting and carcinogenic agents increasing the incidence of total cancers including lung, breast, and hematologic cancers and soft-tissue sarcomas. In particular, a compound designated 2,3,7,8-T2CDD (TCDD) has been classified as a Group 1 carcinogen according to the IARC classification (IARC, 1997). TCDD is often called the most toxic manmade compound; it can cause a plethora of toxic effects. Dioxins are developmental and reproductive toxicants, and they cause immunotoxicity, dermal and hepatic toxicity, and different endocrine effects. They are inducers of stress responses altering cellular communication and proliferation (reviewed recently by Matsumura, 2003) as well as of aryl hydrocarbon hydroxylase (AHH), which is strongly implicated in yielding chemical intermediates that are carcinogenic to the human organism. Moreover, as animal studies suggest, prenatal exposure of rats to TCDD increases the sensitivity of the pups to dimethylbenzanthracene (DMBA)-induced mammary cancer, while neonatal exposure increases the number and incidence of methylnitrosourea (MNU)-initiated tumors (Birnbaum and Fenton, 2003).
Since dioxins and furans exhibit varying levels of toxicity, the toxic effects of combined PCDDs and PCDFs are usually expressed in TEQs (Toxic Equivalents). TEQs relate the toxicity of all dioxins and furans to the known toxicity of 2,3,7,8-T₄CDD using a weighting scheme adopted by the EPA and most European countries (USEPA, 1989).

### 6.3.3 Other Organic Pollutants

Simple phenols are probably not carcinogenic, but their methylated derivatives are both carcinogenic and mutagenic, whereas the majority of nitrophenols are mutagenic but not carcinogenic (Moore and Ramamoorthy, 1984). Chlorinated phenols have been shown to give rise to an increased incidence of lymphomas, leukemias, and hepatocellular carcinomas in mice and rats (Bull, 1985).

### TABLE 6.7

<table>
<thead>
<tr>
<th>Food Item</th>
<th>Content of PCDDs/Fs (pg TEQ/100 g)</th>
<th>Daily Intake (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetable, wet weight</td>
<td>2.0</td>
<td>219</td>
</tr>
<tr>
<td>Fruit, wet weight</td>
<td>1.5</td>
<td>136</td>
</tr>
<tr>
<td>Pork</td>
<td>27.7</td>
<td>8.3</td>
</tr>
<tr>
<td>Beef</td>
<td>260</td>
<td>14.6</td>
</tr>
<tr>
<td>Chicken</td>
<td>229</td>
<td>1.7</td>
</tr>
<tr>
<td>Eggs</td>
<td>150</td>
<td>3.0</td>
</tr>
<tr>
<td>Cow milk</td>
<td>211</td>
<td>6.5</td>
</tr>
<tr>
<td>Butter and cheese</td>
<td>221</td>
<td>10.5</td>
</tr>
<tr>
<td>Fish and shellfish</td>
<td>3353</td>
<td>0.34</td>
</tr>
</tbody>
</table>

*Source: Falandysz, J., Polychlorinated Biphenyls (PCBs) in the Environment: Chemistry, Analysis, Toxicity, and Risk Assessment, Fundacja Rozwoju Uniwersytetu Gdanskiego, Gdansk, Poland, 1999 (in Polish).*

### TABLE 6.8

<table>
<thead>
<tr>
<th>Compound</th>
<th>No Fish Consumption</th>
<th>Normal Fish Consumption</th>
<th>High Fish Consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,7,8-TCDD</td>
<td>1.8</td>
<td>2.5</td>
<td>8.0</td>
</tr>
<tr>
<td>TEQ</td>
<td>17.5</td>
<td>25.8</td>
<td>63.5</td>
</tr>
</tbody>
</table>

Polycyclic aromatic hydrocarbons (PAHs) were the first class of compounds whose carcinogenic potential was demonstrated in experimental animals. Benzo[a]pyrene, benzo[e]pyrene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, benzo[a]fluorene, chrysene, fluorene, perylene, and pyrene are classified as promoters or known or suspected carcinogens. The relative carcinogenicity of each compound is directly proportional to its half-life. The carcinogenicity of benzo[a]pyrene is best recognized. Its concentrations resulting from environmental pollution in uncooked seafood (main source) vary within the range of 0 to 20 μg/kg. However, PAHs are mainly formed in food upon thermal processing; this issue is discussed in other chapters of this volume. Drinking water accounts for less than 1% (about 0.01 μg/day) of the total benzo[a]pyrene food intake (1.01 μg/day) (Zoeteman, 1985).

6.4 ENVIRONMENTAL POLLUTANTS IN POTABLE WATER

The major anthropogenic pollutants in tap water are volatile organohalogen compounds. These compounds are commonly used as solvents, cleaning and degreasing agents, blowing agents, polymerization modifiers, and heat-exchange fluids. Once discharged with wastes, they find their way into lakes and rivers, and then into seas and oceans. Their concentrations in water and air are very variable and depend upon atmospheric conditions due to washing by rain and evaporation from water during long periods of warm weather. Apart from the production of organohalogen solvents, which amounts to several million tons annually, one of the most important sources of organohalogen compounds, particularly volatile ones, is water disinfection by chlorination (de Leer, 1987; Biziuk and Przyjazny, 1996). The actual disinfecting agent is hypochlorous acid formed in the course of a disproportionation reaction taking place when chlorine dissolves in water. During chlorination, harmless humic and fulvic precursor compounds naturally occurring in water are converted into organohalogens, which are dangerous to human life and health. The largest group of compounds formed during chlorination is that of trihalomethanes (THMs). THMs comprise chloroform, the most abundant compound, bromodichloromethane, dibromochloromethane and tribromomethane. Organobromine compounds are formed when the water being chlorinated contains substantial amounts of bromides or when the chlorine used for disinfecting is contaminated with bromine. Hypobromous acid formed in the reaction of bromide ions with hypochlorous acid reacts with an organic matrix about 200 times faster than hypochlorous acid does. The amount and kind of organohalogen compounds formed depend upon the water pH, the amount of chlorine used, and the content of organic matrix (Total Organic Carbon; TOC). Other volatile organochlorine products of water chlorination, such as tetrachloromethane, chloroethylene, 1,1-dichloroethylene, 1,1,2-trichloroethylene, tetrachloroethylene, 1,1,1-trichloroethane, and 1,2-dichloroethane, are also commonly found. Koch and Krasner (1989) estimated that among organohalogen compounds formed during chlorination of water, 77% are trihalomethanes, 15% are haloacetic acids, 3% are halonitriles, 4% is trichloroacetaldehyde hydrate, and 1% are the remaining com-
pounds (chlorinated acetone, chlorinated ethers and diethers, chlorinated acetonitrile, chloropicrine, chloral, chlorophenols, chlorinated ketones, etc.) (de Leere, 1987).

Several volatile organohalogen compounds fall into category of known or suspected carcinogens, e.g., chloroform, carbon tetrachloride, 1,1-dichloroethylene, 1,1,2-trichloroethylene, 1,1,2,2-tetrachloroethylene, dibromomethane, 2-bromoethylpropane, 1,1,2,2-tetrachloroethane, hexachlorobutadiene, and vinyl chloride (Moore and Ramamoorthy, 1984). Correlations between concentrations of THMs in drinking water and risks of bladder or colon/rectal cancer for humans were found (Cotrouvo, 1985). Chloroform causes hepatocellular carcinomas and liver and kidney tumors in mice and renal tumors in rats after chronic exposure (Bull, 1985). Carbon tetrachloride can induce preneoplastic changes in the liver of rats, liver tumors in male rats, and liver tumors in mice. Trichloroethylene also causes increases in the incidences of hepatocellular carcinomas, pulmonary carcinoma, and malignant lymphomas in mice. Tetrachloroethylene causes a high incidence of nephropathy in the mice and rats and hepatocellular carcinomas in mice (Condie, 1985). Haloacetonitriles have the ability to increase the incidence of lung tumors in mice, but this class of compounds possesses weak carcinogenic properties (Bull, 1985).

The concentration of toxic, mutagenic, and carcinogenic volatile organohalogen compounds in tap water formed upon chlorination is relatively high and reaches up to 100 \( \mu g/l \) and more. Therefore, the World Health Organization, European Union, and most countries have introduced a maximum admissible concentration (MAC) for some organohalogen compounds present in tap water. The MAC values vary from 0.3 \( \mu g/l \) for 1,1-dichloroethene (WHO, Norway) to 100 \( \mu g/l \) (EU, WHO, EPA, U.K.) for the sum of trihalomethanes (TTHM) and 350 \( \mu g/l \) for the sum of trihalomethanes (Canada). The typical concentration of trichloromethane (chloroform), the most frequently present compound in water treated by chlorination, is from 1 to 30 \( \mu g/l \). The latter value corresponds to the MAC in tap water according to WHO recommendations. Since many THMs are either known or suspected carcinogens, it has been suggested that their recommended concentration in water for maximum protection of human health should be null.

Semivolatile and volatile monocyclic aromatic compounds (benzene, 2,6-nitrotoluene, 2,4,6-trinitrotoluene, azobenzene, and dodecylobenzene) comprise another group of anthropogenic tap water pollutants. They were found to act as either promoters or suspected carcinogens (Moore and Ramamoorthy, 1984). From the BTEX group of contaminants (benzene, toluene, ethylbenzene, and xylene), only benzene is considered carcinogenic — Group I in the IARC classification. It is toxic to the hematopoietic system, causing hematological changes, including leukemia (Nollet, 2000).

6.5 CARCINOGENIC INORGANIC CONTAMINANTS OF FOOD

Although studies on chemical carcinogenesis concentrate mostly on organic compounds, the earliest definitive correlation between exposure and cancer was estab-
lished for inorganic chemicals — chromium, nickel, and arsenic in particular. Recent investigations have provided valuable insights into the molecular mechanisms of metal carcinogenesis as well as of other inorganic pollutants found in the human environment.

Asbestos is a major mineral suspected carcinogen. It occurs in nature in a fibrous form of two main types: chrysotile and amphibole. Asbestos is introduced to the human body mainly by two routes: to the lung by inhalation and to the gastrointestinal tract with water. It can be transferred to tap water from contaminated supplies and as a result of corrosion of pipe materials consisting of asbestos-cement compounds. Webber described episodes in Woodstock, New York, when 10,000 million fibers/L were detected in some samples of tap water and 0.12 to 0.04 fibers/mL were detected in household air (Webber et al., 1988). Asbestos fibers in water are usually shorter than 10 μm. Such small needles can cause cancer of the gastrointestinal and urinary systems when swallowed and lung and throat cancer upon inhalation. Moreover, asbestos can accumulate in lung tissue, and symptoms can develop many years after exposure. Since carcinogenic effects of this mineral result from inflammation caused by mechanical irritation of tissues exposed, fibers longer than 8 to 10 μm (length/diameter greater than 3:1) affecting larger areas seem more carcinogenic. Diseases caused by asbestos have been the subject of review (Falchi and Paolletti, 1994).

Heavy metals belong to one of the most dangerous groups of anthropogenic nonbiodegradable environmental pollutants. This is due to their toxicity, bioaccumulation, persistence in the environment, and biomagnification in the food chain. Metals can be transported with water and air and hence can enter the human organism through breathing as well as with water and food (Table 6.9). The highest concentrations of metals (Pb and Cd) were observed in vegetables cultivated in the vicinity of roads; concentrations decreased as the distance from the road increased. The concentrations of lead in vegetables grown in the vicinity of roads are too high for human consumption. Thus, traffic has proven to be an important source of environmental pollution directly affecting food.

Regarding cancer risk, metals represent a diverse class of carcinogens bringing about an array of biological perturbations associated with cancer development. At present, the mechanisms responsible for carcinogenicity of metals are far from clear. Some metal-related effects described below might be of biological significance.

Metals and their compounds are not mutagenic in bacterial systems and are weakly mutagenic in mammalian cell test systems. Nonetheless, they are able to interact with cellular biomolecules, including proteins and DNA (Hartwig, 1995). Genotoxic effects of metals, observed at cytotoxic doses, include induction of single- and double-strand DNA breaks, interstrand crosslinks, and DNA-protein crosslinks. Metal ions may alter interactions between DNA and proteins, thereby leading to chromatin damage such as sister chromatid exchange or chromosomal aberrations (Ohshima, 2003). In addition, at low, nontoxic doses, metals exert a strong co-genotoxic effect in the presence of various types of mutagens, including typical food carcinogens. Recent studies have provided evidence that DNA repair systems are important targets of metal action resulting in increased frequencies of endogenous and exogenous DNA damage, which in turn may increase the frequency of tumor formation (Hartwig, 1998; Schwerdtle et al., 2003). In addition, the redox properties
of metals make them potent generators of reactive oxygen species (Kasprzak, 2002; Pourahmad et al., 2003). The induction of reactive oxygen species elicits intracellular signal transduction and gene activation (Tully et al., 2000). However, this type of response independent of reactive oxygen is observed, which may in part explain the tumorigenic properties of metals (Chen and Shi, 2002; Barchowsky and O’Hara, 2003). Another potential mechanism of metal carcinogenicity was reported for trivalent inorganic arsenic. As(III) compounds were demonstrated to stimulate angiogenesis, resulting in enhanced tumor growth and metastasis in the mouse model (Soucy et al., 2003). Recapitulating, cellular and molecular mechanisms of metal carcinogenic action involve complex pleiotropic effects, all of which deserve great concern as they may potentially increase human cancer incidence.

The interest in carcinogenic metals has currently increased and numerous reviews addressing this issue in general or for selected metals have appeared in the literature recently; so here only a brief description of the most notorious metal pollutants will be provided. The estimated daily intakes of metals discussed below are given in Table 6.10.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Total Input to Fresh Water (thousands metric tons/year)</th>
<th>Main Food Sources</th>
<th>Concentration (mg/kg)</th>
<th>Dietary Intake (µg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>41</td>
<td>Seafood — organic</td>
<td>3 to 37</td>
<td>10 to 20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Seafood — inorganic</td>
<td>1.0 to 2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wine</td>
<td>0.02 to 0.11</td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>2.1 to 17</td>
<td>Meat, fish, fruits</td>
<td>0.005 to 0.010</td>
<td>30 to 40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peanuts, spinach</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pork kidney</td>
<td>0.18 to 1.0</td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>45 to 239</td>
<td>Nuts</td>
<td>0.14</td>
<td>42 to 78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Egg yolk</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cheeses</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td>33 to 194</td>
<td>Cacao products</td>
<td>Up to 9.8</td>
<td>100 to 800</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nuts</td>
<td>Up to 5.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other products</td>
<td>&lt;0.5</td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>97 to 180</td>
<td>Dairy products</td>
<td>0.003 to 0.083</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vegetables</td>
<td>0.005 to 0.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Meat, fish, poultry</td>
<td>0.002 to 0.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cereals, cheese</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

Carcinogenic and Anticarcinogenic Food Components

The main sources of arsenic are domestic wastewater, sewage sludge, manufacturing processes (metallurgic and chemical, pulp and paper industry), and smelting and refining as well as steam electrical production. Inorganic arsenic causes skin, liver, prostate, and lung cancers (Pershagen, 1981; Rojas et al., 1999) and is classified by IARC in Group I.

Beryllium is introduced to the human body mainly with food and drinking water, with a very small contribution from the air. The main source of beryllium is coal combustion. Beryllium and some of its compounds are carcinogenic to experimental animals and are suspected of being carcinogenic to the lung in humans (Moore, 1991).

Cadmium occupies a unique place among metals because of its low rate of excretion and thus its long half-life reaching approximately 20 to 30 years in humans, predominant storage in soft tissues, and a plethora of adverse effects (Waalkes and Misra, 1996). In the human environment, this metal originates from domestic wastewater, sewage sludge, manufacturing processes (metals, chemicals), smelting and refining, base metal mining and dressing, and steam electrical production. Heavy smoking causes a fourfold increase of cadmium levels in the blood and a threefold

<table>
<thead>
<tr>
<th>Food Product</th>
<th>Amount Consumed</th>
<th>As</th>
<th>Cd</th>
<th>Cr</th>
<th>Ni</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breakfast:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>200 g</td>
<td>—</td>
<td>2</td>
<td>—</td>
<td>2</td>
<td>0.7</td>
</tr>
<tr>
<td>Fruit</td>
<td>200 g</td>
<td>—</td>
<td>0.2</td>
<td>—</td>
<td>—</td>
<td>1.6</td>
</tr>
<tr>
<td>Cereals</td>
<td>100 g</td>
<td>—</td>
<td>—</td>
<td>5</td>
<td>—</td>
<td>3.2</td>
</tr>
<tr>
<td><strong>Lunch:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinach</td>
<td>100 g</td>
<td>—</td>
<td>6</td>
<td>2</td>
<td>10</td>
<td>3.1</td>
</tr>
<tr>
<td>Broccoli</td>
<td>50 g</td>
<td>—</td>
<td>0.5</td>
<td>1</td>
<td>10</td>
<td>1.6</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>50 g</td>
<td>—</td>
<td>0.7</td>
<td>1</td>
<td>—</td>
<td>1.6</td>
</tr>
<tr>
<td>Egg</td>
<td>80 g</td>
<td>—</td>
<td>0.8</td>
<td>—</td>
<td>16</td>
<td>1.6</td>
</tr>
<tr>
<td>Fish</td>
<td>100 g</td>
<td>100</td>
<td>1.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Water</td>
<td>300 ml</td>
<td>3</td>
<td>0.03</td>
<td>—</td>
<td>—</td>
<td>1.4</td>
</tr>
<tr>
<td><strong>Dinner:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat</td>
<td>200 g</td>
<td>—</td>
<td>2</td>
<td>8</td>
<td>—</td>
<td>6.3</td>
</tr>
<tr>
<td>Carrots</td>
<td>50 g</td>
<td>—</td>
<td>0.9</td>
<td>1</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>50 g</td>
<td>—</td>
<td>0.5</td>
<td>1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Rice</td>
<td>80 g</td>
<td>—</td>
<td>0.4</td>
<td>4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Potato</td>
<td>100 g</td>
<td>—</td>
<td>2</td>
<td>—</td>
<td>2</td>
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<td>Chocolate mousse</td>
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<td><strong>Total</strong></td>
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increase in the kidneys of smokers compared to nonsmokers (Pocock et al., 1988). The IARC classified Cd(II) and its compounds in Group 1 in 1993. Since then, a number of epidemiological studies were carried out in exposed workers that confirmed the carcinogenic potential of this metal in the case of human lung cancer but not prostate cancer. The cancer risk increased in the presence of arsenic and nickel (Verougstraete et al., 2003).

The main sources of chromium are domestic wastewater, sewage sludge, manufacturing processes (metals, chemicals, pulp and paper, petroleum products), smelting and refining, and base metal mining and dressing. Chromium in biological systems can occur in two stable oxidation states: Cr(III) and Cr(VI). Cr(III) compounds are nontoxic. In fact, they play a crucial role in sugar and fat metabolism and therefore are considered essential trace element nutrients in humans. In contrast, Cr(VI) compounds are extremely toxic; they are also genotoxic and induce DNA crosslinks and breaks. Inside cells, Cr(VI) undergoes rapid reduction to two chemically unstable intermediates, Cr(V) and Cr(IV), until it is finally reduced to Cr(III). The oxidative stress induced upon this stepwise reduction is proposed to be responsible for tissue damage and chromium toxicity as well as carcinogenicity (Cohen et al., 1993).

Lead was one of the first metals used in human history. Its compounds are liberated to the environment from different manufacturing processes (cement, metals, chemicals, pulp and paper, petroleum products). The most common source of this metal is combustion of leaded gasoline. Lead can cause several dysfunctions, among them anemia, insomnia, hypertension, renal dysfunction, sperm count suppression, and damage to the peripheral nervous system. The IARC has classified Pb and its compounds in Group 2B (Moore, 1991).

Nickel, like other metals discussed above, is released into the environment as an exhaust from different industries. In humans, the highest concentrations of nickel are found in the liver, kidney, and brain. It can cause dermatoses, including contact dermatitis, atopic dermatitis, and allergic sensitization (Moore, 1991). In model systems, a variety of genotoxic effects, exerted either directly by nickel compounds or via induction of reactive oxygen species, have been reported. Nickel is carcinogenic in humans; among nickel refinery workers, a statistically significant elevation in the incidence of respiratory cancers has been found (Coogan et al., 1989), but there are no evidence of carcinogenicity of nickel taken in either with food or water.

As a class of carcinogens, metals are unique, widespread environmental pollutants. They can neither be created nor destroyed by humans; hence, they are only transported and transformed into various products. Therefore, a decrease of human exposure to carcinogenic metals cannot be achieved by banning their usage; instead, it requires constant monitoring and specific measures.

6.6 SUMMARY AND CONCLUSIONS

The environmental pollutants discussed above are omnipresent in human surroundings; exposure to such chemicals occurs predominantly through the food chain. Many of these compounds display mutagenic and carcinogenic properties in experimental models; some have been classified as human carcinogens. It has
long been believed that the amounts of environmental pollutants occurring in human diet are too small to pose a significant carcinogenic hazard. Evidence suggesting that former assessments underestimated actual risk is growing, however. On one hand, current methods of sample preparation and analysis enable accurate measurements of pollutants in the human environment and human body. It has also been recognized that past contacts with environmental pollutants, i.e., pre- or neonatal, might sometimes sensitize the human organism to future exposures to carcinogenic factors. On the other hand, development of biomarkers and better understanding of molecular mechanisms leading to neoplastic transformation help to relate cancer incidence to chemical exposure. For example, Hardell and Ericsson (2003) suggest that the current decline of increasing incidence of non-Hodgkin’s lymphoma in Sweden (from about 3.15% during 1971 to 1990 to 0.5% on average during 1991 to 2000) and other countries may be a result of decreased exposure to organochlorine compounds. This exposure was the highest in the 1960s and 1970s but then decreased substantially owing to regulations imposed in the late 1970s prohibiting use of some of these chemicals. The change in the incidence of non-Hodgkin’s lymphoma serves as a good example of how limitation of exposure may be reflected in cancer statistics some decades later. Some reports also show that consumption of organic fruits, vegetables, and juices may shift pesticide exposure of young children from a range of uncertain risk to a range of negligible risk (Curl et al., 2003).

The ubiquitous presence of environmental pollutants in food raises still another question. It has been convincingly documented in experimental models that edible plants contain numerous cancer chemopreventive substances. It has been thus somewhat surprising that recent epidemiological studies carried out in the United States and Europe failed to demonstrate clearly the benefits of diet rich in fruits and vegetables regarding cancer incidence (Hertog et al., 1994; van den Berg et al., 2001; Moller et al., 2003). However, the contents of environmental pollutants in foods consumed by subjects recruited for these studies were not taken into account. Could it happen that the toxic residue compounds outweighed or at least leveled off the benefits associated with chemoprotective substances produced by plants? What levels of, for example, organochlorine compounds in plantborne foods may be allowed to keep the proper risk/benefit balance? These problems hopefully will be resolved in the near future.

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Carcinogenic and Anticarcinogenic Food Components


Carcinogenic and Anticarcinogenic Food Components


7 Dietary Anti- and Prooxidants: Their Impact on Oxidative DNA Damage and Cancer Risk

Ryszard Olinski, Daniel Gackowski, Marcus S. Cooke, and Joseph Lunec

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7.1 INTRODUCTION

It is widely believed that a link exists between diet and cancer incidence. Although a plethora of epidemiological studies have focused upon a possible protective effect of diet rich in fruits and vegetables, data also exist pointing to a diet rich in fat derived from animal products appearing to promote the development of certain
cancers (Halliwell and Gutteridge, 1999). The mode of action of dietary micronutrients is complex and not fully understood. However, one possible mechanism by which a protective effect is exerted is via the antioxidative activities of such plant food constituents as vitamins A, C, and E or phenolic compounds. These antioxidants are effective free radical scavengers and should protect biomolecules, such as proteins, lipids, and nucleic acids, from oxidative damage. On the other hand, the procarcinogenic effect of some food components may be responsible for an increased formation of oxidative DNA damage.

In view of the importance of DNA damage in carcinogenesis, it is conceivable that any agent capable of reacting with DNA and chemically modifying it could be carcinogenic. It is likely that reactive oxygen species (ROS) belong to this group of agents. ROS are the products of partial reduction of oxygen (Scheme 1). These species, which include superoxide anion, hydrogen peroxide, and hydroxyl radical, are continuously produced in living cells as byproducts of normal metabolism. They can also be generated by exogenous factors, such as ionizing radiation. Diets rich in fat may be a source of lipid hydroperoxides that may undergo metal-dependent decomposition to unsaturated aldehyde genotoxins that in turn may react with DNA bases to form ethenoaducts. It has been shown that free radical attack upon DNA generates a whole series of DNA modifications, among them modified bases (Scheme 2). Hydroxyl radical (·OH) attack of DNA leads to a large number of pyrimidine- and purine-derived damaged bases (Dizdaroglu, 1998). Some of these modified DNA bases have considerable potential to alter the integrity of the genome (Floyd, 1990).

### 7.2 Mutagenic and Carcinogenic Properties of DNA Base Damage

8-Oxo-7,8-dihydroguanine (8-oxoGua) appears to be one of the most prevalent lesions. The presence of 8-oxoGua residues in DNA leads to GC→TA transversions,
unless repaired prior to DNA replication. Therefore, the presence of 8-oxoGua may lead to mutagenesis. Furthermore, many observations indicate a direct correlation between 8-oxoGua formation and carcinogenesis in vivo. ROS-induced mutation has been reported at hotspot codons of the human tumor suppressor gene \( p53 \) and \( Ha-Ras \) oncogenes, specifically. In agreement with this finding, GC→TA transversions have been frequently detected in the \( p53 \) gene and in the Ras proto-oncogene in cases of lung carcinomas and primary liver cancer. Likewise, Olinski et al. (2003) have demonstrated elevated levels of free radical–induced DNA base modification, including 8-oxoGua, in human cancerous lung tissues when compared with cancer-free surrounding tissues.

The mutagenic and carcinogenic potential of any modified DNA base is reflected in its miscoding properties. Recently, it has been demonstrated that several modified bases have miscoding potential. The presence of 2-hydroxyadenine (2-OH-Ade) in DNA may induce A→C and A→T transversions and A→G transition; 2-hydroxy-deoxyadenosine-triphosphate (2-OH-dATP) is a substrate for misincorporation by DNA polymerase; 8-oxoadenine (8-oxoAde) has miscoding properties

**Scheme 2** Modified DNA bases.
and induces mutation in mammalian cells; and 5-hydroxycytosine (5-OH-Cyt) is potentially mutagenic, leading to GC→AT transition and GC→CG transversion. In addition, 5-OH-Cyt appears to be more mutagenic than is any other product of oxidative DNA damage. It is also possible that derivatives of guanine other than 8-oxoGua have miscoding properties. In contrast, biological consequences of other base modifications, such as 4,6-diamino-5-formamido-pyrimidine (fapyadenine), 5,6-dihydroxyuracil (5,6-di-OH-Ura), and 5-hydroxy-5-methylhydantoin (5-OH-5-MeHyd), have received limited attention (reviewed in Olinski et al., 2003). It is conceivable that these lesions may also be mutagenic. Therefore, the background oxidative damage to DNA may be critical to the appreciation of the importance of the oxidative stress in the development of cancer (reviewed and discussed in Wang et al., 1998).

### 7.3 ANALYSES OF 8-OXOGUA IN HUMAN SAMPLES

The background level of 8-oxoGua in cellular DNA represents a dynamic equilibrium between the rates of oxidative DNA damage and repair in that specific tissue/cells. That repair enzymes that specifically recognize and remove 8-oxoGua have evolved is a clear indication of the biological importance of this lesion, but exactly how much 8-oxoGua is present at the background level is the subject of controversy. The European Standards Committee on Oxidative DNA Damage (ESCODD) was set up to resolve the problems associated with the measurement of background levels of oxidative DNA damage, in particular 8-oxoGua, in human cells. As a result of this endeavor, the analysis of this lesion has become more precise and accurate (ESCODD, 2000; ESCODD, 2002).

An alternative approach to the assessment of oxidative DNA damage *in vivo* is the measurement of urinary levels of 8-oxoGua and 8-oxodG (8-oxo-7,8-dihydro-2-deoxyguanosine). It is generally accepted that the products of repair of 8-oxoGua in cellular DNA are excreted into the urine without further metabolism. Some data suggest that 8-oxodG may be derived from nucleotide excision repair (NER). However, it seems that oxidatively damaged DNA bases are mainly repaired by the base excision repair (BER) pathway, although the NER pathway may also play a role in the repair of some oxidized DNA bases (reviewed and discussed in Cooke et al., 2002a). Therefore, simultaneous determination of the level of 8-oxodG as well as 8-oxoGua in urine may better reflect oxidative damage of cellular DNA.

The analysis of 8-oxoGua in urine presents particular difficulties, and until recently there has been no reliable assay for its detection. New techniques employ mass spectrometry (MS) for simultaneous determination of 8-oxodG and 8-oxoGua in the same urine sample (Ravanat et al., 1999; Gackowski et al., 2001b; Weimann et al., 2002). After sample purification by high-performance liquid chromatography (HPLC) and analysis by gas chromatography with stable isotope dilution, Gackowski et al. (2001b) have found that urinary excretion of 8-oxoGua and 8-oxodG by humans does not depend on diet but may reflect involvement of different repair mechanisms (BER and NER respectively) and sanitization of the
deoxynucleotide pool. It cannot be entirely excluded that processes other than repair contribute to 8-oxoGua and 8-oxodG levels in human urine, e.g., a portion of 8-oxodG may be derived from dead cells (Lindahl, 1993). On the other hand, a fraction of the excreted 8-oxoGua may include a contribution from oxidized RNA. However, this possibility is less likely for the following reasons:

1. To date, no enzymatic activity has been identified that can recognize and liberate 8-oxoGua from RNA.
2. It is also unlikely that 8-oxoGua is liberated from RNA via spontaneous hydrolysis of the N-glycosylic bond of the nucleoside (depurination), since it was demonstrated that the bond is much more stable than in the unmodified nucleoside (Bialkowski et al., 1996).

### 7.4 ENDogenous FACTORS THAT INFLUENCE THE BACKGROUND LEVEL OF 8-OXOGUA IN CELLULAR DNA AND THEIR RELEVANCE TO CARCINOGENESIS

#### 7.4.1 Antioxidant Vitamins and 8-Oxogua Level in Cellular DNA

Many epidemiological studies have reported an inverse association between vegetable and fruit consumption and occurrence of cancer and other degenerative diseases. One possible explanation of this protective effect concerns the antioxidant activities of such plant food constituents as vitamins A, C, and E. These antioxidant vitamins are effective free radical scavengers and therefore should protect biomolecules such as proteins, lipids, and nucleic acids from oxidative damage, hence decreasing the quantity of potentially mutagenic oxidatively modified DNA bases. Duthie et al. (1996), using single cell gel electrophoresis (comet assay), found that supplementation of healthy volunteers with vitamin C (100 mg/day), vitamin E (280 mg/day), and β-carotene (25 mg/day) significantly reduced base damage in lymphocyte DNA. Collins et al. (1998) demonstrated a significant negative correlation between basal concentration of serum carotenoids and oxidatively modified pyrimidines, although supplementation with carotenoids did not influence oxidative DNA damage. The authors did not find any correlation between levels of damage and serum concentrations of vitamins E and C. Another study investigating oxidative DNA damage also demonstrated no effect of supplementation with carotenoids or vitamin C or E (for detailed discussion of this subject see Moller and Loft, 2002). Therefore, at present, it is difficult to reach a firm conclusion whether supplementation with antioxidant vitamins protects against oxidative DNA damage. It is possible that a preventive effect of the vitamins may be only seen when basal levels are very low, for example, due to severe oxidative stress. Indeed, Jaruga et al. (2002) demonstrated that vitamin supplementation of HIV-infected patients, who had very low levels of antioxidant vitamins and significantly increased amounts of 8-oxoGua and other base modifications in lymphocytes, restored the vitamin levels to those characteristic for the control.
subjects. Concomitantly, the authors noted a significant decrease in the levels of the modified bases, compared to patients who received placebo.

In another study, Gackowski et al. (2002a) have found that plasma levels of antioxidant vitamins of colon cancer patients were significantly lower than those of the control group (Figure 7.1), which might be prompted by the differences in dietary habits between these groups. However, it is noteworthy that the human subjects were chosen randomly and were matched according to an interview, for both dietary habits and living conditions. Therefore, it is likely that severe oxidative stress, characteristic of colon cancer, is responsible for the reduced levels of the antioxidant vitamins. The decreased plasma concentration of uric acid (another low-molecular-weight antioxidant) in colon cancer patients also supports this assumption. This prooxidative environment resulted in a significantly increased \( p = 0.0034 \) level of 8-oxodG in lymphocyte DNA of cancer patients, compared to the control group (Gackowski et al., 2002a). These findings suggest that oxidative stress is present not only in tumors but also in other tissues of the cancer patients (for further discussion of this issue see Section 7.5.2). Taken together, these results suggest that patients with advanced stages of cancer may benefit from treatment with antioxidants, which may slow the progression of the disease. However, at present it is difficult to reach a firm conclusion whether supplementation with antioxidant vitamins protects against oxidative damage to DNA and other biomolecules. Special precautions should be taken with
cancer patients undergoing radiotherapy and/or chemotherapy, since these treatments influence oxidative processes and can affect redox balance in vivo. There are some data that suggest that the use of antioxidants during therapy can shorten survival time compared with matched controls (Seifried et al., 2003).

7.4.2 Vitamin C and DNA Repair

Vitamin C (ascorbate) is of particular interest given its longstanding relationship with oxidatively damaged DNA. Conventionally, ascorbate has been regarded solely as a highly effective scavenger of free radical species, given its low one-electron reduction potential of 282 mV. In addition to being an antioxidant, vitamin C is also a reducing agent, capable of reducing ferric ions to ferrous ions and hence promoting the Fenton reaction (Scheme 3), which may have important biological consequences since it results in the highly reactive hydroxyl radical. In this capacity as a reducing agent, vitamin C is easily oxidized and can decompose to form hydrogen peroxide, which again in combination with ferrous ions may give rise to the prooxidant and genotoxic effects of vitamin C. Recently, it has been suggested that vitamin C may even be toxic to cells in culture, independently of iron, although these properties appear to be mainly associated with in vitro experimentation (Halliwell, 1996).

One route by which vitamin C may exert its genotoxic effect is via oxidation and decomposition to form glyoxal, a reactive aldehyde (Scheme 3). Once inside the cell, dehydroascorbic acid (DHA), the reduced form of ascorbate, is converted back to ascorbate at the expense of glutathione. Further oxidation of DHA, or its reduction, may cause oxidative damage to lipids. In either case, glyoxal may result from these reactions.

Although there is evidence to suggest that dietary vitamin C insufficiency may give rise to increased levels of oxidative DNA damage in vivo, there have been few reports supporting this hypothesis. Indeed, Duthie et al. (1996), despite showing a reduction in oxidative DNA damage upon supplementation with a mixture of anti-
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oxidants, suggested that vitamin C was not responsible for this effect. This finding was consistent with that reported by Daube et al. (1997), who showed no association between plasma vitamin C concentration and placental 8-oxodG levels. In contrast, Podmore et al. (1998) demonstrated that dietary supplementation with vitamin C reduces 8-oxoGua levels in the DNA of circulating peripheral blood mononuclear cells (PBMC), albeit with a simultaneous increase in 8-oxoAde (illustrated in Figure 7.2). The potential for vitamin C to decrease levels of 8-oxodG is supported by Fraga et al. (1991). It has been suggested that this reduction in oxidative damage to

FIGURE 7.2 Changes in plasma vitamin C, peripheral blood mononuclear (PBMC), and serum and urine levels of 8-oxo-2'-deoxyguanosine (8-oxodG; ○, □, ×, respectively), in healthy human subjects (n = 30) supplemented with 500 mg/day vitamin C. PBMC levels of 8-oxo-adenosine (8-oxoAde; ●) are also shown. Asterisks indicate a significant (* P < 0.05 and ** P < 0.001) difference from baseline and placebo measurements. Figure based upon data reported by Cooke, M.S., Evans, M.D., Podmore, I.D., Herbert, K.E., Mistry, N., Mistry, P., Hickenbotham, P.T., Hussieni, A., Griffiths, H.R., and Lunec, J., FEBS Lett., 439, 363, 1998.
DNA may partially explain the anticarcinogenic properties of vitamin C, although this in itself may be overstated (Cooke et al., 2002b). More recent publications support the proposal that the ability of vitamin C to reduce the levels of oxidative DNA damage is due not merely to the free radical scavenging effect of an antioxidant, but to up-regulation of DNA repair.

A study by Cooke et al. (1998) examined the effect of dietary supplementation with vitamin C on PBMC DNA levels of 8-oxoGua, measured either as the base or as the deoxynucleoside, along with serum and urinary levels of 8-oxodG. This study was the first report examining the effect of vitamin C supplementation upon both serum and urinary levels of 8-oxodG in healthy individuals. Previously, Loft et al. (1992) reported that the intake of vitamin C does not influence urinary 8-oxodG excretion. It was the examination of both urine and serum that led to the surprising results noted. After vitamin C supplementation for 6 weeks, urinary 8-oxodG levels increased (Figure 7.2), becoming significantly raised during the washout period (7 weeks after cessation of supplementation). Similarly, serum levels of 8-oxodG increased significantly upon supplementation (Figure 7.2). These changes were concomitant with a decrease in 8-oxodG in the DNA of PBMC (Figure 7.2), implying a link between the decrease in PBMC levels of 8-oxodG and the increase in serum and urinary 8-oxodG. This finding is without precedent, although a report by Priemé et al. (1997), involving supplementation with 250 mg vitamin C (twice daily for 2 months), showed a modest increase in 8-oxodG excretion following supplementation. Similarly, Cooke et al. (1998) noted a modest increase following a vitamin C regimen of 500 mg/day for 6 weeks, and further sampling revealed a significant increase (30.0%) in urinary 8-oxodG 7 weeks after cessation of supplementation.

The overall conclusion of Cooke et al. (1998) was that, rather than acting as an antioxidant by scavenging free radicals, and hence preventing the formation of 8-oxoGua, vitamin C appeared to promote the removal of 8-oxodG from DNA and/or the nucleotide pool, via up-regulation of repair enzymes, as postulated by Rehman et al. (1998). Furthermore, it was suggested that these enzymes may have been induced in response to a prooxidant effect of vitamin C, noted in a previous report (Podmore et al., 1998). Indeed, there is a precedent for the redox regulation of DNA repair enzymes (Lee et al., 1998). Furthermore, the increase in 8-oxoAde (Podmore et al., 1998) may be explained if a repair activity toward this lesion had not been stimulated.

Most recently, findings from the same group have supported this hypothesis and provided further information concerning the action of vitamin C and DNA repair. The authors measured a recently described lesion, deoxycytidine glyoxal (gdC), which might be derived from lipid peroxidation, glucose autoxidation, free radical attack on deoxyribose, or autoxidation of vitamin C, as well as mechanisms involving hydroxyl radical or iron-oxo (ferryl) species attack on DNA deoxyribose. Cooke et al. (2003) reported an initial, significant increase in gdC (Figure 7.3A) and, given similar increases in fapyguanine and fapyadenine (Rehman et al., 1998), 8-oxoAde (Podmore et al., 1998), and 8-oxodG (Cooke et al., 2002b), this is consistent with vitamin C acting as a prooxidant in vivo. Despite this initial increase in gdC, the overall trend was for a significant (p = 0.001) decrease in gdC levels (Figure 7.3A).
FIGURE 7.3 Changes in plasma vitamin C concentration (■) and (A) peripheral blood mononuclear cells (PBMC) levels of deoxycytidine glyoxal (gdC, ●); and (B) PBMC levels of thymine dimer (T<>T, ●), in healthy human subjects (n = 40) supplemented with 400 mg/day vitamin C. Asterisks indicate a significant (*** P < 0.001) difference from baseline and placebo measurements. Figure based upon data reported by Cooke, M.S., Mistry, N., Ahmad, J., Waller, H., Langford, L., Bevan, R.J., Evans, M.D., Jones, G.D., Herbert, K.E., Griffiths, H.R., and Lunec, J., Free Radic. Biol. Med., 34, 218, 2003.
In contrast to earlier studies, Cooke et al. (2003) also measured a nonoxidative DNA lesion, cyclobutane thymine dimer (T<>T), derived from ultraviolet radiation exposure and measurable immunochemically at basal levels in PBMC. Were it not for the measurement of this lesion, the decrease in gdC may have been attributed solely to the induction of antioxidant defenses and/or free radical scavenging. The reduction in T<>T levels (Figure 7.3B), coincident with the decreases in gdC, can be explained by the induction of DNA repair, and NER specifically. Similarly, while a pathway for the repair of gdC has not been defined, the decrease in gdC levels, arising from the induction of NER, seems entirely plausible. The overall conclusion was that vitamin C may modulate DNA repair, and NER, specifically, prompted by a small prooxidative effect, akin to an adaptive or stress response (Cooke et al., 2003).

The hypothesis that cells exposed to a low-level insult respond more favorably when faced with a subsequent, more significant challenge is typified by the so-called stress response. This phenomenon is well-established for physical stressors such as ultraviolet light, hyperthermia, and anoxia, and it has been suggested that damage to proteins triggers the induction of stress proteins. Enhanced excision repair activity has been noted in mammalian cells after exposure to ionizing radiation. Although not identified, the induced enzyme(s) had a pronounced activity towards an 8-oxodG-containing duplex oligomer. There now are growing numbers of reports that describe the up-regulation of repair activity in mammalian cells through an adaptive response to cellular insult.

The molecular mechanism responsible for the findings from the biomarker studies in humans described above has begun to be elucidated in vitro. Lunec et al. (2002) have shown that vitamin C can modulate the DNA binding of the transcription factors activated protein 1 (AP-1) and nuclear factor kappa binding (NF-κB). Once AP-1 is activated, this dimer of c-Jun and/or c-Fos gene products mediates the expression of a class of proto-oncogenes, known as immediate-early response genes, which includes c-Fos and c-Jun themselves. It can be hypothesised that such a mechanism is a means by which the adaptive (repair) response may be induced; indeed, AP-1 activation is associated with increases in the expression of excision repair cross complementing genes (ERCC1), a potential marker of NER capacity. Utilizing NER-deficient (Xeroderma Pigmentosum complementation group A) and proficient cell lines, Lunec et al. (2002) provided further support for NER being induced by vitamin C.

Using microarray analysis, Duarte et al. (2002) showed that vitamin C exposure of human fibroblasts caused the up-regulation of a whole host of genes categorized into “early,” “intermediate,” and “late” response genes. Following 2 hours of exposure to doses of ascorbate simulating in vivo exposure, a transient induction of many early stress response genes including haemoxygenase-1, MAP kinase phosphatase 1, interleukin 8, cyclooxygenase-2, growth arrest and DNA damage (GADD) 45B, and genes associated with cell cycle progress in G1/S was noted. An intermediate response was observed at 8 hours after exposure and involved a cluster of 13 genes implicated in lipid metabolism, such as down-regulation of the low-density-lipoprotein receptor. A late response, after 24 hours of exposure, included induction of a set of cell cycle control genes. Temporary gene expression changes to oxidative stress were also found in cells transiently exposed to low levels of oxidants, such
as hydrogen peroxide, which are akin to the genes induced by vitamin C. More recently, Catani et al. (2002) showed that expression of the mismatch repair gene mutL homologue 1 and its downstream target p73, can be modulated by ascorbate in human keratinocytes. The data supported the concept that the anticarcinogenic activities of ascorbate may relate to the induction of DNA repair genes. Previously, the same authors had shown that ascorbate modulates ultraviolet B (UVB)-induced AP-1 activity by preventing the phosphorylation of c-Jun protein. Collectively, these data suggest that vitamin C protects against genotoxic cell damage and cell death by modulating the expression of AP-1 regulated genes.

Cursory examination of the literature suggests that vitamin C is not alone in its proposed ability to modulate DNA repair; other dietary components may also have such an effect. Caloric restriction and specific dietary factors such as selenium, ethanol, and coenzyme Q10, as well as intake of Fushimi sweet pepper, kiwi fruit, tomato, and carrot juices, have been associated with increases in DNA repair activity. Clearly, many of these dietary components may be acting in the absence of an antioxidant effect, and these findings, coupled with the discovery that vitamin C may up-regulate DNA repair, have placed the reported anticarcinogenic properties of antioxidants in an entirely new light.

7.4.3 Iron and 8-Oxogua Accumulation

As is described above, several studies have demonstrated that antioxidant vitamins may decrease the amount of 8-oxodG in cellular DNA. One important element in the establishment of prooxidative status of the cell, and by implication in the elevation of the background level of 8-oxodG in cellular DNA, is iron. Iron has the capacity to accept and donate electrons easily, changing between ferric (Fe[III]) and ferrous (Fe[II]) iron. Due to this feature it is a critical component of cytochromes and oxygen-binding molecules such as hemoglobin and myoglobin. However, within the cell, iron can exist in another form, as “free” or “labile” iron (LIP, iron not bound to proteins). LIP-associated iron is in dynamic equilibrium with other, sequestered iron forms in the cell and is bound to cytosolic low-molecular-weight ligands that have not yet been identified. This form of iron is catalytically active and participates in the reaction involved in the production of harmful ROS (the Fenton reaction) (Halliwell and Gutteridge, 1999). However, proteins sequester iron to reduce this threat. Iron ions circulate bound to plasma transferrin, whereas ferritin serves to accumulate it. Although iron is an essential nutrient, it may be harmful in many circumstances. Even in individuals with normal iron stores, this element upon release from protein complexes may participate in reactions generating oxidatively modified DNA bases.

Gackowski et al. (2001a; 2002b) analyzed a broad spectrum of components that affect iron metabolism, as well as their possible association with the endogenous level of 8-oxodG. No correlation was found between plasma concentrations of ferritin or transferrin saturation and the amount of 8-oxodG in lymphocyte DNA (Gackowski et al. 2002b). In contrast, a positive correlation was established between LIP and the oxidatively modified nucleoside \( r = 0.57008, p = 0.000066, n = 43 \). This suggests that under physiological conditions LIP is available for catalyzing Fenton-type reactions, in close proximity to cellular DNA. However, the exact
chemical nature of the complex between iron and DNA is not known, nor is it established how iron reaches the nucleus.

Experimental data demonstrate the existence of a free iron pool in sera of patients with hemochromatosis (de Valk et al., 2000), a disease that predisposes to cancer. Epidemiological data also indicate that dietary iron intake and elevation of the body iron level may increase the risk of cancer, especially of the colon (Nelson et al., 1994). The Western diet has a high intake of red meat, a rich source of bioavailable iron, and is low in fiber, which contains the iron-chelating compound phytate, and hence provides perfect substrates for the Fenton reaction. Gackowski et al. (2002a) suggest a mechanism that may directly link iron overload and carcinogenesis. Specifically, iron overload may favor the persistence of harmful LIP, which may catalyze generation of the potentially carcinogenic 8-oxodG moiety in cellular DNA. It is also noteworthy that some antioxidants (vitamin C) can exert prooxidant effects via interaction with iron (Halliwell and Gutteridge, 1999). The physiological importance of this effect would depend on the availability of “catalytic iron” (see above).

7.5 ACCUMULATION OF 8-OXOGUA IN CANCER PATIENTS

7.5.1 CANCER TISSUES

We and others have demonstrated that elevated levels of typical free radical–induced DNA base modifications, including 8-oxoGua, exist in human cancerous tissues when compared with the cancer-free surrounding tissue (reviewed in Olinski et al., 2003). Several of these DNA lesions possess mutagenic properties. It is not known whether these elevated levels of DNA lesions play a causative role in carcinogenesis, or are merely the result of the disease. However, treatment of laboratory animals with carcinogenic agents (nickel [II]) causes a similar pattern of oxidative base modification in their target organs before tumor formation occurs. These data suggest an important role for oxidative DNA base damage in carcinogenesis (reviewed in Olinski et al., 2003).

Investigations of benign tumors by Foksinski et al. (2000) showed that oxidative DNA damage might be a causative factor in cancer progression. A higher endogenous level of 8-oxoGua in uterine myoma tissues was observed when compared to tumor-free tissues. Uterine myomas are common gynecological benign tumors derived from a single mutated myometrial cell. One of the factors that may predispose to malignant transformation is the greater size of the tumor. The positive correlation found in our work between the size of the tumor and the amount of 8-oxoGua per mg DNA suggests that a higher level of 8-oxoGua, and possibly other base lesions, in benign tumors may be a risk factor that may determine the transformation of tumors from benign to malignant. The increased levels of modified DNA bases may also contribute to the genetic instability and metastatic potential of tumor cells in fully developed cancer (reviewed in Olinski et al., 2003).

It has been estimated that most human cancers contain a large number of mutations. At least 11,000 individual DNA mutations exist in a single carcinoma cell of colorectal tumors (Ionov et al., 1993). It is possible that a proportion of these
mutations may arise during the development of the disease and may contribute to the metastatic potential of tumor cells. Our results suggest that one potential source of this unusually large number of mutations may be via damage to DNA by ROS. This explanation has also been suggested by Malins et al. (1993), who showed that the potential of breast cancer to metastasize increased together with increases in the level of oxidatively modified DNA bases.

7.5.2 Urinary Excretion of 8-OxoGua and 8-OxodG in Cancer Patients

Since the level of the modified nucleosides/bases in urine may be an indicator of oxidative insult on DNA and a general marker of oxidative stress, Rozalski et al. (2002) investigated whether the amounts of 8-oxoGua and 8-oxodG excreted into the urine of cancer patients differed from that of a control group. The amount of the modified base, but not that of the nucleoside, excreted into urine was approximately 50% higher in cancer patients compared to the control group.

The level of the DNA lesions in urine may depend on the type of oxidative DNA insult and reflect different repair mechanism(s). Although it is feasible that DNA repair capacity may differ between cancer patients and healthy subjects, the higher level of 8-oxoGua in the urine of cancer patients may be explained, at least partially, by the reported oxidative stress in cancer tissue (Olinski et al., 2003). However, the amount of the modified base/nucleoside excreted into urine should represent the average rate of DNA damage/repair in whole body. Therefore, it is doubtful that the elevated level of the base product in cancerous cells alone could account for the observed 50% increase of 8-oxoGua in urine. Our results suggest that oxidative stress, represented by the increased amount of the relevant compound in urine, may be derived not only from the tumor tissue but also from other tissues (or processes in cancer patients). The precise mechanism(s) of the oxidative stress is still unknown. However, some factors may be suggested:

1. Cancer patients showed signs of extensive granulocyte activation with a release of ROS followed by a dramatic increase of 8-isoprostane, one of the biomarkers of oxidative stress.
2. Some malignant cells produce hydrogen peroxide at levels as high as those characteristic for polymorphonuclear leukocytes engaged in phagocytosis; hence, the observed oxidative stress in advanced stages of cancer may be associated with a release of the large number of cancer cells into the bloodstream and their penetration into other tissues.
3. Exposure of target human cells to the activated leukocytes causes oxidative DNA base modifications, among them 8-oxoGua.
4. Some tumors may stimulate the defense systems of the body to produce antitumor cytokines, some of which may be responsible for ROS production. It has been shown that elevated plasma level of TNF-α (tumor necrosis factor alpha) is responsible for the increased oxidative DNA damage in cellular DNA (reviewed in Olinski et al., 2003).
It is also possible that the prooxidant environment is characteristic for advanced stages of cancer and that oxidative stress is a result, rather than a cause, of the disease progression.

### 7.6 CALORIC-RESTRICTED DIET AND OXIDATIVE DNA DAMAGE

Epidemiological evidence suggests that unbalanced diets are major contributors to cancer and are likely to be as important as smoking. It has been estimated that 30% of all cancer incidence is related to diet, and that the main problem is a dietary imbalance of too few fruits and vegetables or too much fat. In this context it is worth noting that in rodents, a high-caloric diet appears to be carcinogenic. A caloric-restricted (CR) diet, on the other hand, significantly increases the life span of rats and mice and dramatically decreases the cancer risk (Halliwell and Aruoma, 1993). The main effect of CR is reduced energy intake, which in turn may result in a decrease of oxygen consumption, lower rate of oxidative stress and hence, decreased damage to DNA. With regard to oxidative DNA damage, a decrease in 8-oxodG in the mitochondrial DNA of caloric-restricted rats and small decreases of this lesion in brain, heart muscle, and liver have been reported. In another study, Gredilla et al. (2001) demonstrated that 40% caloric restriction profoundly depresses the rate of hydrogen peroxide generation in rat heart mitochondria and specifically lowers oxidative damage to DNA in this organelle as well as in nuclear DNA. Interestingly, a similar decrease in caloric intake also decreased the aging rate and increased maximum life span of rats by 30 to 50%.

### 7.7 REACTIVE NITROGEN SPECIES AND DNA DAMAGE

Many dietary nitrates are found in vegetables. Nitrites and nitrates are used as preserving agents. In the acidic environment of the stomach, dietary NO$_2^-$ can form nitrous acid (HNO$_2$), which can decompose to oxides of nitrogen (Halliwell and Gutteridge, 1999). Moreover, nitrogen oxide chemistry is associated with the formation of carcinogenic nitrosamines.

Nitric oxide (NO) and nitrogen dioxide (NO$_2$) are free radicals. Together with peroxynitrite, a product of the reaction of NO with superoxide, they form reactive nitrogen species (RNS). Similarly to ROS, RNS may also be generated at high concentrations by activated phagocytes and other cells, at sites of inflammation and within tumors (Weitzman and Gordon, 1990). NO is mainly synthesized in living organisms by the action of a group of enzymes called nitric oxide synthases (NOS) that convert l-arginine into NO and l-citrulline. NO is chemically rather unreactive towards most cellular constituents, except for the heme centers that are its prime biological target in controlling vascular tone. However, NO can react with O$_2^-$ to form peroxynitrate (ONOO$^-$). Peroxynitrite exists in dynamic equilibrium with its conjugate acid (ONOOH$^-$) under physiological conditions. ONOOH$^-$ is a very pow-
erful oxidizing agent, and at physiological pH it rearranges forming \( \cdot \)OH and NO\(_2\) (Halliwell and Gutteridge, 1999).

RNS can induce various DNA lesions, including nitration, deamination, and oxidation of bases, as well as abasic sites and strand breaks (Wink et al., 1998; Halliwell and Gutteridge, 1999). Furthermore, in rat lung cells exposed in vivo to RNS, mutation and chromosomal aberrations are also reported (Isomura et al., 1984). High concentrations of NO have also been reported to generate DNA strand breaks and mutations in the hypoxanthine phosphoribosyltransferase (HPRT) gene and TK locus of TK6 in human lymphoblasts (Nguyen et al., 1992). Drugs that can generate RNS were also tested for their mutagenic properties. DEA/NO (NO donor diethylamine) was strongly mutagenic in *Salmonella typhimurium* (Wink et al., 1998), while another report failed to show mutation induction at the HPRT gene in human epithelial cells by the same drug (Felley-Bosco et al., 1995). More importantly, RNS, together with ROS derived from activated inflammatory leukocytes, were shown to be mutagenic in transgenic Chinese hamster ovary cells (Kim et al., 2003). Therefore, the aforementioned evidence indicates that similarly to ROS, RNS may also be genotoxic and have mutagenic properties.

It has been shown that 8-nitroguanine (8-NO\(_2\)-Gua) is the main modification arising from the reaction of RNS with DNA (Wink et al., 1998). However, other types of modifications were generated after the reaction of peroxynitrite with nucleobases and nucleosides (Douki et al., 1996). Since 8-NO\(_2\)-Gua decomposes spontaneously, with a half life of several hours, and is not repaired by Fpg (formamidopyrimidine glycosylase) (Tuo et al., 2000), it is possible that the biological significance of this base product may be limited, and other types of modification may be more important in the mutagenicity of RNS. An alternative mechanism for NO-mediated genotoxicity may be associated with inhibition of DNA repair processes. It was found that DNA repair enzymes could be inhibited through nitrosation of amino acids at amounts of RNS 100 times lower than that required to damage DNA (Laval, 1996).

### 7.8 CONCLUSIONS

ROS/RNS are continuously produced in the human body and can get into the cell from exogenous sources. The endogenous antioxidant system is inadequate to scavenge all of them, and their reaction with cellular components may result in damage to biomolecules, including DNA. Oxidatively damaged DNA has mutagenic potential, and its accumulation is proposed to have an important role in numerous pathological conditions, such as cancer. Therefore, dietary input of plant-based antioxidants, most notably antioxidant vitamins, is needed to protect against this disease. Moreover, some antioxidants, such as vitamin C, may reduce levels of oxidatively damaged DNA not only via an antioxidant, free radical–scavenging effect, but through the up-regulation of DNA repair. On the other hand, high dietary intake of iron may increase the risk of cancer, especially of colon cancer as a result of generation of the potentially carcinogenic 8-oxodG moiety in cellular DNA.

ROS and RNS may also act as signal molecules that regulate vital cell functions such as proliferation and differentiation. Since antioxidant nutrients may influence
ROS and RNS concentration and redox status of cells, by implication they may also affect normal cell functioning and survival. Therefore, the assumption that ROS/RNS should be avoided at any cost is simply misguided.

REFERENCES


8 Dietary Polyunsaturated Fatty Acids, Eicosanoids, and Intestinal Tumorigenesis

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8.1 INTRODUCTION

Colorectal cancer is the second leading cause of cancer death in the United States, with a reported 57,155 deaths in 1999 (Hoyert et al., 2001). There is sufficient evidence showing that environmental factors contribute to the development of this disease. The risk of colorectal cancer varies greatly from country to country (Parkin and Muir, 1992), and dietary fat intake is positively correlated with colon cancer death rates (Carroll, 1991). More recently, the impact of dietary polyunsaturated fats, in particular those derived from fish oils, has received increasing attention (as reviewed by Larsson et al., 2004). This chapter explores the relationship of this class of lipids with intestinal neoplasia.

8.2 DIETARY POLYUNSATURATED FATTY ACIDS AND THEIR METABOLISM

There are two major families of polyunsaturated fats in the Western diet, the n-3 and n-6 families (Figure 8.1). The n-6 polyunsaturated fatty acids (PUFAs), fatty acids containing two or more double bonds, are derived from the parent compound linoleic acid (LA, 18:2 n-6). LA is the major dietary PUFA in the United States, with median intakes of 17 and 12 g/day for men and women, respectively (Food and Nutrition Board, 2002). Metabolically, LA is converted to γ-linolenic acid (GLA, 18:3 n-6) via the Δ-6 desaturase, rate-limiting enzyme in the formation of highly unsaturated fatty acids (HUFA), those fatty acids containing four or more double bonds (Figure 8.1). GLA can be obtained by diet and is found primarily in specialty oils, such as evening primrose oil and borage oil. Metabolically, GLA is elongated to dihomo-γ-linolenic acid (DGLA, 20:3 n-6) following the addition of two carbons and subsequently desaturated to arachidonic acid (AA, 20:4 n-6) via the Δ-5 desaturase. AA is arguably the most important PUFA associated with membrane phospholipids. AA can also be obtained from dietary sources, where it is only found in food products of animal origin (Taber et al., 1998). The U.S. diet contains ~150 to 200 mg/day of AA; however, the accuracy of this number is questionable due to the unreliability of food composition databases (Taber et al., 1998).

The n-3 family of PUFAs is derived from the parent compound α-linolenic acid (ALA, 18:3 n-3), and the daily median intake for ALA is estimated to be 1.6 and 1.1 g per day for men and women, respectively (Food and Nutrition Board, 2002). The n-3 PUFAs are metabolized in parallel with the n-6 PUFAs and, like LA, ALA is also a substrate for Δ-6 desaturase, which converts it to stearidonic acid (SDA, 18:4 n-3). The levels of SDA are normally quite low in the diet. SDA is found primarily in fish oils, some specialty oils such as echium (Guil-Guerrero et al., 2001) and black currant oils (Del Castillo et al., 2004; Ruiz del Castillo et al., 2002), and a genetically modified (GM) canola oil that was specifically developed to be a plant-based substitute for the more highly unsaturated n-3 fatty acids (Ursin, 2003). When SDA is consumed, it is rapidly converted to eicosapentaenoic acid (EPA, 20:5 n-3) because it enters the metabolic pathway after the rate-limiting step controlled by Δ-6 desaturase (James et al., 2003; Petrik et al., 2000b). Through a sequential series of elongases, action by Δ-6 desaturase and peroxisomal beta-oxidation, EPA is
converted to docosahexaenoic acid (DHA, 22:6 n-3). EPA and DHA are commonly referred to as the “fish oil” n-3 PUFAs. The typical intake for the combination of these fatty acids is estimated to be ~200 mg per day. But as stated previously, unreliability of food composition databases makes this value an educated guess (Taber et al., 1998).

As previously mentioned, Δ-6 desaturase is the rate-limiting step in the conversion of LA and ALA to their HUFA derivatives. In the absence of dietary n-3 and n-6 PUFAs, LA and ALA are rapidly converted to AA and EPA/DHA, respectively. However, under typical Western intakes, the conversion of these parent PUFAs to their respective HUFA derivatives is dramatically attenuated. It has been reported that LA intakes greater than 2.5% of calories do not result in an increase of AA in human neutrophil phospholipids (typical intakes are ~5 to 7% of energy (Food and Nutrition Board, 2002; James et al., 1993). Similarly, dietary supplements of ALA are poorly converted to DHA when given to subjects already consuming n-3 PUFAs (Brenna, 2002; Pawlosky et al., 2003). However, PUFAs with four or more double bonds (i.e., AA, SDA, EPA, DHA) are preferentially utilized because they enter the biochemical pathway after the Δ-6 desaturase step.

The PUFAs of the n-3 and n-6 families utilize the same enzymatic machinery for their metabolism; however, these families of fatty acids are not interconvertible.
and as such, consumption of one family of fatty acids can attenuate the metabolism of the other. Hence, the competition between these two fatty acid families defines the role of dietary HUFAs and the risk of colorectal cancer. Dietary n-3 HUFAs appear to be antitumorigenic, and it has been suggested that this effect is mediated via their antagonism of AA and its downstream metabolites, the eicosanoids.

8.3 EICOSANOIDS

8.3.1 CYCLOOXYGENASE PATHWAY

Following the release of AA from membrane phospholipids by a variety of phospholipase A\textsubscript{2}s, AA is enzymatically oxidized to a multitude of biologically active compounds (Figure 8.2). Conversion of AA to prostaglandins (PG), thromboxane A\textsubscript{2} (TXA\textsubscript{2}), and prostacyclin (PGI\textsubscript{2}) occurs via the endoperoxide intermediate PGH\textsubscript{2}. This is a two-step process involving cyclooxygenase (COX). Two known isoforms

**FIGURE 8.2** Following stimulation, cells release arachidonic acid and/or linoleic acid from membrane phospholipids via phospholipase A\textsubscript{2}. AA can be metabolized by cyclooxygenases to produce prostaglandins, thromboxane (TXA\textsubscript{2}), and prostacyclin (PGI\textsubscript{2}), or by lipoxygenases (LOXs) to form hydroxy derivatives (HETEs). LA, on the other hand, is not a substrate for PG biosynthesis but can be acted upon by 15-LOX-1 to generate the hydroxy derivative 13(S)-HODE. The biochemical pathway beginning with the biosynthesis of AA through its metabolism to eicosanoids (COX and LOX derivatives) is commonly referred to as the arachidonic acid cascade. Abbreviations: ALA, -linolenic acid; AA, arachidonic acid; Ca\textsuperscript{2+}, ionized calcium; cAMP, cyclic adenosine monophosphate; COX, cyclooxygenase; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; HETE, hydroxy-eicosatetraenoate; HODE, hydroxy-octadecadienoate; LA, linoleic acid; LOX, lipoxygenase; PG, prostaglandin; PGI\textsubscript{2}, prostacyclin; TXA\textsubscript{2}, thromboxane.
of COX, COX-1 and COX-2, catalyze this committed step. COX-1 is constitutively expressed in most tissues and appears primarily to have a housekeeping function. COX-2 is not normally expressed in most tissues and is considered to be the inducible form of the enzyme. Its expression is upregulated in monocytes/macrophages, fibroblasts, and epithelial cells in response to cytokines, growth factors, mitogens, and tumor promoters. However, it should be noted that basal COX-2 expression has been observed in the kidney, brain, testes, and tracheal epithelium. COX-2 has been detected in virtually all cancers, including colorectal cancer, although its expression is variable and dependent upon the stage of the neoplasia (Dannenberg and Subbaramaiah, 2003). It appears that COX-2 induction is important in maintaining tumor integrity and contributes to the metastatic process (Cao and Prescott, 2002; Prescott and Fitzpatrick, 2000; Tsujii et al., 1997; 1998; Wang and DuBois, 2004). While COX-1 expression in colorectal tumors does not differ from that in nonneoplastic mucosa, genetic studies indicate that this COX enzyme is also important in tumorigenesis (Chulada et al., 2000). AA is the preferred substrate for both COX-1 and COX-2, but EPA is also a good substrate for COX-2 (Rieke et al., 1999) and effectively antagonizes the conversion of AA to PGs. LA, ALA, SDA, and DHA are not substrates for PG biosynthesis.

8.3.1.1 Prostaglandin $E_2$

Prostaglandin $E_2$ ($PGE_2$) is produced from $PGH_2$ by PGE synthase and is the most abundant COX metabolite in human and rodent intestinal tumors. Expression of COX-2 in nonneoplastic stroma (macrophages and fibroblasts) of benign adenomas and malignant epithelium of carcinomas is attributed with driving the production of most of this excess $PGE_2$, which then acts locally in a paracrine or autocrine manner to mediate its pathobiologic effects (Cao and Prescott, 2002). There are four G protein-coupled E-prostaglandin (EP) receptors that variously increase cytoplasmic calcium or modulate cAMP levels (reviewed in Narumiya and FitzGerald, 2001). The final outcome of AA cascade signaling through $PGE_2$ is dependent on which EP receptor(s) is expressed by the target cell. Three of the EP receptors (EP1, EP2, and EP4) have been implicated in mediating the protumorigenic $PGE_2$ activity in rodent intestinal tumorigenesis (Mutoh et al., 2002; Sonoshita et al., 2001; Watanabe et al., 1999). EP2 and EP4 stimulate adenylate cyclase to increase cAMP levels, while EP1 mobilizes intracellular calcium. Such cytoplasmic fluxes in either cAMP or calcium can have broad downstream effects, and the exact molecular mechanism of tumorigenic $PGE_2$ signaling in colorectal neoplasia remains to be determined. Nevertheless, it is a participant in a signaling concert involving the stroma, tumor epithelial cells, and vascular endothelium in tumor development by promoting growth, inhibiting apoptosis, modifying the immune response, increasing invasiveness, and stimulating angiogenesis (Wang and DuBois, 2004).

8.3.1.2 Prostacyclin

In addition to $PGE_2$, prostacyclin ($PGI_2$) appears to also be important in carcinogenesis; however, its role is less understood. The nuclear transcription factor peroxisomal
proliferator-activated receptor-δ (PPAR-δ) is up-regulated in colorectal tumors, and activation of PPAR-δ promotes tumorigenesis (Gupta et al., 2000; 2004). PGI2 has been identified as a ligand for PPAR-δ (Gupta et al., 2000) and may contribute to neoplastic progression by inhibiting apoptosis (Cutler et al., 2003).

### 8.3.2 Lipoxygenase Pathway

AA can also be oxidized by a number of lipid oxidases, referred to as lipoxygenases (LOX) (Brash, 1999). Of importance are two human lipoxygenase isozymes, 15-LOX-1 and 15-LOX-2 (Figure 8.2). 15-LOX-1 is mainly expressed in reticulocytes, eosinophils, and macrophages and in the skin and colon (Kamitani et al., 1998; Shureiqi et al., 2000), whereas 15-LOX-2 mRNA is normally detected in prostate, lung, skin, and cornea, but not the colon (Shappell et al., 1999). AA is the preferred substrate for 15-LOX-2, generating 15(S)-hydroxy-eicosatetraenoate [15(S)-HETE], and 15-LOX-1 preferentially acts on LA to form 13(S)-hydroxy-octadecadienoate [13(S)-HODE]. AA is a relatively poor substrate for 15-LOX-1.

There is some controversy as to where 15-LOX-1 is prominently expressed. Both normal intestinal mucosa and colorectal tumors express 15-LOX-1 (Ikawa et al., 1999; Nixon et al., 2004; Shureiqi et al., 1999). In the normal colon, expression of 15-LOX-1 is highly localized to the mucosal epithelium (Nixon et al., 2004), whereas tumors express 15-LOX-1 in both the neoplastic epithelium and in infiltrating inflammatory cells (Nixon et al., 2004; Shureiqi et al., 1999). Increased expression in tumors would seem to suggest a protumorigenic activity; however, overexpression of 15-LOX-1 in xenograft tumors reduces growth, suggesting an antitu- morogenic effect (Nixon et al., 2004). 15-LOX-1 expression can be induced with nonsteroidal antiinflammatory drugs (NSAIDs), inhibitors of COX. The use of sulindac (a nonselective COX-1/2 inhibitor) or NS398 (a selective COX-2 inhibitor) increased 15-LOX-1 protein expression in two colorectal tumor cell lines (RKO and HT-29 cells), resulting in growth inhibition and increased apoptosis (Shureiqi et al., 2000). These effects were abrogated by inhibition of 15-LOX-1 and subsequently rescued when the inhibition was bypassed with the concomitant addition of 13(S)-HODE (Shureiqi et al., 1999; 2000). 13(S)-HODE formation has antitumorigenic effects and is positively correlated with 15-LOX-1 expression (Nixon et al., 2004; Shureiqi et al., 1999; 2000; 2003).

As mentioned previously, PPAR-δ activation may have positive affects on colorectal neoplasia, and LA metabolites may modify transcriptional activity of these receptors (Desvergne and Wahli, 1999; Shureiqi et al., 2003). It has been reported that 13(S)-HODE (at relatively high levels) can bind to PPAR-δ, decreasing its activation and expression (Shureiqi et al., 2003). Although one can speculate, it is not known whether diet can modify neoplasia by this pathway.

Another PPAR isoform, PPAR-γ, also appears to have a role in intestinal tumorigenesis, but not without controversy. The 15-LOX-1 products of LA can act as activating ligands for PPAR-γ in macrophages (Nagy et al., 1998), but HETEs and HODEs enhance mitogen-activated protein (MAP) kinase activity, causing phosphorylation and down-regulation of PPAR-γ in HCT116 colon cancer cells (Hsi et al., 2001). Although the more notable action of PPAR-δ is its association with a reduction
in atherosclerosis (Chawla et al., 2001), several mutated isoforms have been identified in 55 sporadic colon cancers (Sarraf et al., 1999). If 15-LOX-1 products have the ability to shut down PPAR-γ activity, this would have essentially the same net effect as a loss-of-function mutation in PPAR-γ, representing a novel and potentially important effect of 15-LOX-1 metabolites in promoting colorectal carcinogenesis.

However, the relative contribution of each metabolic pathway for 15-LOX-1 in this complex lipid signaling network will clearly require further evaluation to determine which has a dominant effect in neoplastic intestinal tissues and which can be beneficially modified by dietary intervention. Unfortunately, little attention has been focused, beyond speculation, on how diet can manipulate lipoxygenase product profiles and whether these manipulations have selective effects on intestinal neoplasia and risk of colorectal cancer.

8.4 DIETARY HIGHLY UNSATURATED FATTY ACIDS, THE ARACHIDONIC ACID CASCADE, AND INTESTINAL TUMORIGENESIS

The AA cascade refers to a set of biochemical events beginning with LA and ending with eicosanoids, affecting cell behavior through their interaction with specific receptors or transcription factors. The earliest indications that the AA cascade had a role in colorectal cancer came from epidemiological studies that demonstrated that the use of NSAIDs, inhibitors of COX-1 and -2 activities, was inversely related to mortality from this disease (Thun et al., 1991). This protective effect has been confirmed in many experimental settings, where inhibition of COX-1 and/or COX-2 by a variety of NSAIDs has repeatedly reduced tumor load. However, it has also been reported that NSAIDs may inhibit tumorigenesis by increasing AA (Cao et al., 2000; Chan et al., 1998). Treatment of cultured cells with NSAIDs inhibits COX activities, thereby increasing cytosolic levels of free AA (Chan et al., 1998). It has been suggested that increasing cytosolic levels of AA induces apoptosis through a ceramide-mediated pathway. Treatment of the same cultured cells with exogenous AA produced similar results, solidifying the hypothesis that AA is apoptotic in tumor cell lines. However, when EPA was tested in cultured cells, results were identical to those observed with AA and were found to be associated with a prooxidant environment (Dommels et al., 2003). Furthermore, feeding trials using animal models produce different effects. Classical animal feeding studies demonstrated that the n-6 PUFA LA (in the absence of HUFA) was protumorigenic, while substitution of fish oil for the LA-containing corn oil inhibited neoplasia (Reddy et al., 1991). The conflicting results observed in these studies could be due to the fact that in vivo systems respond differently to these fatty acids versus cultured cells. As such, the invariable link between dietary n-3 PUFAs and colorectal cancer inhibition is related to their negative effects on cellular AA and its metabolism.

Therefore, if de novo AA biosynthesis, AA levels in tissues, and its subsequent conversion to prostaglandins (in particular PGE₂) contribute significantly to intestinal neoplasia, then manipulating the AA cascade by dietary intervention should modify the tumorigenic process. This was clearly established in a series of studies using
Carcinogenic and Anticarcinogenic Food Components

Apc\textsuperscript{Min/+} mice (Hansen-Petrik et al., 2002a; 2002b; Petrik et al., 2002a; 200b). The Apc\textsuperscript{Min/+} mouse model was derived from a C57BL/6J male mouse that had been exposed to the mutagen ethyl nitrosourea, resulting in a mutation in the adenomatous polyposis coli (Apc) gene (Moser et al., 1990). The resultant offspring who are heterozygous for this mutation spontaneously develop 35 to 50 tumors throughout the intestinal tract following a loss of heterozygosity. Most human colorectal cancers occur spontaneously (i.e., sporadic forms), and in the great majority of these cases mutational inactivation of the APC gene appears to be the earliest driving event. In a relatively small number of colorectal cancer cases, an individual is predisposed to the disease due to a germline mutation in one allele. These individuals, familial adenomatous polyposis patients, develop hundreds to thousands of tumors throughout the intestinal tract following loss of the wild-type allele. As such, Apc\textsuperscript{Min/+} mice recapitulate this human condition and are considered a good model for studying the effects of diet on intestinal neoplasia.

In an effort to more clearly define the role of dietary PUFAs in tumorigenesis and determine whether manipulation of the AA cascade by diet is sufficient to modify tumorigenesis, we instituted a number of studies:

- We conducted feeding trials using an appropriate \textit{in vivo} model, the Apc\textsuperscript{Min/+} mouse.
- Using this \textit{in vivo} model, we enhanced and inhibited prostaglandin biosynthesis and linked these changes to neoplastic changes.
- We inhibited the AA cascade at multiple points to establish a direct association with this metabolic pathway and intestinal neoplasia.
- We established that bypassing each inhibited step attenuated the antitumorigenic effects of each intervention.
- Each intervention was designed to mimic at least one aspect of how dietary n-3 PUFAs and dietary AA modify the AA cascade and tumorigenesis.

Collectively, these results would establish that the antineoplastic effects of dietary n-3 HUFA act by antagonizing AA metabolism.

8.5 DIETARY N-3 POLYUNSATURATED FATTY ACIDS AND INTESTINAL TUMORS

8.5.1 Effects of Alpha-Linolenic Acid, Stearidonic Acid, Eicosapentaenoic Acid, and Docosahexaenoic Acid on Intestinal Tumors

Apc\textsuperscript{Min/+} mice were fed diets supplemented with four different n-3 PUFAs (3%, wt/wt), ALA, SDA, EPA, and DHA (Figure 8.3). The design of this experiment was important in a number of ways. The background diet mimicked the Western diet with regards to macronutrient and fatty acid composition (Petrik et al., 2000b). Furthermore, each HUFA supplemented to the diet replaced an equal amount of
Polyunsaturated Fatty Acids, Eicosanoids, and Intestinal Tumorigenesis

Oleic acid, a neutral lipid with respect to the AA cascade and tumorigenesis. Therefore, any dietary effects on tumorigenesis could only be attributed to the individual n-3 PUFA added to the diet. Dietary n-3 PUFAs have multiple effects on the AA cascade. They are very effective inhibitors of Δ-6 desaturase, they replace AA in tissue phospholipids with EPA, and they reduce AA-derived PG formation by inhibiting COX activities (Figure 8.3). Importantly, dietary addition of ALA and SDA tests the effects of n-3 PUFA that normally precede or follow the Δ-6 desaturase step, allowing us to compare these results to the fish oil-derived n-3 HUFA, EPA, and DHA. After 7 weeks on diet, ALA had no effect on tumor load (tumors per mouse and tumor size). In contrast, the n-3 HUFAs SDA and EPA significantly reduced tumor numbers by 47 and 48%, respectively. DHA supplementation resulted in a 30% reduction in tumor number. These results suggest that n-3 PUFA with four or more double bonds (HUFAs) were most efficacious, and the fact that ALA had no effect on tumor multiplicity implicates the important regulatory effect of ALA conversion to SDA and EPA via Δ-6 desaturase. Both SDA and EPA were very effective in reducing tissue AA content (73 and 62% reduction, respectively) with concomitant reductions in PGE2 (Petrik et al., 2000b). PGE2 has been identified as very important in carcinogenesis (Cao and Prescott, 2002; Prescott and Fitzpatrick, 2000). Hence, it is logical to suggest that dietary n-3 PUFAs act by antagonizing AA metabolism and reducing PG formation. However, it is important to point out that these data do not rule out additional PG-independent effects, such as modulation of other AA pathways (e.g., lipoxygenases) or effects mediated by changes in membrane phospholipid PUFA content that could directly affect cell signaling pathways (Fan et al., 2003; Lee et al., 2003).

### FIGURE 8.3 The effects of dietary n-3 PUFAs on AA metabolism and tumorigenesis. Dietary n-3 PUFAs can inhibit the biosynthesis of AA from LA and reduce tissue AA content and downstream derivatives, such as prostaglandins (PGs). ApcMin mice were fed diets supplemented with four different n-3 PUFAs (3%, wt/wt), ALA, SDA, EPA, or DHA, for 7 weeks (Petrik et al., 2000b). Of the four, only ALA failed to inhibit intestinal tumorigenesis. Values are means ± SEM and were compared using Fischer’s least significant difference multiple comparison method. Different superscripts within the row indicate that the values are significantly different among groups at P < 0.05. Abbreviations: ALA, α-linolenic acid; AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; PGE2, prostaglandin E2; PUFAs, polyunsaturated fatty acids; SDA, stearidonic acid.
The U.S. diet contains more than enough LA (5 to 7% of calories) to enrich tissues with AA. Similarly, typical rodent diets contain sufficient levels of this essential nutrient to adequately maintain levels of AA to meet cellular needs. Since it is hypothesized that a reduction in AA and its metabolism has antineoplastic effects, it was important to ascertain what effect augmenting tissue AA content and PG levels above baseline levels would have on this process. We previously demonstrated that dietary AA dramatically enriched tissue phospholipids with AA and elevated eicosanoid levels approximately two- to threefold in vivo (Li et al., 1994). With this in mind, we fed ApcMin/+ mice a baseline diet containing LA with and without AA supplementation (Figure 8.4). The addition of AA recapitulated the earlier findings with regard to tissue phospholipid AA and PG levels, but had no significant impact on tumor number, suggesting that the level of LA in the base diet was more than sufficient to produce enough AA for a maximum response on tumorigenesis.

Our earlier study demonstrated that feeding EPA dramatically attenuated phospholipid AA content and eicosanoid levels in vivo, and supplementing AA to the EPA-containing diet (AA + EPA) abrogated any of the effects attributed to EPA (Li et al., 1994). This study demonstrated that of the two fatty acids, AA confers a
dominant effect biologically when both are provided concurrently in the diet. Similar results also have been observed in humans (O’Dea and Sinclair, 1982).

Therefore, to test whether the antitumorigenic effect of dietary n-3 HUFAs could be reversed by increasing AA and its metabolism, mice were fed diets containing either EPA, AA, or a combination of EPA and AA (1.5%, wt/wt) (as depicted in Figure 8.5). As observed previously, consumption of AA had no effect on tumor number despite elevated levels of tissue AA and PGs, and EPA reduced tumor number by more than 50% with concomitant reductions in tissue AA content, PGE$_2$, and PGF$_{1\alpha}$ (measured as 6-keto PGF$_{1\alpha}$) levels (Table 8.1). However, when AA was added to diets containing equivalent amounts of EPA, the effects of EPA were eliminated with regards to tumor number, tissue AA content, and PG levels. These results suggest that the antitumorigenic effect of dietary EPA is, in part, mediated through its antagonism of AA metabolism by inhibiting Δ-6 desaturase, displacing

**TABLE 8.1**
The Effects of Feeding Apc$^{Min/+}$ Mice Diets Supplemented with AA, EPA, or AA Plus EPA on Tumor Number, Intestinal Fatty Composition, and PGE$_2$ and 6-keto-PGF$_{1\alpha}$ Levels

<table>
<thead>
<tr>
<th>Dietary Group</th>
<th>Control Diet</th>
<th>AA</th>
<th>EPA</th>
<th>AA + EPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA content (mol %)</td>
<td>23.7 ± 0.7$^b$</td>
<td>15.3 ± 0.4$^c$</td>
<td>26.7 ± 1.1$^a$</td>
<td>17.3 ± 0.3$^c$</td>
</tr>
<tr>
<td>AA content (mol %)</td>
<td>16.7 ± 0.2$^c$</td>
<td>28.2 ± 0.7$^a$</td>
<td>6.2 ± 0.3$^d$</td>
<td>25.1 ± 0.7$^b$</td>
</tr>
<tr>
<td>EPA content (mol %)</td>
<td>0.3 ± 0.03$^c$</td>
<td>0.2 ± 0.06$^c$</td>
<td>7.1 ± 0.41$^a$</td>
<td>1.0 ± 0.04$^b$</td>
</tr>
<tr>
<td>PGE$_2$ level (pg/mg protein)</td>
<td>22.5 ± 7.6$^b$</td>
<td>53.6 ± 7.4$^a$</td>
<td>6.1 ± 1.3$^c$</td>
<td>28.0 ± 5.0$^b$</td>
</tr>
<tr>
<td>6-keto-PGF$_{1\alpha}$ level (pg/mg protein)</td>
<td>44.4 ± 11.7$^b$</td>
<td>82.3 ± 8.2$^b$</td>
<td>19.4 ± 4.0$^c$</td>
<td>61.7 ± 12.2$^{a,b}$</td>
</tr>
<tr>
<td>Tumors/mouse</td>
<td>68 ± 9$^a$</td>
<td>48 ± 9$^a$</td>
<td>22 ± 4$^b$</td>
<td>48 ± 6$^a$</td>
</tr>
</tbody>
</table>

*Note: Values are means ± SEM and were compared using Fischer’s least significant difference multiple comparison method. Different superscripts within each row indicate that the values are significantly different among groups at $P < 0.05$. Abbreviations: AA, arachidonic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; PGE$_2$, prostaglandin E$_2$; PGF$_{1\alpha}$, prostaglandin F$_{1\alpha}$.**
AA from membrane phospholipids, and inhibiting AA-derived PG biosynthesis. Therefore, beneficial effects of dietary PUFAs are observed when AA metabolism is impaired, and the levels of LA typically provided in animal diets are sufficiently converted to AA such that enriching tissues above this level does not necessarily enhance tumor load (Larsson et al., 2004). This putative threshold level of AA is easily maintained by typical levels of LA in the Western diet and may therefore be a key target for dietary cancer prevention.

The presence of HUFAs in the diet results in predictable changes in fatty acid composition (Table 8.1). When n-3 HUFAs are consumed, they target phospholipid pools containing AA by substituting AA with EPA (Li et al., 1994; Petrik et al., 2000a; 2000b). However, dietary AA targets two pools of fatty acids, LA and EPA (when present in tissues) (Li et al., 1994; Petrik et al., 2000a; Whelan et al., 1992). Dietary AA is significantly enriched in all phospholipid classes and is readily incorporated into phosphatidylcholine at the direct expense of LA (Whelan et al., 1992). Phosphatidylcholine is a prime substrate for phospholipase A2 and the release of AA from membrane phospholipids. Enriching tissues with AA at the expense of LA would modify the product profiles of both the COX and 15-LOX pathways, with increases in 2-series PGs (Petrik et al., 2000b) and the HETE/HODE ratio.

8.6 ARACHIDONIC ACID CASCADE AND INTESTINAL TUMORIGENESIS

The premise of the previous experiments was that the AA cascade is important in intestinal tumorigenesis. To clearly establish this fact, a number of experiments were performed \textit{in vivo} to test the ability of specific components of the cascade to rescue tumorigenesis. Each experiment targeted a step in the pathway that is affected by dietary n-3 PUFAs (as depicted in Figure 8.3), and each inhibition was bypassed to demonstrate that targeting each step had an impact on tumorigenesis.

8.6.1 INHIBITION OF \textit{De Novo} ARACHIDONIC ACID BIOSYNTHESIS

In the first experiment, we inhibited \textit{de novo} biosynthesis of AA by selectively inhibiting Δ-6 desaturase with SC-26196 (Figure 8.6) (Hansen-Petrik et al., 2002a). When Δ-6 desaturase was inhibited, tumor number was reduced by ~37%, and this effect was reversed when AA was concomitantly added to the diet with the inhibitor. While the attenuated tumorigenesis observed in the SC-26196-treated mice suggests Δ-6 desaturase involvement, this interconnection is strengthened by the fact that concomitant AA supplementation abrogated the antitumorigenic efficacy of SC-26196. The n-3 PUFAs, like SC-26196, are inhibitors of Δ-6 desaturase (Garg et al., 1988).

8.6.2 INHIBITION OF NONSTEROIDAL ANTIINFLAMMATORY DRUG-INDUCED TUMOR REDUCTION WITH PROSTAGLANDIN E\textsubscript{2}

The above study was followed up with experiments inhibiting both COX isoforms, COX-1 and COX-2, using two different nonselective inhibitors (piroxicam or sulin-
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dac), and bypassing the inhibition with simultaneous administration of two PGE2 analogues, 16,16-dimethyl PGE2 and 17-phenyl-trinor PGE2 (Figure 8.7) (Hansen-Petrik et al., 2002b). In each case, the animals were allowed to develop ~50 tumors, and treatment with the NSAIDs for less than a week reduced tumor multiplicity by 95 and 55% following piroxicam or sulindac administration, respectively. The tumor-suppressing effects of NSAIDs were mediated by a reduction in tumor cell mitosis and concomitant increases in apoptosis. When the COX inhibition was bypassed with short-term administration (4 days) of the PGE2 analogues, these antitumorigenic effects were significantly attenuated, demonstrating the importance of PGE2 in maintaining tumor integrity. To more clearly demonstrate this point, ApcMin/+ mice with preexisting intestinal tumors were administered a monoclonal antibody against PGE2 to immunologically sequester PGE2 systemically (Figure 8.8). If NSAIDs and n-3 PUFA inhibit tumorigenesis in part by reducing PGE2 formation, then immunological neutralization of PGE2 activity should have a similar effect. After 4 days of treatment, tumor number was reduced by 34% (p < 0.02), confirming the role of PGE2 in maintaining tumor integrity (Hansen-Petrik et al., 2002b). Similar results have been reported in mice bearing Lewis lung carcinoma xenografts after treatment with the same monoclonal antibody (Stolina et al., 2000). These results and those discussed previously suggest that PGE2 mediates intestinal tumorigenesis and can promote the carcinogenic process.

8.7 SUMMARY AND CONCLUSIONS

In summary, dietary n-3 HUFAs possess antineoplastic properties that act at least in part by concomitantly inhibiting a number of steps in the AA cascade. This was confirmed with add-back studies involving dietary EPA and AA, and inhibitors of

FIGURE 8.6 The effect of selectively inhibiting Δ-6 desaturase with SC-26196 on intestinal tumor number in the absence and presence of dietary AA. ApcMin/+ mice were fed diets with and without the Δ-6 desaturase inhibitor SC-26196 and with and without AA for 7 weeks. Inhibition of Δ-6 desaturase, the rate-limiting step in AA biosynthesis, significantly reduced tumor number and the concomitant addition of dietary AA eliminated this effect. Values are means ± SEM and were compared using Fischer’s least significant difference multiple comparison method. Different superscripts within the row indicate that the values are significantly different among groups at P < 0.05. Abbreviations: AA, arachidonic acid; LA, linoleic acid.
selective steps in the biochemical pathway with the concomitant addition of down-stream compounds to bypass the inhibitions. These studies more clearly defined the link between dietary n-3 PUFA, with the AA cascade (including PGE2), and intestinal tumorigenesis using an in vivo model. These data show that while dietary PUFAs can modify intestinal tumorigenesis, they nevertheless lack the full efficacy observed.

**FIGURE 8.7** Intestinal tumor multiplicity in *ApcMin/+* mice treated with and without sulindac or piroxicam, in the presence or absence of the PGE2 analogues 16,16-dimethyl-PGE2 and 17-phenyl-trinor-PGE2 (Hansen-Petrik et al., 2002b). *ApcMin/+* mice were allowed to generate intestinal tumors for 80 days, after which time they were treated with piroxicam or sulindac for 4 to 6 days, in the presence or absence of two prostaglandin E2 analogues. Piroxicam and sulindac significantly inhibited tumorigenesis, and these effects were attenuated with the concomitant addition of the PGE2 analogues. Values are means ± SEM and were compared using Fischer’s least significant difference multiple comparison method. Different superscripts within the row indicate that the values are significantly different among groups at P < 0.05. Abbreviations: AA, arachidonic acid; COX, cyclooxygenase; PGE2, prostaglandin E2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Piroxicam</th>
<th>Piroxicam + PGE2 analogues</th>
<th>Sulindac</th>
<th>Sulindac + PGE2 analogues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumors/mouse</td>
<td>48 ± 6a</td>
<td>2 ± 0.8c</td>
<td>19 ± 5b</td>
<td>22 ± 6b</td>
<td>38 ± 6a</td>
</tr>
</tbody>
</table>

**FIGURE 8.8** Intestinal tumor load in *ApcMin/+* mice treated with the anti-PGE2 monoclonal antibody 2B5 (Mab2B5) for 4 days (Hansen-Petrik et al., 2002b). *ApcMin/+* mice were allowed to generate intestinal tumors for 80 days, after which time they were treated with MOPC21 control antibody or an anti-PGE2 monoclonal antibody 2B5 (intraperitoneally). Immunologic sequestration of PGE2 (systemically) significantly reduced tumor number as compared to the control antibody. Values are means ± SEM and were analyzed using a pairwise comparison. * indicates a significant difference at P < 0.05. Abbreviations: AA, arachidonic acid; COX, cyclooxygenase; PGE2, prostaglandin E2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Mab2B5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumors/mouse</td>
<td>59 ± 6</td>
<td>39 ± 6*</td>
</tr>
</tbody>
</table>
with the use of NSAIDs even though they appear to act via a similar mechanism — inhibition of PG biosynthesis.

These studies also address a number of important issues related to diet and colorectal cancer and proposed mechanisms. By carefully controlling the composition of the diets, we have been able to target the effects of individual fatty acids, a process made more difficult when these fatty acids are delivered in the form of vegetable or fish oils. As a result, n-3 PUFAs with at least four double bonds appear to be the most effective fatty acids with regards to antineoplastic activity (Petrik et al., 2002; 200b). Furthermore, we found that:

1. Dietary AA content is positively associated with tissue AA content.
2. Tissue AA content is positively associated with PGE$_2$ and PGI$_2$ levels ($p < 0.0003$).
3. PGE$_2$ and PGI$_2$ levels are positively correlated with tumor number.

Alternatively, others have suggested that an increase in lipid peroxidation may be responsible for the antitumorogenic effects of n-3 PUFAs or that increasing cellular AA content is proapoptotic. We have indirectly addressed each of these hypotheses within the context of our model (Petrik et al., 2000a). Increasing the unsaturation index of a dietary PUFA increases its peroxidation potential. AA has an unsaturation index similar to that of EPA; thus, the peroxidation potential would be similar. Dietary EPA reduced tumor number. If the peroxidation hypothesis was valid, adding AA to the diet should have resulted in similar reductions, and doubling the unsaturation index by adding AA to the EPA-containing diet should have resulted in further reductions. In contrast, the addition of AA to EPA more than doubled tumor number (compared to the EPA group) rather than reducing tumorigenesis as might be predicted by the peroxidation hypothesis.

Finally, if tissue AA is important in the tumorigenic process, it remains unclear whether dietary LA, at current levels, is an important factor (Larsson et al., 2004). Given the fact that tissue AA levels in humans are poorly correlated with LA intakes above 3% of calories, it seems that moderate changes in current levels of LA consumption (5 to 7% of calories) would not be expected to modify AA levels. As such, increasing the consumption of n-3 HUFA would more likely result in the greatest changes of AA and its metabolism (de Deckere et al., 1998). Because dietary AA is able to abrogate the beneficial effects of n-3 HUFAs and its consumption is comparable to that of n-3 HUFA, it has been suggested that epidemiological studies looking at the ratio of AA to n-3 HUFA in diet or tissues may be warranted (Larsson et al., 2004).

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9 Chemoprevention of Cancer: Basic Mechanisms and Molecular Targets

Wanda Baer-Dubowska and Ewa Ignatowicz

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9.1 INTRODUCTION

Despite significant advances in the treatment of many tumors, the battle against cancer is far from being won. It has become increasingly obvious that the problem of cancer cannot be solved by treatment alone, and an alternative approach is needed. Although the carcinogenic process may be driven by mutations, many epigenetic variables can
also be important determinants during the 20-year (or more) latent period before invasion and metastasis occur (Sporn, 2000). Modulation of these regulatory pathways, as well as blocking mutagenic damage to DNA, offers great potential for the prevention of cancer. The traditional approach to cancer prevention has consisted of attempts to eliminate carcinogenic agents and to detect and remove precancerous lesions. Currently, efforts are increasingly focused on interrupting, reversing, or delaying the neoplastic process. This approach not only complements therapeutic modalities currently in use but may provide alternatives for combating tumors that are unresponsive to treatment (Itri, 1993). Hence, cancer chemoprevention or reversal of carcinogenesis in the premalignant phase can be defined as the use of natural or synthetic chemicals to suppress, delay, or prevent the process of carcinogenesis.

Food is a rich source of potential anticarcinogenic agents, and it is reasonable to postulate that dietary components may prevent cancer from reaching its invasive and metastatic stages. The importance of dietary constituents in cancer prevention was pointed out during the 1960s and 1970s by experiments of Wattenberg and associates (Wattenberg, 1966), in which they demonstrated that various compounds, especially those present in fruits and vegetables, such as indoles and isothiocyanates, inhibit chemically induced tumors in laboratory animals. Alternatively, components of the diet may reduce the risk of second primaries or modify the behavior of established cancer (Gescher et al., 2001).

9.2 CONCEPTUAL BASIS FOR CHEMOPREVENTION

Cancer begins through the multistage process of carcinogenesis resulting from exposure to a wide variety of carcinogenic insults. The major stages of carcinogenesis were deduced over the past 60 years, primarily from animal model studies. These stages are termed initiation, promotion, and progression (Weinstein et al., 1995; Yuspa and Shields, 1997). Tumor initiation begins when DNA in a cell or population of cells is damaged by exposure to exogenous or endogenous carcinogens. If this damage is not repaired, it can lead to genetic mutations. The responsiveness of the mutated cells to their microenvironment can be altered and may give them a growth advantage over normal cells. In the classic two-stage carcinogenesis system in mouse skin, a low dose of an initiator (a polycyclic aromatic hydrocarbon, 7,12-dimethylbenz[a]anthracene, DMBA) causes permanent DNA damage (the initiating event) but does not give rise to tumors over the lifespan of the mouse unless a tumor promoter, such as a phorbol ester derivative (a naturally occurring plant constituent), 12-O-tetradecanoyl-phorbol-13-acetate (TPA), is repeatedly applied (DiGiovanni, 1994). The tumor promotion stage is characterized by selective clonal expansion of the initiated cells, a result of TPA-induced oxidative stress, and the altered expression of genes whose products are associated with hyperproliferation, tissue remodeling, and inflammation. During tumor progression, preneoplastic cells develop into tumors through a process of clonal expansion that is facilitated by progressive genomic instability and altered gene expression.

Most animal models used in carcinogenesis research were developed before the identification of the major cancer-related genes, the recognition of the importance of host susceptibility to a carcinogenic insult, or the realization that mitogenesis and
apoptosis together regulate cell number. The temporal sequence implied in this scheme is now thought to be overly simplistic and somewhat misleading. Human carcinogenesis, rather than occurring in three discrete stages in a predictable order, is best characterized as an accumulation of alterations in genes regulating cellular homeostasis such as oncogenes, tumor suppressor genes, apoptosis-regulating genes, DNA repair genes, and the epigenome (Hursting et al., 1999; Jones and Baylin, 2002).

Nonetheless, these animal models have contributed significantly to our understanding of carcinogenesis and the ways to interfere with that process. Moreover, these models allow convenient categorization of chemopreventive agents into those that can block initiation (blocking agents) and those that suppress promotion and progression (suppressing agents) (Manson et al., 2000). Potential targets or strategies for nutritional modulation or chemoprevention are shown in Figure 9.1. Early solid cancers are generally detected as intraepithelial neoplasia or carcinoma in situ, which correspond to the promotion and progression stages, respectively. Therefore, anti-promotion and antiprogression agents are of particular clinical interest. Ultimately, such agents prevent the growth and survival of cells already committed to becoming malignant (secondary and tertiary chemoprevention). Figure 9.2 presents the levels of chemoprevention application and target populations. According to this scheme, antiinitiating agents might be applied to “primary chemoprevention.”

9.3 MOLECULAR TARGETS FOR CHEMOPREVENTIVE DIETARY BLOCKING CONSTITUENTS

9.3.1 MODULATION OF XENOBIOTIC-METABOLIZING PHASE I AND PHASE II ENZYMES

Most chemical carcinogens are genotoxic, causing DNA damage by reacting with DNA bases and forming adducts. Endogenous carcinogens, which are often reactive oxygen species (ROS) generated as a part of normal oxidative metabolism or as a
result of metabolism of xenobiotic compounds, or induced by ultraviolet and gamma radiation, can also cause extensive DNA damage. Chemical carcinogens usually require metabolic activation in order to exert their mutagenic and carcinogenic activities. The key role in the activation of chemical carcinogens is played by the cytochrome P450 (CYP) family of enzymes (Phase I monooxygenases). These enzymes transform chemicals to biologically inactive metabolites as well as to chemically reactive electrophilic intermediates that covalently bind to specific sites in DNA, initiating a carcinogenic response. The active metabolites may undergo additional metabolism by Phase II enzymes, e.g., glutathione-S-transferase (GST), UDP-glucuronosyltransferase, epoxide hydrolase, and NAD(P)H:quinone reductase, to biologically inactive products (Conney, 2003).

To date, 19 CYP gene families comprising over 500 enzymes have been identified in mammalian species (http://drnelson.utmem.edu/cytochromeP450.html). Members of the CYP 1 to 3 families are mostly involved in the activation of chemical carcinogens. The other P450 proteins catalyze many important endogenous processes, including steroid hormone and prostaglandin biosynthesis (Smith et al., 1998).

Dietary chemicals may affect the level of CYP isozymes by altering, e.g., the rates of transcription. They may also degrade P450 through protein turnover or by suicide inhibition. Moreover, many dietary chemicals are substrates of the P450-dependent monooxygenase system. They or their metabolites may inhibit or enhance the activities of this system by binding to CYP species or to NADPH:reductase, or by affecting key steps in the catalytic cycle (Yang et al., 1992; 1994). Although Phase I enzymes metabolize procarcinogens to biologically active and inactive products, induction of Phase I enzymes is usually but not always associated with inhibition of carcinogenesis.

The induction of CYP-dependent monooxygenases by diet was demonstrated in pioneering work by Wattenberg (Wattenberg et al., 1971). In studies in which dry vegetable powder was added to a purified diet, Brussels sprouts, cabbage, turnips, and other vegetables were found to be inducers of intestinal benzo[a]pyrene hydrox-

![Figure 9.2](image_url)
ylase in rats. Further studies on cruciferous vegetables showed that autolytic products of indolylmethyl glucosinolate, particularly indole-3-carbinol, were responsible for this effect. It was demonstrated that after an oral dosage of indole-3-carbinol to rats, P450 1A1 mRNA was elevated several-fold in the liver and colon, and P450 1A2 mRNA was also elevated in the liver (Vang et al., 1990). The mRNA transcripts for hepatic CYP1A1, 1B1 and 2B1/2 in liver and mammary gland were also up-regulated after oral treatment of rats with indole-3-carbinol (Horn et al., 2002). Another example of transcriptional regulation is the induction of P450 2B1 by diallyl sulfide, a component of garlic oil (Yang et al., 1992). Inactivation of P450 was demonstrated for several dietary chemicals. The inactivation of CYP2E1 by diallyl sulfide is the result of its initial activation and then conversion by this enzyme to a reactive intermediate that modifies the heme moiety of P450 2E1 (Brady et al., 1991). A similar effect was demonstrated for resveratrol, a constituent of grapes and red wine (Mikstacka et al., 2002). Mechanism-based inactivation of CYP1A1 by naturally occurring coumarins was demonstrated in mouse liver (Cai et al., 1996).

Dietary constituents can bind to the active sites of P450 enzymes, serving as substrates or competitive inhibitors. Diallyl sulfide and phenethyl isothiocyanate are competitive inhibitors of CYP2E1. Many dietary flavonoids and phenolic acids have been shown to inhibit CYP1A1- or 3A4-mediated reactions in human or mouse microsomes (Buening et al., 1981; Guengerich et al., 1991; Baer-Dubowska et al., 1998). More recently, CYP1B1 was intensively studied as a target for chemopreventive strategies. This P450 is expressed in a number of human tissues in which cancer occurs; it not only activates many chemical carcinogens but also catalyzes hydroxylation of estrogens, considered to be an important step in hormone-related carcinogenesis. Resveratrol and its substituted derivatives were shown to be effective inhibitors of CYP1B1, which may be useful in preventing cancer caused by estrogens and xenobiotics (Guengerich et al., 2003).

The production of reactive metabolites is largely dependent on primary metabolism by cytochrome P450 enzymes. Secondary metabolism via conjugation is principally but not invariably detoxifying. Elevated levels of Phase II enzymes help to protect organisms from chemical carcinogens and from other toxic effects of electrophiles. The importance of GST for polycyclic aromatic hydrocarbons was emphasized by a study indicating enhanced DMBA-induced skin carcinogenesis in mice lacking the \( \pi \) class of GST, when compared with DMBA-induced skin carcinogenesis in wild-type mice (Henderson, 1998). In humans, genotype \( GSTM1*0 \) is considered a cancer risk factor (Alexandrov et al., 2002). Thus, induction of individual classes of GST might be particularly important for some individuals since these forms are involved in conjugation of specific chemicals.

Inducers of Phase II enzymes belong to two families: monofunctional inducers that elevate Phase II enzymes selectively and bifunctional inducers that upregulate both Phase I and Phase II enzymes (Talalay et al., 2003). Bifunctional inducers are large planar polycyclic aromatic compounds that are potent ligands for the aryl hydrocarbon (Ah) receptor and thereby activate the transcription of the gene battery, which includes \( CYP1A \) and certain Phase II enzymes such as GST (Nebert et al., 1993). In sharp contrast, monofunctional inducers of Phase II genes are chemically highly diverse and lack any obvious identifying structural characteristics. The finding
that electrophilic acceptors in the Michael reaction are inducers of Phase II enzymes, and that their induction potencies correlate closely to their reactivity in this reaction, provided the first systematic insight into the chemical nature of these inducers. It was therefore perplexing that most classes of inducers were electrophiles. Whereas some inducers are oxidants, others are antioxidants, and a third group (e.g., dietary isothiocyanates) are neither.

Many Phase II genes contain the consensus sequence designated the antioxidant response elements (ARE) (Talalay et al., 2003). Monofunctional inducers are more desirable candidates for chemoprevention since, as was mentioned earlier, the overwhelming function of Phase II enzymes is the inactivation of electrophiles and reactive oxygen intermediates. However, not many dietary pure monofunctional inducers have been described so far. Isothiocyanate sulforaphane, the hydrolysis product of its thioglucoside glucoraphanin, a constituent of cruciferous vegetables, is considered a monofunctional inducer of Phase II enzymes (Fahey et al., 1997). Surprisingly, recent studies by Paolini et al. (2004) have shown that glucoraphanin in rat lungs only slightly induced Phase II enzymes but powerfully induced Phase I carcinogen-activating enzymes. Concomitant with Phase I induction, glucoraphanin generated ROS. Thus, the regular administration of this compound could actually increase rather than decrease cancer risk, especially in individuals exposed to environmental mutagens and carcinogens, such as those found in tobacco smoke.

Many chemical carcinogens are metabolized by P450 enzymes to noncarcinogenic as well as to proximate and ultimate carcinogenic metabolites. The effects of inducers of these enzymes as well as inhibitors on the carcinogenicity of chemicals will depend on the effect on the ratio of metabolism of the carcinogen to inactive and active metabolites by both Phase I and Phase II enzymes.

9.3.2 Scavenging of Reactive Metabolites

Besides interfering with carcinogen activation and detoxification pathways, dietary compounds may act as scavengers of reactive metabolites of carcinogens and prevent their binding to DNA. A common feature of the ultimate metabolites of all procarcinogens, as well as directly acting carcinogens, is their electrophilicity. The electrophilic metabolites may themselves be reactive oxygen species (ROS) and interact as such with DNA. Moreover, oxygen-derived free radicals may also be involved in a step required for activation of a procarcinogen, and thus the reactions involved in metabolic activation of carcinogens may release ROS that can in turn attack DNA (Perchellet et al., 1995). Scavenging DNA-reactive intermediates with antioxidants or other agents that can trap electrophiles presents a plausible strategy to modulate the early stages of carcinogenesis. Such a mechanism of anticarcinogenic activity has been proposed for dietary phenols such as ellagic acid, tannic acid, and epigallocatechin-3-gallate (EGCG), the major polyphenolic antioxidant found in green tea. The latter reportedly can trap the activated metabolites of several procarcinogens (Stoner and Mukhtar, 1995). Tannic acid selectively inhibited the formation of DMBA–dAdo DNA adducts \textit{in vitro} and \textit{in vivo} in mouse epidermis (Ignatowicz et al., 2003; Szaefer et al., 2004). This finding might further affect the Ras signaling pathway since dAdo adducts to a greater extent than dGuo are responsible for Ras gene mutation.
9.3.3 Enhancement of DNA Repair

Although the gene products and general mechanisms of DNA repair in prokaryotes are fairly well characterized, mammalian repair systems have only recently been elucidated, and relatively little is known about the influence of dietary factors on these processes. EGCG, lycopene, and selenium enhance unscheduled DNA synthesis and other measures of repair capacity such as inhibition of base substitution. Dietary energy restriction has also been shown to increase apoptotic cell death in heavily damaged cells, thereby accelerating the elimination of cells with irreparable DNA damage (Hursting et al., 1999; Klein et al., 2003). Examples of chemopreventive dietary blocking constituents are presented in Table 9.1.

<table>
<thead>
<tr>
<th>Proposed Mechanism of Action</th>
<th>Dietary Constituent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition of formation/activation of carcinogen by Phase I enzymes</td>
<td>Indole-3-carbinol and other sulfur-containing constituents of cruciferous plants, diallyl sulfoxide and other garlic oil constituents, coumarins, polyphenols, resveratrol, epigallocatechin-3-gallate</td>
</tr>
<tr>
<td>Induction of Phase II conjugation pathways</td>
<td>Isothiocyanates, indoles, tea polyphenols, resveratrol</td>
</tr>
<tr>
<td>Antioxidant activity and scavenging of ROS</td>
<td>Provitamins and vitamins (carotenoids, α-tocopherol, ascorbic acid), flavonoids and phenolic acids, selenium</td>
</tr>
<tr>
<td>Scavenging of reactive metabolites and/or protection of DNA nucleophilic sites</td>
<td>Ellagic, tannic, and other plant phenolic acids, retinoids</td>
</tr>
<tr>
<td>Enhancement of DNA repair</td>
<td>Epigallocatechin-3-gallate, selenium, soy protease inhibitors, and genistein</td>
</tr>
<tr>
<td>Induction of apoptosis of cells with irreparable DNA damage</td>
<td>Retinoids, genistein, flavonoids</td>
</tr>
</tbody>
</table>

9.4 Molecular Targets for Chemopreventive Dietary Suppressing Constituents

9.4.1 Signal Transduction Modulators

The tumor promotion phase of multistage carcinogenesis involves the clonal expansion of the initiated cells. Tumor-promoting agents are not mutagenic as carcinogens are but rather alter the expression of genes whose products are associated with hyperproliferation, apoptosis, inflammation, and tissue remodeling. Changes in gene expression as a consequence of external tumor promoter stimuli usually activate (sometimes inactivate) specific signal transduction pathways (Hursting et al., 1999).

There are 17 known signal transduction pathways plus at least two stress response pathways (Nebert, 2002). The transduction pathways are controlled by cascades of cellular transduction molecules triggered by hormones or growth factors, e.g., epi-
dermal growth factor (EGF) or platelet-derived growth factor (PDGF), and in carcinogenesis they are either faultily expressed or activated by tumor promoters. Thus, many of the substances that are pivotal in cell signaling are possible chemopreventive targets (Table 9.2). One example of such a substance is curcumin, a major constituent of the spice turmeric, which inhibits the activity of several kinases involved in cell signaling, such as protein kinase C (PKC), epidermal growth factor receptor kinase, and mitogen-activated protein kinases. Such inhibition is not necessarily a corollary of direct enzyme interaction but can reflect interference with activating elements upstream of the kinase (Gescher et al., 2001 and references therein).

Many promoters work through receptor-mediated mechanisms. For example, TPA interacts with specific receptors that are isoforms of PKC. Others, such as okadaic acid, are potent inhibitors of phosphatases and increase the level of phosphorylated proteins, an effect similar to activation of kinases (Fischer and DiGiovanni, 1995). PKC can be modulated by dietary compounds such as the flavonoids quercetin or curcumin; however, inhibition of this enzyme does not seem to be the major mechanism of antipromotional activity of these compounds since they also affect other kinases (DiGiovanni and Fischer, 1995; Gescher et al., 2001).

Regardless of the promoter character, promoters trigger a cascade of events that include, among others, increased DNA synthesis and induction of ornithine decarboxylase (ODC). The inhibition of ODC induction by the plant flavonoids quercetin and apigenin was related to inhibition of tumor promotion by TPA in mouse skin (Wei et al., 1990).

Beside signaling pathways initiated by G-protein–coupled receptors and mediated by PKC, activation of receptor tyrosine kinases (RTK) is an equally important pathway in the control of cell growth and differentiation. A key component of many RTK reaction cascades is the protein Ras, the product of protooncogene ras. Its activation

<table>
<thead>
<tr>
<th>Proposed Mechanism of Action</th>
<th>Dietary Constituent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modulation of signal transduction</td>
<td>PKC inhibitors (curcumin, genistein, retinoids), farnesylation inhibitors (limonene, perillyl alcohol), ODC inhibitors (flavonoids, e.g., quercetin and apigenin)</td>
</tr>
<tr>
<td>Modulation of cell cycle control and induction of apoptosis</td>
<td>Flavonoids (e.g., quercetin, flavopiridol, and 2-thiophenylfluoridol), retinoids</td>
</tr>
<tr>
<td>Hormone modulations</td>
<td>Aromatase inhibitors (soy isoflavonoids), inducers of estradiol hydroxylation (indole-3-carbinol)</td>
</tr>
<tr>
<td>Suppressing of inflammation and scavenging of ROS</td>
<td>Curcumin, flavonoids, genistein, antioxidants (see Table 9.1)</td>
</tr>
<tr>
<td>Modulation of transcription factors’ expression or activation</td>
<td>Curcumin, green tea polyphenols, anthocyanins, isothiocyanates</td>
</tr>
<tr>
<td>Inhibition of angiogenesis</td>
<td>Flavonoids, soy isoflavonoids</td>
</tr>
<tr>
<td>Correction of DNA methylation imbalances</td>
<td>Folic acid</td>
</tr>
</tbody>
</table>

TABLE 9.2
Examples of Dietary Suppressing Agents in the Chemoprevention of Cancer
Chemoprevention of Cancer: Basic Mechanisms and Molecular Targets

through point mutation is a target for many carcinogens and leads to constitutive signaling of the RTK pathway (Cantley et al., 1991). Oncogenic mutations in the ras allele occur in approximately 30% of human cancers. Ras, like a variety of other proteins involved in cancer development, undergoes post translational modification, farnesylation (Rowinsky et al., 1999). Several agents have been identified that specifically block post translational Ras farnesylation. At least one natural product, \( \alpha \)-limonene from citrus fruits, with the reported ability to disrupt Ras signaling mechanisms through inhibition of Ras post translational modification, has been shown to inhibit tumorogenesis in rats (Crowell et al., 1994). The primary function of activated Ras (Ras-GTP) is to recruit and activate another protein, Raf, that initiates a chain of mitogen-activated protein kinases (MAPKs) in a cascade. Once activated, the last protein kinase in the signaling cascade translocates to the nucleus, where it phosphorylates and activates specific transcription factors that bind to promoter regions of a number of genes controlling cell differentiation and proliferation.

Cell-cycle regulatory proteins and checkpoints are downstream elements of cellular signaling cascades crucial for cell proliferation; they are the other targets for chemopreventive suppressing agents. Alterations of the cyclin gene family, as well as cyclin-dependent kinase inhibitors and tumor suppressor proteins such as Rb and p53, which underlie the development of many human tumors, might be particularly important targets for potential chemopreventive agents (Johnson et al., 1995). Examples of such compounds include the flavonoids quercetin, flavopiridol, and 2-thioflavopiridol described in this volume (Chapter 13).

For example, monoterpenes such as limonene and perillyl alcohol, which are common compounds derived from the essential oils found in fruits and vegetables, selectively inhibit proliferation via a G1 block, inducing apoptosis (Willis and Colburn, 2002; Ripple, 2000). Similarly to cell proliferation, which depends on activation of a signal transduction cascade that ultimately leads to an altered pattern of gene expression, apoptosis appears as a cellular response to extracellular signals, such as growth factor deprivation. Apoptosis emerged as a critical target for prevention when it became clear that apoptosis and mitogenesis are equally important in the homeostasis of cell number. Moreover, the growth advantage manifested by initiated cells during promotion is usually the net effect of increased proliferation and decreased apoptosis. Evidence suggests that the Ras signal transduction pathway may also play a role in the triggering of the programmed cell death pathway (Trent and Ananthaswamy, 1995). Blocking Ras farnesylation and inducing apoptosis by limonene seem to confirm the relationship between these two signaling pathways. Kong et al. (2000), using mammalian cell lines, have shown that naturally occurring phenolic compounds and isothiocyanates affect both MAPK cascade activation and apoptosis. At low concentrations, they activate MAPKs, leading to expression of survival and defensive genes. Increasing the concentrations of these compounds activates the caspase pathway, leading to apoptosis.

### 9.4.2 Hormone Modulators

Hormones affect carcinogenesis mostly by epigenetic mechanisms, such as stimulation of cell proliferation of estrogen-dependent target cells and reprogramming of
cellular differentiation. In addition, significant evidence exists that certain estrogens can also cause genetic alterations by mechanisms not involving the classic estrogen receptor (Barrett, 1991).

Epidemiological and experimental evidence strongly supports a role for estrogens in the development and growth of breast and prostate tumors. Therefore, one chemopreventive strategy for breast and prostate cancers is to decrease estrogen production and activity in these tissues. Particularly interesting are two groups of compounds: antiestrogens and aromatase inhibitors. Antiestrogens compete with estrogen for estrogen receptor binding sites. Aromatase inhibitors inhibit an enzyme complex that consists of the specific cytochrome P450 (CYP19) and flavoprotein NADPH cytochrome P450 reductase that catalyze the synthesis of estradiol (Kelloff, 2000). Isoflavonoids from soy are an example of dietary compounds interacting with these enzyme systems (Chapter 16).

9.4.3 Suppressing Inflammation and Scavenging Reactive Oxygen Species

In addition to inducing changes in gene expression by activating specific signaling pathways, tumor promoters can elicit the production of proinflammatory cytokines (such as tumor necrosis factor and several interleukins) and nonprotein factors (such as nitric oxide and ROS) involved in inflammation and carcinogenesis (Hursting et al., 1999). Chronic inflammation and oxidative stress contribute to experimental and human carcinogenesis (Ohshima and Bartsch, 1994; Frenkel, 1997). Release of arachidonic acid from cellular membranes and its metabolism to eicosanoids are of critical importance to cancer promotion (Hursting et al., 1999). Eicosanoids, which include the prostaglandins (PGs) and hydroxy forms of arachidonic acid, are involved in inflammation, the immune response, tissue repair, and cell proliferation.

Prostaglandin synthesis is regulated by cyclooxygenase (COX) gene expression. Two separate gene products, COX-1 and COX-2, have similar cyclooxygenase and peroxidase activities, although they are differently regulated. COX-1 is a constitutive isoform present in most tissues; it mediates the synthesis of PGs required for normal physiological functions. COX-2 is not detectable in most normal tissues, but it is induced by cytokines, growth factors, oncogenes, and tumor promoters and is involved in several experimental and human cancers (Subbaramaiah et al., 1997; Prescott and Fitzpatrick, 2000). Treatment with selective inhibitors of COX-2 such as celecoxib, a novel nonsteroidal antiinflammatory drug (NSAID), caused the regression of polyps in patients with familial colon adenomatous polyposis, which is a precancerous condition (Willis and Colburn, 2002).

COX-2 seems to be the target for many dietary constituents, of which curcumin has been the most extensively studied. This phytochemical also inhibits the PKC pathway and gene expression mediated by the transcription factors AP-1 and NFκB (Subbaramaiah et al., 1997; Gescher et al., 2001). Thus curcumin may affect the transcription of the COX-2 gene at more than one level. A similar mechanism of eliciting biological effects was suggested for retinoids. These naturally occurring and synthetic analogs of vitamin A suppress carcinogenesis in various tissues by stimulating differentiation, apoptosis, and immune recognition of aberrant cells.
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Nitric oxide (NO), which is produced by constitutive and inducible forms of nitric oxide synthase (NOS), mediates many physiological (smooth muscle relaxation, vasculature tone and circulation, neurotransmission, apoptosis) and pathological (inflammation) processes. Two constitutive forms of NOS, endothelial and neuronal, produce “puffs” of NO in a highly controlled and transient manner and are regulated by exogenous Ca^{2+} and calmodulin. Inducible NOS (iNOS) is not dependent on the exogenous Ca^{2+}/calmodulin system and is regulated primarily at the transcriptional level for hours to days after induction/stimulation (e.g., bacterial infection) (Kelloff, 1999). iNOS is responsible for the overproduction of NO that is observed during inflammation (MacMicking et al., 1997) and tumor development (Rao et al., 1999). Increased iNOS expression and activity were reported in human gynecological (Thomsen et al., 1994), breast (Thomsen et al., 1995), and central nervous system (Cobbs et al., 1995) tumors.

Several lines of evidence indicate a specific association between iNOS and COX-2. For example, inflammatory cytokines induce both iNOS and COX-2 in different cell lines, and these isozymes are concurrently induced in inflammatory tissues in vivo. Moreover, nitric oxide induces COX-2 activity, thus indirectly it augments the PG synthesis. NO additionally exerts cytotoxic effects, a feature that is not attributed to PGs. The feasibility of suppressing tumor growth by diminishing iNOS activity in tumor cells was demonstrated using the highly specific iNOS inhibitor 1400W (Thomsen et al., 1997). Genistein, curcumin, and EGCG suppress induction of iNOS. Direct scavenging of NO by flavonoids and curcuminoids may also contribute to the chemopreventive actions of these compounds; however, inhibition of iNOS activity appears at present to be the most useful and specific approach for regulating NO production (Kelloff, 2000).

ROS, besides the role mentioned above in carcinogenesis initiation, are involved in important events related to promotion and progression, e.g., in cell-cycle progression induced by Ras (Irani et al., 1997). As can be expected from their involvement in redox reactions, the NO and ROS systems can interact. Concurrent local increased production of ROS, e.g., by immune cells, can generate even more reactive compounds such as ONOOH, which instantly decomposes to hydroxyl radical, the most aggressive DNA and lipid oxidant (Pryor and Squadrito, 1995). Thus, the balance between the NO and ROS generating systems may play a key role in regulating cell growth and integrity, which implies that manipulating the redox status of the cell may be an effective way to control NO production.

Chemopreventive antioxidants suppress ROS formation by direct quenching of free radicals, inhibition of enzymes involved in ROS generation (e.g., NADPH-oxidase, xanthine oxidase, COX), and transient metal sequestration, which prevents the formation of hydroxyl radical from more benign precursors. Many naturally occurring antioxidants including ascorbic acid, carotenoids, and polyphenolic compounds found in green tea, spices, fruit, and vegetables have been shown to effectively inhibit both tumor initiation and promotion (Hurting et al., 1999; Kelloff, 2000). Some compounds, e.g., curcumin, may act as potent antioxidants but also as prooxidants. The balance between these properties depends on the presence of
metal ions. When copper (II) ions are present, curcumin can lead to the formation of ROS (Ahsan et al., 1999). Therefore, at high ingested doses in organs accessible to it, curcumin’s beneficial antioxidant properties may be masked by unwanted prooxidant effects.

9.4.4 TRANSCRIPTION FACTORS AS TARGETS FOR CHEMOPREVENTION

Transcription factors participate in the final stages of all transduction and stress pathways by causing the up- or down-regulation of specific genes (Nebert, 2002). Among them, two are particularly interesting as targets for chemoprevention: activator protein-1 (AP-1) and nuclear factor-κB (NFκB) (Li et al., 1997).

AP-1 activation occurs in response to many external stimuli, resulting in induction of AP-1 target genes involved in mitogenesis, differentiation, transformation, or inflammation, depending on the stimulus and tissue type (Willis and Colburn, 2002). For example, the UV irradiation signal is transduced from the plasma membrane via a phosphorylation cascade to the AP-1 transcription factor (Rosette and Karin, 1996). A variety of other stimuli, such as phorbol esters (Dong et al., 1994), growth factors (Lamb et al., 1997) and oxidative agents (Pinkus et al., 1996), can stimulate AP-1 activity by MAPKs. AP-1 regulates transcription of many genes involved in carcinogenesis and is itself up-regulated during tumor promotion and progression (Dong et al., 1994; Domann et al., 1994; Saez et al., 1995). The AP-1 transcription factor complex is made of Jun homodimers and Jun-Fos heterodimers, which bind to and transactivate a cis element called the TPA-response element. Agents that inhibit AP-1 activity have been shown to be efficacious against carcinogenesis (Bair et al., 2002). These include green tea polyphenols and their major constituent EGCG (Barthelman, 1998) and anthocyanins from purple sweet potato and red cabbage (Hou et al., 2004). Identification of the functionally significant AP-1 target genes is expected to reveal candidate genes that might alone or in combination constitute even more effective molecular targets for cancer prevention.

Several genes that mediate processes involved in carcinogenesis are regulated by the transcription factor NF-κB. It plays a central role in general inflammatory as well as immune responses. The active NF-κB complex comprises two subunits designated p65 (RelA) and p50 (Gilmore et al., 1996). NF-κB is present in most cells, where it remains in an inactive form in the cytoplasm bound with inhibitory protein IκB. After stimulation with a number of agents, e.g., cytokines, phorbol esters, and viral infections, active NF-κB is released and then is translocated to the nucleus, with the rapid degradation of IκB (Pahl, 1999).

NF-κB has been shown to be a critical regulator of COX-2 expression (Kojima et al., 2000). Moreover, it was shown at least in a mouse skin model that NO induces expression of COX-2 through activation of NF-κB. Thus, curcumin inhibits induced COX-2 expression through inhibition of NF-κB activation (Chun et al., 2004).

Ursolic acid, a triterpenoid derived from several medicinal plants, inhibits NF-κB activation, which correlates with suppression of NF-κB–dependent cyclin D1 (cell cycle regulation), COX-2, and matrix metalloproteinase 9 expression (Shishodia et al., 2003). The latter is a member of endopeptidase family that can selectively
degrade extracellular matrix proteins and nonmatrix proteins and therefore plays a central role in tumor invasion, angiogenesis, and metastasis (John and Tuszynski, 2001). Its gene promoter sequence, similarly to \textit{cox-2}, contains a binding site for NF-\kappaB.

The expression of Phase II enzyme genes is controlled by the Nrf2 transcription factor and its repressor protein Keap 1. Under basal conditions, Nrf2 is retained in the cytosol by binding to Keap 1. The inducer, such as isothiocyanate, disrupts the Nrf2-Keap 1 complex, and the released Nrf2 migrates to the nucleus, where it binds to ARE and stimulates Phase II gene transcription (Talalay et al., 2003). As was mentioned in Section 9.4.1, such compounds as isothiocyanates and green tea polyphenols at low concentrations activate MAPKs and thus lead to activation of Nrf2 and ARE, with subsequent induction of Phase II and other defensive genes. At higher concentrations, these agents activate the caspase pathways, leading to apoptosis (Kong et al., 2000; 2001).

9.4.5 Modulation of the Events Related to the Progression Stage of Carcinogenesis and the Epigenome

Tumor progression involves the accumulation of additional genetic alterations in a neoplastic cell clone. In the progression stage, a focal lesion consisting of a population of initiated and promoted cells ultimately becomes an invasive malignant tumor. One frequently observed genetic alteration that appears to contribute to malignant progression is mutation in the p53 tumor suppressor gene. Its product, p53 protein, regulates the expression of a number of DNA damage and cell-cycle and apoptosis-regulating genes (Harris, 1993). By enhancing transcription of these critical genes, p53 regulates the cellular response to damage and plays a role in maintaining genomic stability (Smith et al., 1995; Guinn and Mills, 1997). Genomic instability, a hallmark of spontaneous malignant progression, is characterized by sequential chromosomal aberrations, such as duplications, deletions, and loss of heterozygosity, which lead to rapid accumulation of unfavorable genetic alterations and eventually to malignant cell growth. Unchecked activity of telomerase (the enzyme responsible for replication of the DNA of telomeres) has been also associated with genetic instability of transformed cells; EGCG and other tea catechins can inhibit this enzyme (Naasani et al., 1998).

As a tumor grows in size, it stimulates the formation of new blood vessels, a process termed angiogenesis. Cancer cells promote angiogenesis by secreting growth factors that act on the endothelial cells of surrounding blood vessels, stimulating them to proliferate. Inhibiting the tumor’s ability to form new blood vessels has become a promising anticancer strategy. Chapter 13 presents examples of flavonoids possessing such activities.

In the postgenomic era of cancer biology, it is also becoming increasingly evident that epigenetic controls of gene expression play an important role in the development of cancer. Alterations in gene expression without changes in DNA coding sequences that are heritable through cell division occur throughout all stages of carcinogenesis and are involved in silencing tumor suppressor genes. Two changes integral to epigenetic transcriptional control are DNA methylation and covalent
modification of histone proteins (Kopelovich et al., 2003). In cancer cells, genome-wide hypomethylation is accompanied by hypermethylation of short CpG-rich regions known as CpG islands, with unique patterns of individual gene methylation exhibited by each tumor type (Esteller, 2002; Costello et al., 2000). Hypermethylation of promoter regions of genes is associated with transcriptional silencing and is at least as common as mutation as a mechanism for inactivation of classic tumor suppressor genes in human cancers (Kopelovich et al., 2003). A broad spectrum of genes aberrantly methylated in cancers includes those associated with carcinogen metabolism and DNA repair, the cell cycle, signal transduction, apoptosis, hormone response, angiogenesis, and invasion and/or metastasis (Esteller et al., 2001; Costello and Plass, 2001).

The second major layer of epigenetic transcriptional control that has been widely studied is modification of histone proteins, particularly acetylation. These two means of epigenetic control, DNA methylation catalyzed by a family of DNA methyltransferases and histone acetylation catalyzed by acetyltransferases, are integrally linked. For example, DNA methyltransferases recruit histone deacetylases, leading to histone deacetylation and transcriptional repression (Kopelovich et al., 2003). Unlike tumor suppressor genes inactivated by genetic alterations, genes silenced by epigenetic mechanisms are intact and responsive to reactivation by small molecules. Many diverse genes hypermethylated in cancers can be reactivated with DNA methyltransferase inhibitors (Goffin and Eisenhauer, 2002). It is clear that some epigenetic changes are among the earliest events observed during cancer development, making them excellent targets for chemoprevention. One dietary compound that can correct DNA methylation imbalance is folic acid, a vitamin provided by various types of food. Moreover, the unique epigenetic fingerprint observed, e.g., in patients with preinvasive esophageal lesions that progressed to more advanced lesions (Eads et al., 2001) underscores the potential use of epigenetic markers in risk assessment and early detection.

9.5 SUMMARY AND CONCLUSIONS

Since the pioneering work of Wattenberg, a wide array of phytochemicals with possible anticancer and other beneficial effects has been recognized. Most of these diet-derived agents show pleiotropic activities. Thus, although convenient, their traditional classification into blocking and suppressing compounds seems not always appropriate. Moreover, these compounds may reveal novel, sometimes unexpected effects, as new mechanisms in carcinogenesis are being discovered. One example is the recently described new role of the aryl hydrocarbon receptor, indicating that this activated receptor acts as an environmental sensor and cell cycle checkpoint that commits cells exposed to adverse environmental stimuli to arrest before the onset of DNA replication (Puga et al., 2002). It follows that compounds such as resveratrol, which inhibit AhR activation, not only diminish carcinogen metabolism but also affect the cell cycle.

The appropriate use of a chemopreventive agent ultimately depends on the understanding of its mechanism of action at all levels, namely at the molecular, cellular, tissue, and organ levels, as well as in the animal as a whole. Based on such
knowledge, the trend in the field of chemoprevention has been to develop new agents based on their mechanism of action (Sporn, 2000). Resveratrol is a dietary prototype that is currently the subject of modifications (Chun et al., 2001, Chapter 12 of this book). Moreover, many of the targets outlined above — angiogenesis, kinases, transcription factors, apoptosis — are intrinsically related to the hallmarks of cancer. Not surprisingly, these targets are also under investigation as targets for molecular or “antisignaling” chemotherapeutic anticancer drugs. Consequently, novel antisignaling drugs warrant clinical evaluation as potential cancer-chemopreventive agents if they lack adverse effects (Gescher, 2001). On the other hand, it is obvious that the human diet contains constituents with various chemopreventive mechanisms in the food matrix, thus offering unique natural combinations. However, diet analysis of the evidence from observational epidemiology allows only very limited conclusions to be drawn about the enhancing or inhibiting effects of particular eating patterns, foods, or food components on cancer risk. The most compelling evidence about benefits or risks of dietary exposure to particular foods will derive from well-designed and controlled human intervention trials. The clinical trials performed so far did not provide clear indications. Some, such as those with β-carotene, yielded negative outcomes, while others, such as with folate, enhanced the credibility of dietary chemoprevention as a practical approach to the control of cancer.

An important fact to recognize is that most of the promising effects of food components seen in experimental models were achieved at concentrations that are unlikely to be achieved in humans. Quantitative pharmacokinetic information, particularly about oral bioavailability, is therefore required. The human diet is known to contain mutagens, carcinogens, and anticarcinogens. The ultimate effect of food on human health will depend on the balance among all these components.

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Carcinogenic and Anticarcinogenic Food Components


10 Chemopreventive Phenolic Compounds in Common Spices

Young-Joon Surh

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10.1 SPICES AS A RESERVOIR OF CHEMOPREVENTIVE PHYTOCHEMICALS

Spices, including dried roots, bark, buds, fruits, seeds, or berries, are plant products used to enhance the flavor, color, and palatability of foods and beverages. Today,
spice use is ubiquitous and decidedly nonrandom, although the frequency of use of individual spices varies among societies. For instance, pepper, ginger, turmeric, cloves, cinnamon, and nutmeg can be found in the cooking of almost every country around the world. The phrase “spice of life” is no mere figure of speech. Spices play a major role in our lives as exotic and aromatic enhancement to food, as folk medicine, and even as modern remedies for today’s ailments. There is increasing evidence supporting the considerable health benefits of spices and their ingredients. Each spice has a unique spectrum of secondary metabolites that have evolved in plants to protect them against biotic enemies including insects, vertebrates, fungi, and parasites. Most spices contain dozens of such self-defensive phytochemicals, collectively called phytoalexins, that are plants’ recipes for survival strategies of their coevolutionary races against biotic enemies. Many phytoalexins in spices are phenolic substances with strong antioxidant and antiinflammatory properties that may confer chemopreventive activity (Fisher, 1992; Nakatani, 1992; 2000; Surh, 2002a). The following sections summarize the chemopreventive effects of selected antioxidant and antiinflammatory phenolics derived from representative spices.

10.2 CURCUMIN FROM TURMERIC

Turmeric (Curcuma longa L.) belongs to the ginger family (Zingiberaceae), and has been used for centuries as a natural food colorant and preservative. Numerous scientific investigations, as well as millennia of experience in folk medicine, reveal the breath of therapeutic potential of this spice in addition to its equally broad flavoring or coloring potential. Turmeric has been shown to ameliorate inflammation, ulceration, platelet aggregation, lipid oxidation, and the growth of microbes. Moreover, whole turmeric extract or standardized preparations containing defined amounts of its ingredients have been shown to exert antitumorigenic effects by inhibiting both initiation and later stages (i.e., promotion or progression) of carcinogenesis.

The ground dried rhizome of turmeric contains curcumin (structure shown in Table 10.1), which provides yellow pigmentation of this plant. As a major yellow-coloring component of turmeric, curcumin exhibits antiinflammatory and antioxidant properties and counteracts malignancies induced by a variety of chemical carcinogens in laboratory animals (vide infra).

10.2.1 CHEMOPREVENTIVE EFFECTS ON EXPERIMENTALLY INDUCED CARCINOGENESIS

Chemopreventive properties of curcumin have been extensively investigated and well defined (reviewed in Conney et al., 1997; Gescher et al., 2001; Nagabhushan and Bhide, 1992; Surh, 1999; 2002a; 2003). Curcumin acts as both blocking and suppressing agent. Thus, curcumin inhibits the development of chemically induced tumors of oral cavity, skin, forestomach, duodenum, and colon in rodents (Conney et al., 1997). Curcumin inhibited the metabolic activation of 7,12-dimethyl-
benz[a]anthracene (DMBA) and subsequent DNA adduct formation by acting as an antagonist of the arylhydrocarbon (Ah) receptor involved in the expression of cytochrome P450 (CYP) 1A1 and by competitively inhibiting this enzyme in human mammary epithelial carcinoma (MCF-7) cells (Cioloño et al., 1998). Curcumin was also found to inhibit benzo[a]pyrene (B[a]P)-induced forestomach tumorigenesis in mice (Singh et al., 1998), which was attributed to its suppression of hepatic CYP1A1 responsible for the activation of B[a]P and up-regulation of the detoxification enzymes, such as glutathione S-transferases and epoxide hydrolase (Manson et al., 2000). In addition, dietary supplementation of curcumin (2%, w/v) for 30 days significantly increased the activities of glutathione peroxidase, glutathione reductase,

### TABLE 10.1
Chemopreventive Spices and Their Active Ingredients

<table>
<thead>
<tr>
<th>Spice</th>
<th>Active Ingredient(s)</th>
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<tr>
<td>Turmeric (Curcuma longa L., Zingiberaceae)</td>
<td><img src="curcumin.png" alt="Curcumin" /></td>
</tr>
<tr>
<td>Ginger (Zingiber officinale Roscoe, Zingiberaceae)</td>
<td><img src="6-gingerol.png" alt="6-Gingerol" /></td>
</tr>
<tr>
<td>Hot red pepper (Capsicum annuum L., Solanaceae)</td>
<td><img src="capsaicin.png" alt="Capsaicin" /></td>
</tr>
<tr>
<td>Rosemary (Rosemarinus officinalis L., Labiatae)</td>
<td><img src="carnosol.png" alt="Carnosol" /> <img src="rosmarinic-acid.png" alt="Rosmarinic Acid" /></td>
</tr>
<tr>
<td>Cloves (Eugenia caryophyllata Thunb. or Syzygium aromaticum [L.] Merr. et Perry, Myrtales)</td>
<td><img src="eugenol.png" alt="Eugenol" /></td>
</tr>
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</table>
glucose-6-phosphate dehydrogenase, and catalase in the liver and kidney of male ddY mice, as compared with corresponding control animals fed a normal diet. Parallel to these changes, curcumin administration resulted in a considerable enhancement of the activity of glutathione S-transferase and quinone reductase (Iqbal et al., 2003).

However, the literature data also indicate that curcumin is likely to elicit tumor-suppressing properties and act in the later stages of carcinogenesis, interfering with cellular processes involved in tumor promotion and progression. Several kinases, telomerase, cyclooxygenase-2 (COX-2), cell cycle regulators, and transcription factors AP-1 and nuclear factor kappaB (NFκB) are among the cellular targets of this chemopreventive phytochemical (Gescher et al., 2001).

10.2.2 COX-2 AS A POTENTIAL TARGET FOR ANTIINFLAMMATORY AND TUMOR-ANTIPROMOTING EFFECTS OF CURCUMIN

The roles of prostaglandins and other eicosanoids derived from arachidonic acid in the development and progression of human cancer have been known for over two decades. Increased prostaglandin synthesis may influence proliferation, survival capability, angiogenesis, and the metastatic potential of tumor cells, as well as carcinogen metabolism. Arachidonic acid metabolites derived from the COX-2 and lipoxygenase (LOX) pathways are hence important mediators of growth-related signal transduction, implying that intervention through these pathways should be useful for arresting tumor promotion and progression. Clinical trials with nonsteroidal antiinflammatory drugs (NSAIDs) have demonstrated that NSAID treatment causes regression of preexisting colon adenomas in patients with familial adenomatous polyposis (Moran, 2002; Thun et al., 2002). NSAIDs, particularly selective COX-2 inhibitors, can retard, block, or reverse colon carcinogenesis and thus, naturally occurring COX-2 inhibitors offer opportunities for effective chemoprevention (Reddy and Rao, 2002; Surh et al., 2001).

Curcumin is a powerful antiinflammatory agent with many properties in common with NSAIDs such as aspirin. Curcumin, like other inhibitors of COX and LOX, is thought to inhibit carcinogenesis by preventing the formation of arachidonic acid metabolites. Curcumin inhibits arachidonic acid metabolism, and therefore prostaglandin production, by inhibiting COX-2 (Rao et al., 1995). Metabolism of curcumin by human and rat hepatocytes yielded hexahydrocurcumin and hexahydrocurcuminol (Ireson et al., 2001). In rats, curcumin administered i.v. (40 mg/kg body weight) disappeared from the plasma within 1 h of dosing, and the major metabolites in plasma were glucuronide and sulfate conjugates, whereas hexahydrocurcumin, hexahydrocurcuminol, and hexahydrocurcumin glucuronide were present in small quantities. Curcumin and four of its metabolites at a concentration of 20 μM were compared in terms of their ability to inhibit phorbol ester-induced prostaglandin E₂ (PGE₂) production by human colonic epithelial cells (Ireson et al., 2001). Curcumin reduced PGE₂ production to constitutive levels, while tetrahydrocurcumin, hexahydrocurcumin, and curcumin sulfate exhibited only weak PGE₂ inhibitory activities.

Topically applied curcumin inhibited 12-Ο-tetradecanoylphorbol-13-acetate (TPA)-induced COX-2 and LOX activities in mouse epidermal microsomes and
cytosol, respectively (Huang et al., 1991a). This may be explained by suppression of TPA-induced COX-2 expression via inactivation of upstream cellular signaling molecules by curcumin, shown in our studies (Chun et al., 2003). Dietary curcumin also reduced COX and LOX activities and the levels of their major products, PGE$_2$ and hydroxyeicosatetraenoic acid, in the colonic mucosa of rats (Rao et al., 1993; 1995; Reddy and Rao, 2002). Curcumin inhibited TPA-mediated induction of COX-2 in several gastrointestinal cell lines (Zhang et al., 1999) and effected growth inhibition of HT-29 human colon cancer cells (Goel et al., 2001). Curcumin markedly inhibited constitutive expression of COX-2 and its mRNA transcript, but not that of COX-1 in these cells.

10.2.3 EFFECTS ON THE ACTIVATION OF NFkB AND AP-1 TRANSSCRIPTION FACTORS

The molecular mechanisms underlying inhibition of COX-2 by curcumin remain poorly understood. Since the COX-2 gene harbors NFkB binding sequences in its promoter region (Tazawa et al., 1994), curcumin may down-regulate COX-2 expression by blocking the NFkB signalling pathway (Figure 10.1). In support of this assumption, curcumin suppressed both COX-2 expression and NFkB DNA-binding activity induced by model colon tumor promoters, including tumor necrosis factor-alpha (TNF-α) and fecapentaene-12 in human colonic epithelial cells (Plummer et al., 1999). Curcumin blocked TNF-α-induced NFkB transactivation as assessed by the transient transfection assay with an NFkB-luciferase construct. Inactivation of NFkB by curcumin was achieved through blockade of phosphorylation of the inhibitory subunit IkB prompted by the NFkB-inducing kinase (NIK)/IkB kinase (IKK) complex.

In human non–small cell lung carcinoma cells, curcumin abolished cigarette smoke–induced DNA binding and transcriptional activities of NFkB, which correlated with suppression of NFkB–dependent expression of COX-2, matrix metalloproteinase (MMP)-9, and cyclin D1. Inhibition of cigarette smoke–induced NFkB activation and NFkB–regulated gene expression by curcumin appears to be mediated through suppression of IKK (Shishodia et al., 2003).

Intragastric infusion of ethanol led to fatty liver, necrosis, and inflammation in rats, which were accompanied by activation of NFkB and the induction of COX-2, inducible nitric oxide synthase (iNOS), and nitrotyrosine formation. Treatment with curcumin prevented both the pathological and biochemical changes induced by ethanol (Nanji et al., 2003), possibly via suppression of induction of NFkB-dependent genes.

Topical application of curcumin suppressed phosphorylation of cytoplasmic IkBα and nuclear translocation and subsequent DNA binding of p65, a functionally active subunit of NFkB, in TPA-stimulated mouse skin (Chun et al., 2003). Curcumin also suppressed DNA binding of NFkB and also AP-1 in cultured human promyelocytic leukemia (HL-60) cells (Han et al., 2002b). Since the NFkB inhibitor pyrrolidine dithiocarbamate attenuated the TPA-induced COX-2 expression as well as NFkB DNA binding in mouse skin, NFkB may be a molecular target of curcumin in its down-regulation of epidermal COX-2 (Chun et al., 2003).
NFκB plays a central role in cell survival and proliferation in many types of malignancies. In human multiple myeloma cells, curcumin decreased the constitutive IκBα phosphorylation through the inhibition of IKK activity, which led to the suppression of translocation of NFκB to nucleus and its subsequent DNA binding (Bharti et al., 2003). The down-regulation of NFκB also induced chemosensitivity to antitumor agents, such as vincristine and melphalan. In addition, curcumin repressed DNA binding of AP-1, another ubiquitous eukaryotic transcription factor, in TPA-treated mouse skin (Surh et al., 2000) and gastrointestinal cell lines (Zhang et al., 1999). Suppression of TPA-induced activation of AP-1 and expression of its components (c-
Jun and c-Fos) has been considered to be responsible for tumor-antipromoting activity of curcumin (Huang et al., 1991b; Kakar and Roy, 1994; Lu et al., 1994). In addition, curcumin is a potent inhibitor of protein kinase C (PKC) and epidermal growth factor (EGF)-receptor tyrosine kinase (Lin et al., 2000; Liu et al., 1993).

10.2.4 Modulation of Nrf2 Signaling

The transcription factor Nrf2, which normally exists in an inactive state as a consequence of binding to a cytoskeleton-associated protein Keap1, can be activated by stimuli that affect cellular redox status. Alteration of the Nrf2-Keap1 interaction enables Nrf2 to translocate to the nucleus, bind to the antioxidant-responsive element (ARE) and initiate the transcription of genes coding for Phase II detoxification/antioxidant enzymes and cytoprotective proteins. Curcumin has been reported to stimulate the expression of Nrf2 in porcine renal epithelial cells; this was associated with a significant increase in the expression and catalytic activity of heme oxygenase-1 (HO-1), a redox-sensitive inducible protein that provides protection against oxidative and nitrosative stress (Balogun et al., 2003).

Curcumin stimulates transcriptional activation of the HO-1 gene by promoting dissociation of the Nrf2-Keap1 complex, leading to increased Nrf2 binding to the resident HO-1 AREs. The p38 mitogen-activated protein kinase (MAPK) appears to be involved in curcumin-mediated HO-1 induction (Balogun et al., 2003). In another study, curcumin caused increased nuclear translocation and ARE binding of Nrf2, thereby inducing expression of glutamate cysteine ligase (Dickinson et al., 2003). Curcumin bears an α,β-unsaturated ketone moiety and can therefore act as a Michael-reaction acceptor capable of modifying cysteine thiols located in Keap1. Alternatively, the compound may phosphorylate specific serine and/or tyrosine residues of Nrf2 through activation of upstream kinase(s), which will facilitate the nuclear translocation and ARE binding of Nrf2.

The induction of antioxidant or carcinogen-detoxifying enzymes by curcumin suggests the potential value of this compound as protective agent against chemical carcinogenesis and other forms of electrophilic toxicity. It is proposed that curcumin may suppress tumor initiation, promotion, and/or progression by modulating one or more of the aforementioned signal transduction pathways in the target cells.

10.3 [6]-Gingerol and Its Analogs From Ginger

Ginger (Zingiber officinale Roscoe, Zingiberaceae) has been used as a condiment throughout the world for more than 2500 years. Besides its extensive use as a spice in seasoning foods, the rhizome of ginger has been used since antiquity in oriental herbal medicine for the management of colds, fever, rheumatic disorders, gastrointestinal discomforts, motion sickness, and leprosy. Ginger has antiemetic, diuretic, antiinflammatory, analgesic, carminative, stimulative, antioxidative, and antipyretic effects. The flavoring substances in ginger reside in its volatile oil (oleoresin), which comprises approximately 1 to 3% of total weight. The active ingredients in ginger oil are sesquiterpenes, including bisapoline, zingiberene, and zingiberol. In addition,
[6]-gingerol (structure shown in Table 10.1) and its homologs are responsible for the characteristic pungent taste of ginger and have a variety of pharmacological properties (Nakatani, 1992; Kiuchi et al., 1992). The chemopreventive effects of ginger and its ingredients have been reviewed (Surh, 2002a; Surh et al., 1999).

A hot water extract of ginger (0.125%) inhibited spontaneous mammary tumorigenesis in mice (Nagasawa et al., 2002). The ginger extract exhibited inhibitory effects on the growth of several human cancer cell lines (Leal et al., 2003). An ethanol extract of ginger applied topically onto the dorsal skin of SENCAR mice, significantly inhibited epidermal activities of COX, LOX, and ornithine decarboxylase (ODC); ameliorated epidermal edema and hyperplasia induced by TPA; and also reduced DMBA-initiated papilloma formation (Katiyar et al., 1996). Application of [6]-gingerol prior to each topical dose of TPA throughout the promotion stage significantly inhibited TPA-induced skin tumor promotion in female ICR mice (Park et al., 1998). [6]-Gingerol also suppressed ODC activity and TNF-α production in TPA-stimulated mouse skin (Park et al., 1998; Surh et al., 1999) and lowered the TPA-induced superoxide production in differentiated HL-60 cells (Surh et al., 1999). Likewise, [6]-paradol, a minor pungent component of ginger but present abundantly in the seed of Grains of Paradise (Aframomum melegueta Roscoe, Zingiberaceae), elicited inhibitory effects on TPA-induced ear edema and production of TNF-α, ODC activity, and tumor promotion in the skin of ICR mice, and it attenuated production of TPA-induced superoxide in differentiated HL-60 cells (Surh et al., 1999). [6]-Paradol and its saturated and dehydrated homologs inhibited tumor promotion, ODC activity, hydrogen peroxide production, and myeloperoxidase activity in TPA-treated mouse skin and reduced oxidative base modification in calf thymus DNA induced by UV and hydrogen peroxide (Chung et al., 2001). Paradol and some of its derivatives induced apoptosis in an oral squamous carcinoma cell line, possibly through a caspase-3-dependent mechanism (Keum et al., 2002). [6]-Paradol as well as [6]-gingerol also induced apoptosis in cultured HL-60 cells (Lee and Surh, 1998). Treatment of mouse epidermal JB6 cells with each of the above compounds blocked EGF-induced anchorage-independent cell growth on the soft agar and AP-1 activation (Bode et al., 2001). Likewise, [6]-gingerol pretreatment attenuated TPA-induced COX-2 expression and activation of p38 MAPK and NFκB in mouse skin in vivo (Kim et al., 2005).

When male F344 rats (6 weeks of age) were fed a diet containing 0.02% gingerol for 3 weeks followed by the basal diet until the termination of the experiment (1 year), the multiplicity of azoxymethane-induced intestinal neoplasms was significantly reduced compared with that in the animals treated with the carcinogen alone (Yoshimi et al., 1992). Moreover, pulmonary metastasis in mice implanted with B16F10 melanoma cells was abolished by gingerol (Suzuki et al., 1997).

Zingiberaceae rhizomes, commonly used in the Malaysian traditional medicine, were examined for tumor-antipromoting activity on the basis of their ability to inhibit TPA-induced activation of Epstein-Barr virus early antigen (EBV-EA) in Raji cells (Vimala et al., 1999). The rhizome extracts capable of suppressing EBV activation without cytotoxic effects in Raji cells include: C. domestica, C. xanthorrhiza, Kaempferia galanga, Z. cassumunar, Z. officinale, and Z. zerumbet.

Helicobacter pylori is the primary etiological agent associated with dyspepsia, peptic ulcer disease, and the development of gastric cancer. Ginger root extracts
containing the gingerols inhibited the growth of some strains of *H. pylori* in culture, and this activity may contribute to the chemopreventive effects of ginger on gastric carcinogenesis (Mahady et al., 2003).

### 10.4 CAPSAICIN IN HOT CHILI PEPPERS

Hot chili peppers that botanically belong to the genus *Capsicum* (Solanaceae) are among the most heavily and frequently consumed spices. While the nutritional values of hot red peppers have been attributable to the relatively high content of carotenoids, vitamin C, and vitamin E, which are potent antioxidants, their pungent and irritant properties come from the alkaloid capsaicin (Table 10.1).

#### 10.4.1 GASTROPROTECTIVE EFFECTS

Although hot spicy foods have been suspected to damage the mucous membranes of gastrointestinal tract and thereby cause or promote gastric cancer in humans, a population-based study conducted in Italy reported that red pepper consumption was associated with a lower rate of stomach cancer (Buiatti et al., 1989). Moreover, researchers in Singapore have reported that hot chili pepper and its pungent ingredient capsaicin have preventive effects on ulceration of the digestive tract in humans (Yeoh et al., 1995) and rodents (Kang et al., 1995; 1996; Teng et al., 1998). Several reports surfaced that hot peppers or capsaicin, by stimulating the flow of digestive juices, may shield the stomach lining against damage from acids and alcohol. Ethanol-induced oxidative and inflammatory damage in the gastric mucosa, hemorrhagic erosion, lipid peroxidation, and myeloperoxidase activity in rats were ameliorated by intragastric capsaicin treatment, which apparently suppressed COX-2 (Park et al., 2000). Capsaicin was found to inhibit the growth of *H. pylori* in culture (Jones et al., 1997); however, jalapeno peppers did not alter the status of *H. pylori* infection *in vivo*.

Capsaicin given at a low intragastric dose (0.1 μg/kg body weight) protected against aspirin- and ethanol-induced gastric mucosal injury, while larger amounts of the compound (10 and 30 mg/kg body weight) invariably aggravated it (Abdel Salam et al., 1995). Overall, the link between red pepper consumption and the increased risk of stomach cancer in humans needs to be clarified. Although large quantities of hot peppers can severely irritate the stomach and esophagus, the amounts found in normal diets are more likely to prevent than to cause cancer. Hot pepper and hot pepper products are on the GRAS (generally recognized as safe) list defined by the U.S. Food and Drug Administration. Nevertheless, consumption of too much hot pepper should be avoided, particularly by people with ulcers, heartburn, or gastritis.

#### 10.4.2 ANTIMUTAGENIC AND ANTICARCINOGENIC ACTIVITIES

Capsaicin has been found to inhibit chemically-induced carcinogenesis and mutagenesis in various animal models and cell culture systems, possibly through the suppression of metabolic activation of mutagens and carcinogens, and antioxi-
dant and antiinflammatory effects (Surh et al., 1998; 2001; Surh, 2002b). Capsaicin inhibited TPA-promoted mouse skin carcinogenesis (Park and Surh, 1997; Park et al., 1998), and TPA-induced activation of NFκB and AP-1 in mouse skin (Han et al., 2001; Surh et al., 2000). NFκB inhibition was attributed to blockade of IκBα degradation and subsequent translocation of NFκB into the nucleus. Similarly, TPA-induced DNA binding of NFκB and AP-1 was suppressed in HL-60 cells in the presence of capsaicin (Han et al., 2002a; Surh et al., 2000). Dietary administration of capsaicin reduced the incidence of azoxymethane-induced colon tumorigenesis in male F344 rats and increased the activities of Phase II detoxification enzymes, such as glutathione S-transferase and quinone reductase in the liver and colon (Yoshitani et al., 2001).

10.4.3 Induction of Apoptosis in Malignant or Transformed Cells

Capsaicin has been found to preferentially suppress the growth of cancerous or transformed cells by inducing apoptosis (reviewed by Surh, 2002b). The mechanisms of capsaicin-induced apoptosis include:

1. Inhibition of plasma membrane NADH-oxidoreductase, which is constitutively activated in tumors and transformed cells (Morre et al., 1995; 1996; 1997; Wolvetang et al., 1996)
2. Inhibition of constitutive and induced activation of NFκB in human malignant melanoma cells, leading to inhibition of cell proliferation (Hail and Lotan, 2002)
3. Generation of ROS and rapid activation of c-Jun NH₂-terminal kinase (JNK) (Macho et al., 1999; Macho et al., 2003)
5. A decrease in the constitutive production of reactive oxygen species (Brar et al., 2001; Lee et al., 2000; 2002)
6. Nuclear condensation and internucleosomal DNA fragmentation in human hepatocellular carcinoma (SK-Hep-1) cells (Jung et al., 2001)
7. Up-regulation of p53 and c-Myc in cultured human gastric cancer cells (Kim et al., 1997)
8. Inhibition of the growth of adult T-cell leukemia cells through the induction of cell cycle arrest (Zhang et al., 2003)
9. The up-regulation of IκBα, resulting in reduced NFκB/p65 DNA-binding activity (Zhang et al., 2003)

10.4.4 Antimetastatic and Antiangiogenic Effects

Direct injection of capsaicin into B16 mouse melanoma transplanted in C57BL/6 mice suppressed the growth of tumors (Morre et al., 1996), and intraperitoneal administration of capsaicin to C57BL/6 mice reduced the pulmonary colonization
of B16-F10 murine melanoma in a dose-dependent fashion (O.H. Kim et al., unpublished observations). Capsaicin inhibited vascular endothelial growth factor (VEGF)-induced proliferation, DNA synthesis, chemotactic motility, and capillary-like tube formation of primary cultured human endothelial cells (Min et al., 2004). The inhibition of both VEGF-induced vessel sprouting in the rat aortic ring assay and vessel formation in the mouse matrigel plug assay and suppression of tumor-induced angiogenesis in the chick chorioallantoic membrane assay by capsaicin have also been demonstrated.

10.5 CARNOsol AND ROSMARINIC ACID FROM ROSEMARY

Rosemary has been one of the most inexpensive spices and herbs. The dried leaves of rosemary (*Rosemarinus officinalis* L., Labiatae) have been extensively used as a spice, a flavoring agent, an abortifacient, etc. Extracts of rosemary have antioxidant, antiinflammatory, and antibacterial effects (al-Sereiti et al., 1999; Aruoma, 1996; Ho et al., 1994; 2000; Leal et al., 2003; Slamenova et al., 2002).

10.5.1 ANTIOXIDANT AND ANTIINFLAMMATORY EFFECTS

Several phenolic diterpenoids with antioxidant properties were isolated from rosemary leaves (Ho et al., 1994; 2000). These include carnosol and rosmarinic acid (Table 10.1). Carnosol and rosemary extract exhibit potent antioxidative properties, including peroxynitrite scavenging activity (Choi et al., 2002). Peroxynitrite is a cytotoxicant with strong oxidizing properties towards various cellular constituents, effecting cell death, lipid peroxidation, carcinogenesis, aging, etc. Carnosol markedly inhibited lipopolysaccharide (LPS)- and interferon-gamma (IFNγ)-induced nitrite production in mouse peritoneal cells (Chan et al., 1995). High concentrations of nitric oxide (NO) are produced by iNOS during proinflammatory and carcinogenic processes. Treatment of the mouse macrophage cell line with carnosol markedly reduced LPS-stimulated iNOS expression and subsequent NO production in a concentration-dependent fashion (Lo et al., 2002). Carnosol also inhibited the LPS-induced iNOS and NFκB promoter activity and phosphorylation, as well as degradation of IkBα. LPS-induced activation of p38 and p44/42 MAPK was likewise diminished by carnosol treatment. These results suggest that carnosol suppresses the iNOS gene expression by blocking NFκB activation, which may provide possible mechanisms for its antiinflammatory and chemopreventive action (Lo et al., 2002). The compound also inhibited the induction of COX-2 by abrogating PKC signaling and thereby the binding of AP-1 to the cAMP response element (CRE) of the COX-2 promoter (Subbaramaiyah et al., 2002).

Rosmarinic acid is another antioxidative and antiinflammatory component of rosemary. Oral administration of rosmarinic acid inhibited diesel exhaust–induced pulmonary injury as well as expression of iNOS mRNA and formation of nitrotyrosine and 8-hydroxy-2’-deoxyguanosine (8-OH-dG) in the lung, and protected against liver injury induced by LPS in mice (Osakabe et al., 2002). More recently, topical application of rosmarinic acid inhibited neutrophil infiltration, myeloperoxidi-
dase activity, expression of COX-2, lipid peroxidation, and 8-OH-dG formation in TPA-stimulated mouse skin (Osakabe et al., 2004).

10.5.2 Antitumorigenic Effects

Rosemary extracts have been reported to inhibit experimentally induced carcinogenesis. Dietary supplementation of rosemary extract significantly reduced the mammary tumor incidence and also inhibited covalent binding of DMBA to mammary epithelial cell DNA (Singletary and Nelshoppen, 1991). Carnosol appears to contribute partly to the antitumorigenic activity of rosemary. Intraperitoneal injection of carnosol as well as rosemary extract resulted in a significant decrease in the number of DMBA-induced adenocarcinomas and the levels of DNA adducts in rat mammary gland (Amagase et al., 1996; Singletary et al., 1996). In addition, intragastric administration of rosemary extract (100 mg/kg/day) for five consecutive days reduced the number and areas of diethylnitrosamine-induced glutathione S-transferase placental form-positive foci in the livers of male F344 rats (Kitano et al., 2000).

Topical application of the rosemary extract to mouse skin attenuated the covalent binding of B[a]P to epidermal DNA and inhibited tumor initiation by B[a]P and DMBA, and also inhibited TPA-induced ODC activity, inflammation, hyperplasia, and tumor promotion (Huang et al., 1994). Likewise, topical application of carnosol or ursolic acid, isolated from rosemary, inhibited TPA-induced ear inflammation, ODC activity, and skin tumor promotion.

10.5.3 Effects on Carcinogen-Metabolizing Enzymes

Whole rosemary extract or an equivalent concentration of its principal antioxidant constituents, such as carnosol or carnosic acid, markedly inhibited B[a]P-DNA adduct formation in cultured human bronchial epithelial cells (Offord et al., 1995). Expression and catalytic activity of CYP1A1 were inhibited by rosemary components, suggesting that the observed suppression of B[a]P-DNA adduct formation by these agents results chiefly from their inhibition of the metabolic activation of B[a]P. Carnosol induced expression of glutathione S-transferase, which is known to detoxify the ultimate electrophilic carcinogenic metabolite of B[a]P including the bay-region diol epoxide, and also that of another important Phase II detoxification enzyme, NAD(P)H:quinone reductase. Hence, rosemary components have the ability to decrease the activation and/or increase the detoxification of an important human carcinogen such as B[a]P. The suppression of aflatoxin B1-derived DNA adduct formation by rosemary components also appears to be mediated via the mechanism that involves inhibition of metabolic activation of the carcinogen and induction of the Phase II detoxification enzymes (Mace, 1998; Offord et al., 1997). Similarly, the NAD(P)H:quinone reductase activity was elevated in a murine hepatoma cell line challenged with rosemary or other spices such as red pepper and basil (Tawfiq et al., 1994). Oral or intraperitoneal administration of rosemary extract and carnosol to female rats caused marked increases in the activities of hepatic glutathione S-transferase and NAD(P)H:quinone oxidoreductase (Singletary et al., 1996). Water-
soluble extract of rosemary upregulated both CYPs and Phase II detoxification enzymes in male rats, whereas the dichloromethane extract of rosemary acted as a monofunctional inducer, effecting the induction of glutathione S-transferase, NAD(P)H:quinone oxidoreductase and UDP-glucuronosyltransferase (Debersac et al., 2001a; Debersac et al., 2001b).

10.5.4 Proapoptotic and Cytostatic Effects

Carnosol induced apoptosis in several acute lymphoblastic leukemia cell lines (Dorrie et al., 2001). In another study, carnosic acid with strong antioxidant activity inhibited proliferation of human myeloid leukemia cells without induction of apoptotic or necrotic cell death (Steiner et al., 2001). Growth arrest occurred concomitantly with a transient cell cycle block in the G1 phase. At low concentrations, carnosic acid substantially augmented (100- to 1000-fold) the differentiation-inducing effects of 1,25-dihydroxyvitamin D3 and all-trans retinoic acid. Furthermore, such combinations of carnosic acid and any of these inducers of differentiation synergistically inhibited proliferation and cell cycle progression (Steiner et al., 2001).

Rosmarinic acid has been found to induce apoptosis in Jurkat and peripheral T cells (Hur et al., 2004). Rosmarinic acid inhibited splenic T-cell proliferation, and prolonged allograft survival synergistically with rapamycin, potentiating the immunosuppressive effects of the latter drug (Yun et al., 2003).

10.6 Eugenol from Clove

Clove (the flower-buds of *Eugenia caryophyllata*, also named *Syzygium aromaticum* [L.] Merr. et Perry, Myrtaceae) has been used in traditional oriental medicine as a vermifuge and an antibacterial agent, and also to treat toothache. Eugenol (4-allyl-2-methoxyphenol; Table 10.1) is one of the major compounds in clove essential oil, being widely used as a flavoring agent and also in cosmetic products. Eugenol is well known for its antioxidant activity (Fujisawa et al., 2002). In an ascorbate- or H2O2/Fe2+-induced lipid peroxidation system, eugenol significantly inhibited lipid peroxidation, possibly due to its free radical scavenging ability (Nagababu and Lakshmaiah, 1992; Reddy and Lokesh, 1992). The inhibitory activity of eugenol against the oxidation of human low-density lipoproteins was the highest among 13 phenolic compounds of essential oils (Teissedre and Waterhouse, 2000). Glutathione S-transferase activity was found to be significantly elevated in eugenol-treated rats (Vidhya and Devaraj, 1999). Among aqueous alcoholic extracts of commonly used spices, including garlic, ginger, onion, mint, cloves, cinnamon, and pepper, clove exhibited the highest inhibitory activity against oxidation of linoleic acid in the presence of soybean LPO (Shobana and Naidu, 2000).

Methanol extract of the cortex of *E. caryophyllata* containing eugenol was found to inhibit the PGE2 production in LPS-activated mouse macrophages (Kim et al., 2003), and eugenol itself suppressed COX-2 gene expression in these cells (Hong et al., 2002) and inhibited the proliferation of human colon cancer cells (Kim et al., 2003). Dietary eugenol decreased inflammation of carrageenan-induced edema in rats (Reddy and Lokesh, 1994). Eugenol was cytotoxic and inhibited DNA synthesis in both a human
salivary gland tumor cell line and normal human gingival fibroblasts (Atsumi et al., 2000). Eugenol also suppressed chemically induced mutagenesis (Rompelberg et al., 1995; 1996; Sukumaran and Kuttan, 1995; Yokota et al., 1986). Its anticarcinogenic activity has been attributable to the induction of glutathione S-transferase in the mouse liver and small intestine (Zheng et al., 1992). Similarly, eugenol and eugenol-rich oil inhibited murine skin papillomagenesis, possibly through modulating xenobiotic detoxifications (Bhide et al., 1991; Singh et al., 1999; Sukumaran et al., 1994). On the contrary, eugenol exerted minimal protection against carcinogenesis in Swiss bare mice (Azuine et al., 1991) and promoted forestomach carcinogenesis in rats (Imaida et al., 1990). Therefore, additional studies are needed to clarify the chemopreventive effects of eugenol and eugenol-containing plant oils such as clove oil.

**10.7 CONCLUDING REMARKS**

A vast variety of edible phytochemicals have been shown to be protective against cancer in numerous animal and cell culture models (Surh, 2003). Many phenolic substances derived from spices have been reported to elicit chemopreventive as well as antioxidative and antiinflammatory activities.

With the rapid progress in molecular medicine and development of the state-of-the-art technologies applied to biomedical research in general, we are now better aware of the intracellular events associated with malignant transformation. Despite this progress, the identification of molecular and cellular targets of chemopreventive phytochemicals remains still incomplete. The chemopreventive effects of spice ingredients have been often attributed to their antioxidant and antiinflammatory properties. Since tumor promotion is closely linked to oxidative and inflammatory tissue damage, spice ingredients capable of blocking prooxidative and proinflammatory damage are anticipated to act as tumor antipromoters. Many of the molecular alterations related to the promotional stage of carcinogenesis occur in cell signaling pathways that regulate cell proliferation. Therefore, it would be important to identify the key molecules in the cell signaling network that can be affected by spice ingredients for better understanding of their underlying mechanisms.

Equally important is the information on bioavailability and metabolism of individual spice ingredients with chemopreventive potential, particularly if they are considered to be included in human intervention trials or to be utilized as lead compounds for the development of chemopreventive agents. The use of chemopreventive spices together with the existing antineoplastic agents may also potentiate their therapeutic efficacy while minimizing the toxicity that often arises in the conventional chemotherapy.

**ACKNOWLEDGMENTS**

Research described in this chapter was supported by the grant for functional food research and development from the Korea Science and Engineering Foundation, Ministry of Science and Technology, Republic of Korea. Mr. Joydeb Kumar Kundu provided excellent editorial services.
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Carcinogenic and Anticarcinogenic Food Components


11 Cancer Prevention by Tea and Tea Constituents

Janelle M. Landau, Joshua D. Lambert, Mao-Jung Lee, and Chung S. Yang

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11.1 INTRODUCTION

During the Tang Dynasty (618 to 906 A.D.), tea became a popular drink in China. In 1644, sailors began bringing tea packages from the Far East to the United Kingdom. Tea replaced ale as the national drink of England. Tea bushes arrived in the United States in 1799, and by 1901, Thomas Sullivan developed the first tea bag. Now tea is second only to water as the world’s most consumed beverage.

All tea comes from *Camellia sinensis*, a warm-weather evergreen. Tea is grown in thousands of tea gardens around the world, resulting in thousands of flavorful variations. Leaves of the *C. sinensis* plant are dried for stability and shelf life. The mode of processing the fresh tea leaves and their extent of exposure to oxygen determine the types of tea. In the manufacturing of green tea, the leaves are steamed, rolled, and dried, avoiding oxidation. In black tea production, the tea leaves are crushed to allow enzyme-catalyzed oxidation. In this process, polyphenolic com-
pounds in tea leaves undergo polymerization and other chemical reactions that result in distinctive color and taste. Oolong tea falls somewhere between green and black teas, in that the polyphenols in leaves are only partially oxidized. Tea is also divided by grades, determined by the leaf size.

Interest in the role of tea in the prevention of disease and promotion of health has grown steadily in recent years. This review discusses recent research on the cancer preventive activities of tea and tea constituents, including their metabolism and availability in the body and potential mechanisms of action. This topic has also been covered in several review articles (Yang and Wang, 1993; Katiyar and Mukhtar, 1996; Yang et al., 2002).

11.2 TEA CHEMISTRY

The green tea leaves are heated to inactivate the enzymes and thus preserve the constituents in the dried tea leaves. A typical brewed green tea beverage (e.g., 2.5 g tea leaves in 250 ml of hot water) contains 240 to 320 mg of catechins and 20 to 50 mg of caffeine. The catechins include (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and (−)-epicatechin (EC), with EGCG as the major catechin in tea (Figure 11.1). Tea leaves also contain flavonols such as quercetin and myricetin as well as the alkaloids caffeine and theobromine. During the production of black tea, the tea leaves are withered, crushed, and allowed to undergo enzyme-mediated oxidation in a process commonly referred to as “fermentation.” During this process, much of the catechin content of the tea leaves is converted to theaflavins and thearubigins. Theaflavins are produced from the oxidation and dimerization of two catechin molecules (Figure 11.1). The structures of the thearubigins, polymers formed by catechins, are poorly characterized. In brewed black tea, catechins, theaflavins, and thearubigins account for 3 to 10%, 2 to 6%, and greater than 20% of the dry weight, respectively. Theaflavins are responsible for the red-brown color and contribute to the taste of black tea.

![The structures of the green tea catechins and black tea theaflavins.](image_url)
Tea catechins and other tea polyphenols are efficient scavengers of free radicals. Several functional groups in their structures appear to be important in conferring their low reduction potentials. All catechins have two hydroxy groups in ortho position in the B-ring (Figure 11.1), which participate in electron delocalization. Both EGC and EGCG have three hydroxy groups in the B ring. In ECG and EGCG, the hydroxy group at the 3 position in the C ring is esterified with gallic acid, thus providing three more hydroxy groups. Both the hydroxy groups in the B ring and those in the gallate moiety have been associated with increased antioxidant activity. There is some evidence that the A ring of EGC and EGCG may also provide an antioxidant site (Zhu et al., 2000).

11.3 INHIBITION OF CARCINOGENESIS IN ANIMAL MODELS BY TEA AND ITS CONSTITUENTS

Cancer preventive activity has been shown for both green tea and black tea against tumors in rodents induced by ultraviolet (UV) light and chemical carcinogens, as well as spontaneous tumors in wild-type and genetically modified mice. The organs for which tea elicited a protective effect include the lung, skin, oral cavity, esophagus, stomach, liver, pancreas, bladder, small intestine, colon, and prostate (Yang and Wang, 1993; Katiyar and Mukhtar, 1996; Yang et al., 2002). Table 11.1 summarizes results of several of these studies.

In most of the studies, different preparations of tea or tea constituents were administered in drinking water. These included freshly brewed green or black tea (e.g., 5 to 20 g of leaves brewed in 1 L of water, referred to as 0.5 to 2% tea); green or black tea solids (dehydrated tea extracts) usually reconstituted with distilled water at concentrations of 0.4 or 0.6%; decaffeinated green or black tea solids prepared as above; and preparations of green tea or black tea enriched in polyphenols, which may contain some caffeine. The tea preparations were given during or after the carcinogen treatment or during the entire experimental period. Tea preparations were found to inhibit 4-(methylnitrosamino)-1-(3-pyridyl)-1-butaneone (NNK)-induced lung tumorigenesis in A/J mice under all three treatment regimens (Yang et al., 1997b). In most of the studies, inhibition of tumor multiplicity (number of tumors/mouse) was observed, although reduction in tumor incidence (number of animals with tumor) was also reported. In the above tumorigenesis model, a black tea preparation administered to lung adenoma–bearing mice significantly inhibited tumor cell proliferation and the progression of adenoma to carcinoma (Yang et al., 1997a). Inhibition of tumor invasion and metastasis in transplanted and spontaneous metastatic tumor models by intragastric infusion of green tea or EGCG was also reported (Sazuka et al., 1995; Taniguchi et al., 1992). However, an inhibition of lung tumorigenesis by tea preparations has not been unequivocally demonstrated. For example, Witschi et al. (1998) reported that green tea extract did not reduce lung tumor multiplicity in male A/J mice treated with one dose of NNK or in a cigarette smoke-induced lung tumorigenesis model. Tea has also been shown to inhibit spontaneous tumorigenesis, e.g., administration of 1 or 2% freshly brewed green or black tea inhibited the spontaneous development of lung adenoma and rhabdomyosarcoma.
in A/J mice (Landau et al., 1997). In this study, the body weights and retroperitoneal fat pad weights of mice drinking 1% green tea were 14 and 35% lower, respectively, than those of the control mice. Likewise, the inhibition of skin tumorigenesis by caffeine or tea was shown to be closely correlated to the reduction of body fat (Conney et al., 2002).

Using a genetically altered mouse model (TRAMP) of prostate carcinogenesis, which closely mimics the pathology and progression of human prostate cancer, it was recently demonstrated that oral consumption of 0.1% green tea polyphenols decreased tumor incidence by 65% (Adhami et al., 2003). In contrast to water-treated animals, which had a high rate of tumor metastasis to other organ sites (25 to 95%), tea-treated mice showed no metastasis. Biochemical and histological analysis showed a significant decrease in proliferating cell nuclear antigen and a tenfold increase in tumor cell apoptosis. Inhibition of matrix metalloproteases (MMPs)-2 and -9 as well as vascular endothelial growth factor (VEGF) was also

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<th>Site</th>
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<th>Negative Studies</th>
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<td>Skin</td>
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<td>Lung</td>
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<td>Oral cavity</td>
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<tr>
<td>Mammary gland</td>
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\(^a\) Reviewed in Yang et al., 2002; Lu et al., 2002.
\(^b\) Reviewed in Yang et al., 2002.
\(^c\) Reviewed in Yang et al., 2002; Li et al., 2002.
\(^d\) Reviewed in Yang et al., 2002; Orner et al., 2003.
\(^e\) Hirose et al., 2002.
\(^f\) Reviewed in Yang et al., 2002; Jung et al., 2001; Jia and Han, 2001; Orner et al., 2002.
\(^g\) Reviewed in Yang et al., 2002; Hirose et al., 2001.
\(^h\) Reviewed in Yang et al., 2002; Sato and Matsushima, 2003; Kemberling et al., 2003.
\(^i\) Reviewed in Yang et al., 2002; Gupta et al., 2001; Nyska et al., 2003.
\(^j\) Kavanagh et al., 2001; Sartippour et al., 2001; Zhou et al., 2004.
observed. These alterations may be underlying mechanisms for the inhibition of tumor development.

The bioavailability of tea constituents is apparently a key factor in determining the effectiveness of tea in inhibiting tumor formation. The oral cavity and digestive tract, which have direct contact with orally administered tea, may receive the most benefit from tea consumption. For example, in the 7,12-dimethylbenz[a]anthracene (DMBA)-induced hamster model of oral carcinogenesis, treatment with 0.6% green tea, as the sole source of drinking fluid, reduced the number of visible tumors by 35% and tumor volume by 57% (Li et al., 2002). Immunohistochemical assays showed that tea increased the apoptotic index (the percent of cells undergoing programmed cell death) of the tumors while it decreased the proliferation index and microvessel density. Tea preparations have been shown to inhibit esophageal, fore-stomach, and intestinal cancer. Tea preparations have also been shown to inhibit colon carcinogenesis in several studies, although such an effect was not observed in others (reviewed by Yang et al., 2002).

Purified tea constituents have also been reported to inhibit tumorigenesis. For example, EGCG inhibited lung tumorigenesis in A/J mice induced by NNK (Chung et al., 2003) or cisplatin (Mimoto et al., 2000). Theaflavins (a mixture of theaflavin, theaflavin-3-gallate, theaflavin-3’-gallate, and theaflavin-3,3’-digallate) reduced NNK-induced lung tumor multiplicity and volume in A/J mice (Yang et al., 1997a). In NNK-induced lung tumorigenesis in F344 rats, inhibition of tumorigenicity by black tea was fully accounted for by a solution containing the corresponding amount of caffeine (Chung et al., 2003).

Caffeine has been shown to play an important role in the inhibition of skin carcinogenesis. Whereas orally administered green tea and black tea were effective in reducing the incidence and multiplicity of UVB-induced skin tumors, orally administered decaffeinated teas were much less or not effective (Huang et al., 1997). The addition of caffeine restored the protective activity to the decaffeinated teas. A topical application of caffeine or EGCG to hairless mice that had been pretreated twice weekly for 20 weeks with UVB decreased the multiplicity of skin tumors by 44 to 72% or 55 to 66%, respectively (Lu et al., 2002). In addition, both compounds were shown to increase the apoptotic index of the tumors by 56 to 92%. In summary, studies conducted in animal models have generally found significant protective effects of both green and black tea as well as the major polyphenolic constituents.

11.4 EPIDEMIOLOGICAL STUDIES OF TEA CONSUMPTION AND CANCER

With so many animal experiments suggesting protective effects of tea against tumorigenesis (Table 11.1), one would expect to see such an effect in humans. The effect of tea consumption on human cancer has been studied extensively, and this topic has been reviewed by many authors (Yang and Wang, 1993; Blot et al., 1996; Kohlmeier et al., 1997; Buschman, 1998; Yang et al., 2002; Higdon and Frei, 2003). Many case-control studies have suggested that subjects who consume large amounts of tea had lower cancer risk; in particular, risk of gastric and esophageal cancers
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was lower among green tea consumers in Japan and China. However, many other studies did not observe this protective effect. For example, of seven case-control studies on the relationship between green tea consumption and gastric cancer risk in Japan and China, four studies found a significant protective effect of tea, two studies found a trend toward a protective effect, and one study in Taiwan found that tea consumption appeared to increase gastric cancer risk (reviewed in Tsubono et al., 2001). In a recent population-based prospective cohort study in Miyagi, Japan, no association between green tea consumption and gastric risk was observed (Tsubono et al., 2001). On the other hand, a population-based study conducted in Yangzhong, China, revealed that a green tea drinker had 48 and 51% reduced risks of gastric cancer and chronic gastritis, respectively, after adjusting for confounding factors such as age, gender, education, body mass index, pack-years of smoking, and alcohol drinking (Setiawan et al., 2001). Similarly, in a population-based case-control study involving 734 esophageal cancer patients, tea consumption, especially among nonsmokers and nonalcohol drinkers, was associated with lower incidence of esophageal cancer (Gao et al., 1994).

The inconsistent results of the epidemiological studies may be due to the type and quantity of tea consumed. For example, studies in Saitama, Japan, have shown that women consuming more than 10 cups of green tea per day had a lower cancer incidence (all sites combined) (Imai et al., 1997). On the other hand, in the Netherlands Cohort Study on Diet and Cancer, consumption of black tea did not affect the risk for colorectal, stomach, lung, and breast cancers (Goldbohm et al., 1996). The most recent studies continue to show inconsistency with regard to the risk of human cancers and tea consumption. For example, one case-control study of colon cancer conducted in North Carolina found no association between tea consumption and colon cancer (Il’yasova et al., 2003), whereas a case-control study in Moscow did find a protective effect against rectal cancer risk in women who consumed black tea (Dora et al., 2003). A pooled analysis of 14 case-control studies conducted in the United States, Europe, and Asia indicated that thyroid cancer risk was not associated with consumption of tea (Mack et al., 2003).

A population-based case-control study of breast cancer was conducted among women of Asian descent living in Los Angeles to investigate the effects of green and black tea (Wu et al., 2003b). Whereas black tea consumption had no effect on breast cancer risk, green tea drinkers had a significantly reduced risk of breast cancer that was maintained even after adjusting for several potential confounding factors including smoking, alcohol, coffee and black tea intake, family history of breast cancer, physical activity, and intake of soy and dark-green vegetables. Among women who carried at least one low-activity catechol-O-methyltransferase (COMT) allele, both green and black tea drinkers showed a significantly reduced risk of breast cancer (Wu et al., 2003a); this finding contrasts with that of the initial study (Wu et al., 2003b), where no effect of black tea was seen. The authors concluded that individuals with a low-activity COMT allele have a reduced risk of breast cancer because they metabolize tea polyphenols less efficiently and therefore, have prolonged exposure to these compounds. A case-control study of prostate cancer in southeast China found that prostate cancer risk declined with increasing frequency, duration, and quantity of green tea consumed (Jian et al., 2003).
These inconsistent results reflect the difficulties in conducting epidemiological studies on tea and cancer. The ability to adjust for all confounding factors such as smoking, alcohol consumption, and other lifestyle factors requires a complete understanding of exactly which factors are involved as well as being able to collect this data accurately from subjects. When these factors are properly adjusted, a clearer picture may be seen. For example, in two studies in Shanghai, a clearly protective effect of green tea consumption against esophageal (Gao et al., 1994) and lung cancer (Zhong et al., 2001) was observed only in nonsmokers or women who were mostly nonsmokers.

The quantification of tea consumption is also difficult. In most studies, information on “how many cups of tea consumed daily” was used. The results are rather inaccurate due to recall bias, the composition of the tea used, the cup size, and how the tea is prepared in terms of the amount of tea leaves, the temperature of water, and brewing time. It is unclear how much of the tea constituents is extracted into the liquid and how much is absorbed into the body or by cells at each organ site. This is especially true with black tea because theaflavins and thearubigins are poorly, or not at all, absorbed. A study of bladder and kidney cancer suggested that only persons who consumed >5 cups/day had a decreased risk of bladder cancer, and no evidence of a dose response was seen (Bianchi et al., 2000). Some studies conducted in Japan suggested that daily consumption of 10 cups of tea is needed to produce a cancer-preventive effect (Imai et al., 1997). Japanese cups are usually smaller, and 10 cups may be equivalent to 6 cups in the United States. Without considering the type and amount of tea used, information on the number of cups is still not accurate.

When the chemical composition of tea and drinking habits in an area are known, as in the Shanghai study (Gao et al., 1994), the estimates of grams of green tea leaves consumed per month may provide more quantitative information. In this study, a protective effect against esophageal carcinogenesis was observed in women who consumed 5 g dried tea leaves per day, which is equivalent to 2 or 3 cups of green tea. A more quantitative approach is to use exposure markers for tea consumption. For example, in a prospective study in Shanghai, urinary EGC levels were used as a marker, and it was found that subjects with this marker had lower incidences of gastric and esophageal cancer (Sun et al., 2002). Such markers account not only for differences in intake but also to some extent differences in metabolism of tea compounds.

11.5 METABOLISM AND BIOAVAILABILITY OF TEA POLYPHENOLS

An understanding of the processes involved in the absorption, distribution, and metabolism of tea polyphenols is essential for determining their potential actions in vivo and their overall significance in human disease prevention. Interindividual variation in the plasma and tissue bioavailability of active tea components is substantial and may result from genetic polymorphisms in the enzymes involved in polyphenol metabolism and individual variation in colonic microflora (Scalbert and Williamson, 2002).

The pharmacokinetics of EGCG and the other catechins have been investigated in rats, mice, and humans (Yang et al., 2002). Studies of [3H]-EGCG in both rats
and mice showed that following a single intragastric (i.g.) dose, radioactivity was distributed throughout the body (Kohri et al., 2001; Suganuma et al., 1998). After 24 h, 10% of the initial dose of radioactivity was found in the blood with about 1% in the prostate, heart, lungs, liver, kidneys, and other tissues of rats and mice. The major route of elimination was through the feces in both species. In the rat, 77% of an intravenous (i.v.) dose of [3H]-EGCG was eliminated in the bile, while only 2% was eliminated through the urine.

The catechins are subject to extensive biotransformation including methylation, glucuronidation, sulfation, and ring-fission metabolism (Figure 11.2). The enzymology and the metabolites have been characterized (Lu et al., 2003a; 2003b; Meng et al., 2002; Lee et al., 2002). Rat liver cytosol had a greater capacity for COMT-catalyzed O-methylation and sulfotransferase-catalyzed sulfation of EGCG than did either mouse or human liver cytosol. By contrast, both mice and humans had a greater ability to glucuronidate EGCG than rats did. The plasma bioavailability of EGCG (total concentration of conjugated and the aglycone forms) in mice following i.g. administration of EGCG was 26.5%. This value was higher than that previously reported for rats (1.6%). Concentrations (per g wet weight) of EGCG in the small intestine and colon were 20.6 and 3.6 μg, respectively, following i.g. administration of 75 mg EGCG/kg body weight to mice. The levels (per g wet weight) in other tissues were less than 0.05 ng. Following i.v. administration of EGCG, its levels were the highest in the liver (1.65 ng/g), lungs (1.24 ng/g), and small intestine (1.10 ng/g). Whereas greater than 50% of plasma EGCG was present as the glucuronide, EGCG was present mainly as the free form in the tissues (Lambert et al., 2003).

Treatment of rats with a green tea polyphenol preparation (0.6% w/v in distilled water) resulted in increased plasma levels of polyphenols over a 14-day period, with levels of EGC and EC higher than those of EGCG (Kim et al., 2000). Plasma levels then decreased over the subsequent 14 days, suggesting an adaptive effect. EGCG levels were found to be the highest in the rat esophagus, intestine, and colon, which have direct contact with tea catechins, whereas EGCG levels were lower in the bladder, kidneys, liver, lungs, and prostate, which depend on systemic bioavailability. When the same polyphenol preparation was given to mice, the EGCG levels in the plasma, lungs, and liver were much higher than in rats. These levels appeared to peak on day 4 and then decreased to less than 20% of the peak values on days 8 to 10 (Kim et al., 2000).

Several studies of the systemic bioavailability of orally administered green tea and catechins in human volunteers have been conducted (Yang et al., 1998; Chow et al., 2001; Lee et al., 2002). Most recently, we have shown that oral administration of 20 mg green tea solids/kg body weight resulted in a maximum concentration (C_max) of 223, 124, and 77.9 ng/mL in the plasma for EGC, EC, and EGCG, respectively (Lee et al., 2002). Time to reach a maximum concentration (T_max) was found to range from 1.3 to 1.6 h with half-lives (t_1/2) of 3.4, 1.7, and 2 h for EGCG, EGC, and EC, respectively. The T_max increased with a greater dose of catechins (Chow et al., 2001). Plasma EC and EGC were present mainly in the conjugated form, whereas 77% of the EGCG was in the free form (Lee et al., 2002). The data support earlier findings that plasma EGC was present as glucuronide (57 to 71%) and sulfate (23 to 36%), with only a small fraction of free EGC (Yang et al., 1998; Chow et al., 2001). Likewise, plasma EC was largely in the sulfated form (66%) with less glu-
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Egc was also methylated (4′-O-methyl-EGC) in humans, and its plasma and urine levels were higher than those of EGC (Lee et al., 2002). EGCG has also been shown to undergo methylation, forming 4′,4″-di-O-methyl-EGCG (Meng et al., 2002). Ring-fission metabolites, 5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone (M4), 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone (M6), and 5-(3′,5′-dihydroxyphenyl)-γ-valerolactone (M6′) were also detected in urine (Meng et al., 2002). Pharmacokinetics and toxicity of EGCG at a dose of 800 mg in pure form or in Polyphenon E (a mixture of the main green tea polyphenols) have been investigated (Chow et al., 2003). These doses were found to be safe and acceptable for humans.

In a Phase II trial investigating the effect of green tea on patients with prostate carcinomas, a daily dose of 6 g green tea (given in 6 1-g doses/day) led to adverse effects in 69% of the subjects, which included nausea, vomiting, insomnia, fatigue, diarrhea, abdominal pain, and confusion (Jatoi et al., 2003). In contrast to previous studies in rodents employing multiple dose regimens, Chow et al. (2003) have shown that daily dosing with 800 mg EGCG to human volunteers for 4 weeks resulted in a 60% increase in the systemic exposure to free EGCG. This increased availability may be due to alterations in drug metabolizing enzymes or the accumulation of EGCG in a nonplasma compartment.

Based on the numerous biotransformation studies that have been conducted, it appears that mice are more similar to humans than rats are in terms of enzymatic ability to conjugate tea catechins. Anaerobic fermentation of EGC, EC, and ECG with human fecal microflora has been shown to result in the production of the ring fission products M4, M6, and M6′ (Figure 11.2) (reviewed in Yang et al., 2002). These compounds also contain a potentially biologically active valerolactone structure, while retaining the polyphenolic character of the parent compound, and may therefore have biological activities.

Theaflavins are poorly absorbed, and very low levels were detected in the plasma (1 ng/mL) and urine (4.2 ng/mL) following ingestion of a large amount of theaflavins (700 mg) (Mulder et al., 2001). Lipinski’s Rule of Five predicts that a compound with a molecular weight greater than 500 and containing greater than five hydrogen bond donors or greater than ten hydrogen bond acceptors will have poor absorption and oral bioavailability (Lipinski et al., 2001). According to this rule, it is predicted that the bioavailability of theaflavins will be quite poor. In addition, the numerous phenolic groups on the theaflavins represent sites for glucuronidation, methylation, and sulfation. Theaflavins and thearubigins may be cleaved in the intestine by microorganisms, resulting in smaller molecules that can be absorbed. Indeed, after ingestion of black tea, most of the phenol group equivalents were recovered in the urine (Higdon and Frei, 2003). Much research remains to be done to determine the role of any of these biotransformation pathways in affecting the bioavailability of the theaflavins and thearubigins.

11.6 MECHANISTIC STUDIES IN VITRO

Numerous potential mechanisms have been proposed for the cancer-preventive activity of tea and tea constituents based on studies with cancer cell lines (Yang et al., 2002; Lambert and Yang, 2003).
11.6.1 **Antioxidant/Prooxidant Activity**

Tea polyphenols have been shown to have strong antioxidant activity *in vitro*, but such activity has been associated with the inhibition of tumorigenesis only in a few cases (reviewed by Higdon and Frei, 2003). The importance of this antioxidative mechanism in the inhibition of carcinogenesis still remains to be demonstrated. On the other hand, recent studies have suggested that the cell-killing activity of tea polyphenols, at least *in vitro*, may be related to their prooxidant activity. For example, we have shown that EGCG-induced apoptosis in H661 human lung cancer cells and Ras-transformed human bronchial cells is completely or partially blocked by the inclusion in the medium of catalase, which catalyzes the decomposition of H₂O₂ (Yang et al., 1998; Yang et al., 2000). When EGCG was added to cell culture medium, production of H₂O₂ was observed (Hong et al., 2002). Preincubation of cells with EGCG has been shown to block signaling systems induced by the epidermal growth factor (EGF) and platelet-derived growth factor (PDGF). Our recent results with esophageal squamous cells indicated that inhibition of the EGF-induced signaling system was associated with the degradation or inactivation of the EGF receptor, and the effect could be abolished by the inclusion in the preincubation system of superoxide dismutase, which converts oxygen radicals to H₂O₂ (unpublished results). The addition of superoxide dismutase also stabilizes EGCG by balancing the donation of electrons from EGCG. Both of these observations suggest a role for EGCG prooxidation in some of the reported activities of EGCG *in vitro*. It is not known whether such reactions occur *in vivo*.

11.6.2 **Specific Protein Kinases Involved in Transcription Factor Activation and Cell Cycle Regulation**

Activation of the transcription factors activator protein 1 (AP-1) and nuclear factor kappa B (NFκB) is commonly seen in carcinogenesis. EGCG and other tea polyphenols have been shown to inhibit the activation of AP-1 and NFκB. Although antioxidative mechanisms have been implicated in this activity, the results can be better explained by the direct inhibition of specific protein kinases by these tea polyphenols. Several studies using different cell lines have shown that EGCG and theaflavin-3,3′-digallate (TFdiG) inhibit the activity of various kinases (Chung et al., 2001; Yang et al., 2001; Pan et al., 2000; Ahmad et al., 2000). These include direct inhibition of MAP kinases, and the results are consistent with previous observations that EGCG and theaflavins (5 to 20 μM) inhibited the phosphorylation of jun N-terminal kinase (JNK), activation of AP-1, and the transformation of mouse epidermal cells as well as the suppression of AP-1 activity by topical application of EGCG to B6D2 transgenic mice.

EGCG and TFdiG have been shown to inhibit the activity of IkB kinase (IKK) in tumor necrosis factor α (TNFα)-stimulated intestinal epithelial cells and lipopolysaccharide (LPS)-stimulated murine macrophages (Yang et al., 2001; Pan et al., 2000). In both cases, there was diminished IkB degradation and NFκB activity in response to stimulation. Likewise, Ahmad et al. (2000) demonstrated that EGCG inhibited the activity of NFκB in TNFα- and LPS-stimulated human epidermoid
carcinoma cells. This effect could also be mediated by inhibition of IκB phosphorylation and degradation.

EGCG and theaflavins have also been reported to inhibit cyclin-dependent kinases (cdk) 2 and 4 (Liang et al., 1999), leading to the inhibition of hyperphosphorylation of the retinoblastoma (Rb) protein and causing G0/G1-phase arrest.

### 11.6.3 Effects on Apoptosis and Angiogenesis

EGCG has been reported to induce apoptosis of cultured cells. As discussed previously, the H2O2 generated in the cell culture system due to autoxidation of EGCG could account for some of the reported apoptotic activity. A recent study suggested that certain green and black tea polyphenols bind to the antiapoptotic proteins Bcl-2 and Bcl-xL, thus preventing inhibition of apoptosis (Leone et al., 2003). Using a combination of advanced physical measurements and computational docking studies, these investigators determined that tea polyphenols with a gallate moiety inhibit the above antiapoptotic proteins at nanomolar levels. If this action does indeed take place in cells, one would expect to observe the induction of apoptosis with nanomolar amounts of EGCG. However, enhanced apoptosis was usually observed with much higher concentrations of EGCG (20 to 100 μM) (Yang et al., 2002).

Cao and Cao (1999) demonstrated the inhibition of endothelial growth and angiogenesis in the chorioallantoic membrane assay by EGCG (20 μM). They also showed that oral administration of 1.25% green tea to mice inhibited corneal neovascularization stimulated by VEGF, a growth factor involved in the formation of blood vessels. Several investigators have demonstrated that EGCG inhibits the expression of VEGF in head and neck, breast, and colon carcinoma cells (Masuda et al., 2002; Sartippour et al., 2002; Jung et al., 2001).

### 11.6.4 Inhibition of Other Enzymes

Berger et al. (2001) demonstrated that EGCG selectively inhibits the activity of topoisomerase I, but not topoisomerase II, in human colon cancer cell lines. The doses of EGCG necessary for this inhibition (10 to 17 μM) are lower than those necessary for inhibition of cell growth (IC50 = 10 to 90 μM). Inhibition of the matrix metalloproteases by EGCG has also been demonstrated at relatively low doses. Garbisa et al. (2001) showed that EGCG inhibits the activity of two gelatinases most frequently overexpressed in cancer and angiogenesis (MMP2 and MMP9) at concentrations of 8 to 13 μM. Additionally, 1 μM EGCG was found to increase the expression of proteins that inhibit the activity of activated MMPs (TIMP-1 and TIMP-2). Others have shown that EGCG also inhibits the activity and expression of the activator of MMPs (MT1-MMP) (Annabi et al., 2002). Because of the physiologically achievable concentrations, these activities represent an attractive mechanism for the observed antiangiogenic and antimetastatic activity of EGCG and green tea in vivo.

EGCG has been reported to inhibit the chymotryptic activity of the 20s proteasome, an enzyme responsible for protein turnover, in leukemic, breast cancer, and prostate cancer cell lines (Nam et al., 2001). This inhibition results in the accumu-
lation of the cell cycle inhibitor p27Kip1 as well as IκB, which results in G0/G1-phase cell cycle arrest and inhibition of NFκB activity, respectively. The IC50 for EGCG-mediated inhibition of the proteasome in cells (1 to 10 μM) is tenfold higher than the IC50 in a cell-free system (86 to 194 nM). This is possibly due to nonspecific binding of EGCG to other cellular components. Such nonspecific binding likely increases in vivo, and therefore a higher dose may be required in animal studies to see the EGCG-mediated inhibition.

11.7 POSSIBLE MECHANISMS OF INHIBITION OF CARCINOGENESIS AND CONCLUDING REMARKS

In spite of the many biological actions of tea constituents discussed above, the mechanisms for the inhibition of carcinogenesis in animals remain unclear. One of the problems in extrapolating results from in vitro studies to animals is that the concentrations used in vitro are usually much higher than those achievable in animal plasma and tissues after tea consumption. Even when activities are inhibited by physiological concentrations of EGCG, as was observed in certain purified enzyme systems (Nam et al., 2001; Garbisa et al., 2001; Leone et al., 2003), caution must be exercised in the extrapolation from purified enzymes to cells and to animals. Therefore, any proposed mechanism of cancer prevention needs to be verified in animal models or human tissues.

Based on information obtained from studies with animal models and cell lines, we propose that multiple mechanisms are involved in the inhibition of carcinogenesis, and both tea polyphenols (mainly EGCG) and caffeine are active compounds. The relative importance of these compounds depends on the model system used. As shown in Figure 11.3, tea polyphenols such as EGCG can directly inhibit specific protein kinases and receptors, thus suppressing the activation of AP-1 and NFκB, the phosphorylation of tumor suppressor Rb, and the expression of cyclooxygenase-2. These actions would inhibit cell growth and transformation, enhance apoptosis, and inhibit angiogenesis. The inhibition of cell proliferation and enhanced apoptosis has been demonstrated in skin, lung, and prostate models of carcinogenesis. The inhibition of angiogenesis due to tea consumption has been observed in NNK-induced lung adenomas (Liao et al, 2004). Both EGCG and caffeine enhance apoptosis, especially in malignant or premalignant cells (Lu et al, 2002).

In many of the experiments that we conducted, treatment of rodents with tea reduced body weight by 5 to 10% without any signs of toxicity. In the UV-induced skin carcinogenesis model, the inhibition of carcinogenesis by tea was correlated with a decrease in the amount of dermal fat, without significantly affecting body weight. The activity has been attributed mostly to the caffeine in tea. An interesting possibility is that fat may secrete TNF or other factors that enhance tumorigenesis or serve as a source of arachidonic acid, the precursor of tumor-promoting prostanoids.

Because of the wide consumption of tea beverages worldwide, the biological activity of tea constituents is an important topic for scientific investigation. The different biological activities discussed in this chapter suggest that tea possesses
cancer-preventive activities. Nevertheless, these activities have not been convincingly demonstrated in humans, in spite of the many epidemiological studies. Recent studies using a more quantitative assessment of tea consumption (Sun et al., 2002) and analyzing subgroups of populations (Wu et al., 2003a) have begun to demonstrate the expected protective effect. Additional positive results are expected when methodology in epidemiological studies is further refined. Another consideration is that at the levels of human tea consumption, which are usually lower than those used in animal cancer chemoprevention studies, the amount of tea polyphenols that reach the target tissues is a limiting factor. For cancer prevention, one possible approach is to increase the amount of tea intake, but this may be limited by the taste and possible undesirable side effects. At moderate levels of tea consumption, the organ sites that are more accessible to tea polyphenols are likely to be protected by tea. This concept could be tested in future epidemiologic and intervention human studies.

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12 Cancer Chemoprevention by Wine Polyphenols and Resveratrol

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12.1 GENERAL PROPERTIES OF GRAPE POLYPHENOLS AND RESVERATROL

Agents that have been evaluated or are proposed to be used in clinical trials for cancer prevention include micronutrients, minerals, synthetic compounds, and natural products. Several chemopreventive agents that have been characterized in this laboratory include brassinin (Mehta et al., 1995), deguelin (Udeani et al., 1997), sulfora-
mate (Gerhäuser et al., 1997), 4’-bromoflavone (Song et al., 1999), brusatol (Mata-Greenwood et al., 2002; Cuendet et al., 2004), and resveratrol (Jang et al., 1997).

12.1.1 Grape Polyphenol Library

Phenolic compounds, including resveratrol, comprise a large group of plant metabolites. Epidemiological studies have suggested an association between frequent consumption of fruits and vegetables and a decrease in cancer incidence. The most widely investigated polyphenols are present in the human diet as components of fruits, vegetables, tea, and wine. All plant phenolic compounds arise from a common intermediate, phenylalanine, or a close precursor, shikimic acid. Some of these compounds are produced to protect the plant from environmental stress, wounds, and herbivores. Early interest in polyphenols concerned their “antinutritional” effects, caused by the decreased absorption and digestibility of food due to binding of polyphenols to proteins and minerals. The astringency of many fruits and beverages is affected by the precipitation of salivary proteins with plant polyphenols.

Grapes and wine contain a variety of polyphenols with different degrees of polymerization. They range in chemical complexity from simple phenolic acids, such as caffeic acid, to very complex high-molecular-weight tannins. Some are mono-, di-, or tri-phenols. Examples include phenol, pyrocatechol, resorcinol, hydroquinone, phloroglucinol, several phenolic aldehydes, and phenolic and benzoic acids. Other grape polyphenols have two or more aromatic rings, e.g., flavanols, flavonols, and anthocyanins. Together with oxidation products of tannins, polyphenols play an important role in the organoleptic characteristics of wine. In particular, tannins confer astringency, flavor, and taste to the beverage by formation of complexes with the proteins of human saliva. Knowledge of these compounds is important to predict the aging quality of wine and its color stability (Flamini, 2003).

More than 500 compounds have been recognized in wine. Based on their general chemical structure, the grape phenolics may be classified into three groups, shown in Figure 12.1:

1. Nonflavonoids (A) derived from hydroxycinnamic and hydroxybenzoic acids, initially synthesized from phenylalanine.
2. Flavonoids (B) (catechin, epicatechin, quercetin, etc.) comprising the largest class (several thousand) of phenolic compounds including flavanols (many polymeric flavanols are present in grape seeds), flavonols, isoflavones, flavanones, and anthocyanins. All are structurally related to the parent flavone (2-phenylbenzopyrone), and exist as free compounds or polymerized to other flavonoids and nonflavonoids. Some of these compounds in grapes and wine are glycosylated with sugars such as glucose, rhamnose, galactose, and arabinose. They are present primarily in skins, seeds, and stems of the fruit.
3. Stilbenes (C) constituting a relatively small group of phenolic compounds that are usually synthesized in plants in response to stress conditions. Their structures contain two aromatic rings connected by a two-carbon
methylene bridge. Resveratrol (3,5,4’-trihydroxystilbene) is the most extensively studied stilbene.

The composition of phenolic compounds in wine varies. Soleas et al. (1997) determined the gross phenol composition estimated in milligrams of gallic acid equivalents (GAE) per liter. Young red wine contained 1060 mg GAE/L of total flavonoids, which was reduced to 706 mg/L with aging. White wine had a lower GAE/L (20 to 30 mg/L), which did not change when the wine was aged. Nonflavonoid concentrations for red and white wines were similar and did not change significantly when wine was aged.

Polyphenols are the principal compounds associated with the health benefits of wine consumption. They are used as active ingredients in some health products, mainly for treatment of circulatory blood disorders such as capillary fragility, peripheral chronic venous insufficiency, and microangiopathy of the retina. In 1999, grape seed extract in the form of tablets, liquids, or capsules was reported to be one of the most popular herbal supplements sold by food, drug, and mass market retail outlets in the United States. A recent estimate for the U.S. grape seed extract market was $40 to 50 million per year (Boswell, 2000).

Pharmacological properties of selected phenols from grape seeds are associated either with some physiological effects, e.g., an increase of tonicity and resistance of capillary walls, or chemical effects, e.g., antioxidative activities and inhibition of superoxide ion formation. Adding phenols to the diet is often followed by a reduction of platelet aggregation and a modulation of eicosanoid synthesis. The capacity of

**FIGURE 12.1** Representative structures of selected classes of wine and grape polyphenols. (A) Nonflavonoids, (B) flavonoids, and (C) stilbenes.
polyphenols to scavenge free radicals by breaking the free radical chain reaction of lipid peroxidation is well known. The ingestion of alcohol-free red wine, which had a relatively high level of phenolics (363.0 ± 48.0 quercetin equivalent [QE] mg/L), caused significant increases in the plasma total radical-trapping antioxidant parameter (TRAP), whereas alcohol-free white wine, containing 31.0 ± 1.0 mg QE/L, had no effect on the TRAP value. Plasma total antioxidant capacity values of subjects who were given red wine demonstrated the maximum peak at 50 min after consumption, while no dynamic changes were observed when white wine was evaluated. Red wine was efficient in protecting low-density lipoprotein from oxidation driven by peroxyl and ferryl radicals (Serafini et al., 2000). The relatively quick appearance of phenolics in plasma suggests that polyphenols may be absorbed in the upper gastrointestinal tract or from small intestine (Kuhnle et al., 2000).

Some active compounds of grape seed extract demonstrated both carcinogenic and anticarcinogenic activities. For example, catechin and caffeic acid induced stomach adenocarcinomas and promoted forestomach and glandular stomach cancers in rats, whereas they inhibited mammary gland tumor formation in rats, and croton oil-promoted skin papillomas and benzo[a]pyrene (BaP)-initiated forestomach tumors in Swiss mice. The predominant grape monomer epicatechin decreased multiplicity of chemically promoted dermal tumors and sarcomas in Swiss mice (NLM CCRIS, 2000), but induced sister-chromatid exchange in human lymphocytes (RTECS, 1999).

The bioavailability of polyphenols varies greatly and plays an important role in antioxidation and other biological activities. Factors influencing bioavailability include the chemical properties of the polyphenols, deconjugation and reconjugation in the intestines, and intestinal absorption. Considering intestinal absorption, a generally accepted concept is that polyphenols are absorbed by passive diffusion. Several classes of colonic bacteria, including Bacteroides spp., which are common in humans, are capable of hydrolyzing some phenolic glucosides and degrading flavonoids further by opening the heterocyclic ring and liberating monophenolic compounds, which are absorbed and excreted in the urine (Gee and Johnson, 2001). Another factor affecting the absorption of polyphenols derived from red wine is their binding to plasma low-density lipoproteins, which may relate to the antioxidant properties of red wine. Daily consumption of 400 ml of red wine by nine subjects during a 2-week experiment resulted in a 20% reduction in the propensity of plasma to undergo lipid peroxidation. Conversely, dietary consumption of white wine resulted in 34% increase in the propensity of plasma to undergo lipid peroxidation (Fuhrman et al., 1995).

**12.1.2 Resveratrol**

Resveratrol (Figure 12.1) is a natural phytoalexin that is expressed in plants as a defensive response against fungal infections and other environmental stressors (Stewart et al., 2003). The word “alexin” is of Greek origin and means to ward off or to protect. Resveratrol may also have alexin-like activity in humans, protecting against degenerative diseases. Synthesis of resveratrol in grapes is most likely associated with natural stress factors such as exposure to ultraviolet radiation (Creasy and Coffee, 1998), injury, or during fungal or mold invasion (Schwekendiek et al.,
Significant amounts of resveratrol were detected in healthy fruit clusters prior to any detectable mold lesions. This suggested that the compound was synthesized soon after the recognition of the pathogen by the plant (Jeandet et al., 2002). Montero et al. (2003) investigated involvement of the plant hormone ethylene in resveratrol synthesis during fruit maturation. High resveratrol content correlated with low ethylene emission. Exogenous application of resveratrol on the fruit surface delayed the increase of ethylene emission and doubled the normal shelf-life of grapes. This response is due to the antifungal activity of resveratrol, indicating the wide potential of such a compound for the control of the microbiota on fruits and practical application as a natural chemical to prolong the shelf life of fruits (Hawksworth, 2003). Possibly, through elucidation of the mechanisms of plant protection, human health benefits of resveratrol could be better understood.

Resveratrol was first recognized as a biologically active compound by Siemann and Creasy (1992). The compound is found in several plants, chiefly in red grapes. The highest concentration (50 to 100 µg/g of grape wet weight) was determined in the grape skin. In wine, cis- and trans- isomers (Figure 12.1) are present in the free or glycosylated forms. cis-Resveratrol was not detected in grape skin and juices. Formation of the cis- isomer by isomerization or breakdown of the trans- form on exposure of wine to light and oxygen has been assumed (Cantos et al., 2000). In dietary supplements, the isomer is not always specified, but in most cases it is the trans- form. In red wine, the concentration of the trans- isomer ranges between 0.1 and 15 mg/L. The ratio of cis- and trans- resveratrol in wines varies by region. Climate, the type of grape, and the length of time the skin is kept with the grape during the winemaking process are some factors that influence the level of resveratrol and the ratio of isomers in wine (Careri et al., 2003). Primarily, the compound is produced in the grape, grape shoots, and vines. Increasing irradiation of harvested grapes by UVB or UVC light enhances yields of resveratrol (Cantos et al., 2000).

Most resveratrol-containing supplements marketed in the United States contain extracts of the root of Polygonium cuspidatum Sieb. and Zucc., also known as the Japanese knotweed. The dried root and stem of this plant are used in traditional Japanese folk medicine (Ko-jo-kon) as a circulatory tonic, against fungal diseases, and against various inflammatory and liver diseases (Nonomura et al., 1963). Moreover, resveratrol synthase genes have been isolated and inserted into plants, creating transgenic varieties of alfalfa, tobacco and other plant species with higher trans-resveratrol concentrations. Phytoalexins inserted into plants may provide defense against different pathogens (Hain et al., 1993). Additionally, transgenic plants, e.g., alfalfa, transformed with resveratrol-synthesizing genes might become an economical source of the compound for scientific research or dietary supplements (Paiva, 1999).

Resveratrol bioavailability might have an effect on biological activity. When 20 mg of resveratrol per kg of body weight (a dose 140 times the amount found in a liter of red wine) was administrated orally to animals, the highest plasma concentration of resveratrol was found in the first 5 min after intake, and this decreased to less than 0.1 µM after 60 min. Modifications of the resveratrol molecule may increase bioavailability (Asensi et al., 2002). An already recognized metabolite of resveratrol, piceatannol, has been studied as an antileukemic agent (Wieder, 2001). At present, resveratrol is available as a food supplement: Resanex, Resveratrol Synergy, Resver-
Carcinogenic and Anticarcinogenic Food Components

The optimal level of intake and precise health benefits are not well defined. Some manufacturers recommend doses from 200 to 600 µg per day. However, metabolic pathways need to be understood more fully before adding resveratrol to human diets can be recommended.

This review summarizes the established activities of resveratrol. The progress, problems, and future efforts to use the agent as an essential component in chemoprevention are also discussed.

12.2 CHEMOPREVENTION OF MULTISTAGE CARCINOGENESIS BY RESVERATROL

Resveratrol has been extensively studied during the last 10 years (Pezzuto, 1995, 1997; Bhat et al., 2001; Bhat and Pezzuto, 2002; Asensi et al., 2002; Aziz et al., 2003; Dong, 2003; Gusman, 2001). The cancer chemopreventive properties of resveratrol were first described by Jang et al. (1997), when it was demonstrated that the compound could act as an antioxidant and antimutagen. The compound induced Phase II drug-metabolizing enzymes involved chiefly in detoxification of carcinogen metabolites (antiinitiation activity), mediated antiinflammatory effects, inhibited cyclooxygenase (antipromotion activity), and induced human promyelocytic leukemia cell differentiation (antiproggression activity). Some protective effects of resveratrol against the multistage process of tumorigenesis are discussed below.

12.2.1 EFFECTS OF RESVERATROL ON INITIATION STAGE

12.2.1.1 Phase I Cytochrome P450 (CYP) Enzymes

Resveratrol inhibits polycyclic aromatic hydrocarbon (PAH)- and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-induced CYP1A1 expression and activity (Ciolo et al., 1998; Ciolo and Yen, 1999). It has been shown that resveratrol is a competitive antagonist for the aromatic hydrocarbon receptor (AhR) and efficiently blocks CYP1A1 induction in various organs (Casper et al., 1999). These investigators hypothesized that resveratrol could serve as both a cardioprotective and antitumor drug through inhibition of AhR-mediated transactivation of CYP genes and radical production. Whereas AhR translocation to the nucleus is promoted, transactivation of dioxin-responsive genes such as CYP1A1 and interleukin is inhibited by resveratrol. There seems to exist some selectivity with which resveratrol distinguishes and inhibits the activity of CYP1A1 over CYP1A2.

Other CYPs, such as CYP1B1, are overexpressed in a variety of human tumors including those of the breast, colon, and lung, and CYP3A4 is predominantly overexpressed in colon and liver cancers. Both of these CYPs were found to be inhibited by resveratrol (Chang et al., 2000; Chan and Delucchi, 2000).

12.2.1.2 Phase II Enzymes

The transcription of Phase II enzymes, e.g., glutathione S-transferase, UDP-glucuronosyl transferase, and menadione oxido-reductase, is not induced through the
classic “xenobiotic responsive element” (XRE), but through an “antioxidant/electrophile responsive element” (ARE/EphRE). This latter DNA binding site interacts with several proteins (including the AhR) in a very complex cross-talk. Resveratrol inhibits XRE-mediated transactivation but is not active on ARE-based mechanisms. Thus, the production of detoxifying enzymes is not hampered by resveratrol treatment (Savouret and Quesne, 2002).

12.2.2 Effects of Resveratrol on Promotion and Progression Stages

The second stage of carcinogenesis, tumor promotion, is slow and reversible. It involves epigenetically controlled clonal expansion of initiated cells that produce a benign tumor (e.g., papillomas in the classical mouse skin model). Tumor progression is the irreversible conversion of a benign tumor to a malignant phenotype (e.g., mouse skin carcinoma) (Hursting et al., 1999). PAHs are commonly employed as initiators and phorbol esters as mediators of tumor promotion and progression stages in mouse skin, a widely studied carcinogenesis model.

12.2.2.1 Protein Kinases

The primary target mediating the tumor-promoting activity of 12-O-tetradecanoxylophorbl-13-acetate (TPA) is the protein kinase C (PKC) isozyme family (Hursting et al., 1999). PKC is a well-established regulatory element in the modulation of a variety of cellular processes such as cell signaling and tumor promotion. Stewart et al. (1999) reported that resveratrol inhibited the PKC-catalyzed phosphorylation of arginine-rich protein substrate in a noncompetitive manner. It exhibited a broad spectrum of inhibitory activity against a variety of PKC isozymes, such as cPKC, nPKC, and αPKC.

In addition, the chemopreventive activity of resveratrol has been linked to its ability to block the NFκB pathway through IκB kinase inhibition (Holmes-McNary and Baldwin, 2000).

NF-B is an inducible transcription factor originally identified as a heterodimeric complex consisting of a 50 kDa subunit (p50) and a 65 kDa subunit (p65). NF-B is strongly linked to inflammatory and immune responses and is associated with oncogenesis in certain models of cancer. A correlation between general protein kinase inhibition and the antitumor properties of natural polyphenols from various plant species has been long known (Chang and Geahlen, 1992). Dong (2003) offered a possible molecular mechanism involving extracellular signal-regulated protein kinases (ERKs) and p38 kinase-mediated p53 activation and induction of apoptosis. Activation of p53 and apoptosis may occur partially through p53 phosphorylation, and resveratrol may interfere with this process.

12.2.2.2 Cyclooxygenase and Lipoxygenase

Key molecular targets implicated in the activity of resveratrol are the cyclooxygenases (COX). Cyclooxygenases produce prostaglandins from arachidonic acid (AA). Prostaglandins stimulate tumor growth by acting on cell proliferation, angio-
genesis, and immunosuppression. COX exists in two isoforms: COX-1 and COX-2, which are implicated in inflammatory reactions. We first identified resveratrol as a potential chemopreventive agent on the basis of COX inhibition (Jang et al., 1997). The antiinflammatory activity of resveratrol was shown through suppression of both the acute and chronic phases of edema in the rat paw-edema model (Bhat and Pezzuto, 2002).

Resveratrol was shown to inhibit the activity of the COX-1 enzyme derived from sheep seminal vesicles (Shin et al., 1998). Further, resveratrol suppressed activation of COX-2 gene expression and activity by interfering with the protein kinase C signal transduction pathway in mammary epithelial cells (Subbaramaiah et al., 1998). Inhibitors of COX are considered valuable therapeutic agents against cancer (O’Byrne and Dalgleish, 2001; Gusman et al., 2001).

AA is also metabolized via lipoxygenase (LOX) to produce hydroxyeicosatetraenoic acids or leukotrienes. AA metabolites derived from LOX pathways play an important role in growth-related signal transduction, implying that intervention through these pathways should be useful for arresting cancer progression. LOX-derived metabolites have an indirect influence on the development and progression of human cancers (Steele et al., 2000). Resveratrol inhibits LOX and COX in chronic myelogenous leukemia cells K-562 (Maccarrone et al., 1999). Reports on a specific role of LOX in cancer growth are limited and overshadowed by the intense interest in COX (Cuendet and Pezzuto, 2000).

### 12.2.2.3 Cell Cycle Effects

Deregulation of cell proliferation in conjunction with inhibition of apoptosis constitutes a basic platform for the progression of neoplastic lesions. The antitumor activity of resveratrol is attributed to effects on the cell cycle, cell proliferation, and apoptosis. Resveratrol triggered arrest of the G1/S transition in prostate cancer cell lines and induced apoptosis (Hsieh and Wu, 1999). The mechanisms for this include inhibition of ribonucleotide reductase and DNA polymerase or topoisomerase II (Galati et al., 2000). Ribonucleotide reductase is a rate-limiting enzyme for DNA synthesis, as it catalyzes the reduction of ribonucleotides to deoxyribonucleotides, the precursors for DNA synthesis.

It appears that resveratrol has the greatest effect on the S-phase, with consequent effects on the S/G2 transition. In the HL-60 cell model system, which has been used to monitor biological activity of large numbers of samples for the discovery of new agents that induce cellular differentiation (Pezzuto, 1995), resveratrol caused an accumulation of cells in the G1/S phase, as seen by the absence of G2/M peaks. After a 24-h treatment, resveratrol caused a significant increase in the levels of cyclins A and E, along with an accumulation of cdc2 in the inactive phosphorylated form (Ragione et al., 1998). Similarly, Hsieh and colleagues noted that resveratrol induced NO synthase in cultured pulmonary epithelial cells with suppression of cell cycle progression through the S and G2 phases. This was accompanied by a concomitant increase in the expression of p53 and p21 and apoptosis (Hsieh, et al., 1999).
Resveratrol showed antiproliferative effects with human prostate cancer cell lines PC-3 and DU145 and was completely ineffective with LNCaP cells. It was proposed that the antiproliferative effect of polyphenols, including resveratrol, is mediated through the modulation of NO production (Kampa et al., 2000). Other studies revealed that in LNCaP cells the effect of resveratrol on DNA synthesis varied depending on the concentration and duration of treatment. LNCaP cells, treated with resveratrol, were induced to enter the S phase, but subsequent progression through the S phase was limited by the inhibitory effect of resveratrol on DNA synthesis at concentrations above 15 \( \mu M \). The unique ability of the compound to exert opposing effects in the cell cycle, induction of S phase and inhibition of DNA synthesis, may be responsible for its apoptotic and antiproliferative effects (Kuwajerwala et al., 2002). The capacity of resveratrol to recruit prostate cancer cells into the S phase raises the possibility of its usefulness in chemotherapy. Prostate cancer is a slow-growing disease, and only a small fraction of its cells are actively proliferating at any given time. This creates a problem for treatment because chemotherapeutic agents and ionizing radiation are most effective in killing cells that are actively proliferating. Therefore, any agent that induces proliferative stimulation of prostate cancer cells can increase their sensitivity to the cytotoxic effects of chemotherapeutic agents and ionizing radiation. In this regard, a preferential effect of resveratrol in recruiting androgen-sensitive PC cells into S phase makes it a potentially attractive adjuvant for chemotherapy or radiation therapy to target proliferating PC cells in S phase (Kampa et al., 2000).

12.2.2.4 Apoptosis

Apoptosis is a normal physiological process, wherein cells undergo programmed cell death with considerable morphological and biochemical changes. Apoptosis is required to maintain a balance between cell proliferation and cell loss. Since misregulation in this balance can lead to malignant transformation, induction of apoptosis in a transformed cell population suppresses the development of cancer. Resveratrol induced apoptosis in HL-60 cells as demonstrated by DNA fragmentation, an increased proportion of subdiploid cell population, and a time-dependent decrease in bcl-2 expression (Surh et al., 1999).

Apoptosis can be induced by UV-mediated DNA damage (Kulms and Schwarz, 2000). The most important mediator of this effect was the tumor suppressor gene p53, a gene that is mutated in about 50% of tumors, and the lack of expression or loss of its function is associated with an increased risk of cancer (Hussain et al., 2000). It has been shown with normal murine epidermis cells JB6 C1 41 that resveratrol suppressed cell transformation and induced apoptosis in a p53-dependent manner. Significantly, apoptosis was induced at the same concentration that was required to inhibit cell transformation. Moreover, resveratrol induced apoptosis in cells expressing wild-type p53, but not in p53-deficient cells (Huang et al., 1999). Further, mechanistic work in this cell line indicated that resveratrol-induced apoptosis and activation of p53 is mediated via a complex formation between ERKs and p38 kinase. It was also shown that stable expression of negative mutants of ERK2 or p38 kinase or their inhibitors impaired resveratrol-mediated apoptosis in...
this cell line (She et al., 2001). Bax, together with the antiapoptotic gene bcl-2, is a transcriptional target for p53. In a rat colon carcinogenesis model, resveratrol induced proapoptotic bax expression in colon aberrant cryptic foci (ACF), but not in the surrounding mucosa. In addition, resveratrol treatment suppressed expression of p21 in normal mucosa but not in ACF (Tessitore et al., 2000).

There have been comparisons of the effect of resveratrol on various breast carcinoma cell lines with different metastatic potentials. Resveratrol caused an accumulation of cells in the S-phase with concomitant reduced expression of Rb and increased expression of p53 and bcl-2 proteins. The compound was most effective against MDA-MB-435 cells, which are highly invasive (Hsieh et al., 1999).

As illustrated in Figure 12.2, resveratrol affects numerous events during the three stages of carcinogenesis.

12.2.3 Antioxidative Effects of Resveratrol

Most plant polyphenols are recognized for their antioxidative activities. Electron acceptors such as molecular oxygen react easily with free radicals to become reactive oxygen species (ROS). They scavenge free radicals, thus breaking the free radical chain reaction of lipid peroxidation. Stivala et al. (2001) have shown that the hydroxy group in the 4 position is required for the antioxidant activity of polyphenols but acts synergistically with 3- and 5-hydroxy groups. Polyphenols also quench or prevent the formation of reactive oxygen and nitrogen species. Another antioxidative mechanism is the chelation of metals such as iron and copper ions, which prevents their participation in Fenton-type reactions and the production of highly reactive hydroxyl radicals (Yang et al., 2001). The antioxidant activity of phenols from 12 different varieties of grapes toward human low-density lipoprotein has been evaluated (Facino et al., 1999). The flavon quercetin blocked the aggregation of human platelets by adenosine-5’-diphosphate (ADP) and thrombin, and this compound has gained considerable prominence as an inhibitor of carcinogenesis (Pace-Asciak et al., 1995). In some experiments with rats, proanthocyanidins from grape seeds reduced susceptibility to heart ischemia and increased the total antioxidant plasma capacity and ascorbic acid plasma level (Facino et al., 1994). Additionally, proanthocyanidins from grape seeds decreased the susceptibility of healthy cells to toxic and carcinogenic agents.

Recent data suggest that absorption of red wine polyphenols by incorporation of polyphenols into low-density lipoproteins contributes to the activity of phenols against oxidative damage. Resveratrol has been evaluated as an antioxidant constituent in the human diet (Fremont, 2000). The antioxidant capacity of the resveratrol molecule is due to the ease with which a hydrogen atom from an aromatic hydroxy group can be donated to a free radical (Duthie and Crozier, 2000). The antioxidant activity and cell cycle effects of resveratrol were investigated by Stivala et al. (2001). For this purpose, derivatives of resveratrol in which a methyl group(s) replaced each single or all hydroxy group(s) were synthesized. Trans-resveratrol showed the best antioxidant activity among the various derivatives. The same authors established that in vitro inhibition of DNA synthesis was stimulated by a direct interaction of resveratrol with DNA polymerases and in murine fibroblast cells. In two other cell
lines, murine mastocytoma P-915 and human myelogenous leukemia K-562 cells, trans-resveratrol exerted an inhibitory effect on the primary components of DNA synthesis, such as DNA polymerase (Sun et al., 1998), and ornithine decarboxylase, a key enzyme of polyamine biosynthesis that is up-regulated during cancer growth (Schneider et al., 2000). The process correlated with a reduction in ROS production, phospholipase A2 translocation, and subsequent AA and PGE2 synthesis stimulated by fetal calf serum or platelet-derived growth factor (Moreno, 2000). ROS have carcinogenic potential and are associated with tumor promotion. Resveratrol may function as a ROS scavenger that suppresses tumor development. In a recent study designed to investigate the antioxidant activity of resveratrol in lymphocyte leukemia cells, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT)-reducing activity was increased without increasing the number of living cells. MTT is a
compound commonly used to detect reductive metabolism in assays of cell viability, proliferation, and cytotoxicity. Concentrations of resveratrol in the range of 5 to 20 \( \mu M \) were not sufficient to induce apoptosis but decreased the rate of cell growth (Bernhard et al., 2003).

### 12.2.4 Hormonal Effects of Resveratrol: Estrogen- and Androgen-Related

Epidemiological studies show that Asian populations, who consume larger amounts of natural polyphenols than North Americans do, have significantly lower incidences of breast and prostate cancer, bowel cancer, and cardiovascular diseases (Wiseman, 2000). Flavonoids and isoflavonoids, such as quercetin and genistein, have been reported to display both estrogenic and antiestrogenic effects (Davis et al., 1999). Similarity in the structure of trans-resveratrol and some synthetic estrogens (diethylstilbestrol) suggested estrogenic activity. Resveratrol has been shown to bind to the estrogen receptor (ER). Gehm et al. (1997), using human breast adenocarcinoma cells, demonstrated that at concentrations of 3 to 10 \( \mu M \) resveratrol competed with 17\( \beta \)-estradiol (E\(_2\)) for binding to the ER. In human breast cancer MCF-7 cells, resveratrol at higher concentrations (20 to 160 \( \mu M \)) inhibited cell proliferation (Mgbonyebi et al., 1998). However, in some in vivo studies, treatment of rats with resveratrol has not confirmed the estrogenic activity indicated by in vitro tests. Oral and subcutaneous administration of resveratrol at concentrations ranging from 1 to 1000 \( \mu g/day/animal \) has been tested in weanling rats (Turner et al., 1999). With lower doses, resveratrol did not affect uterine weight, uterine epithelial cell size, cortical bone cell parameters, or levels of serum cholesterol. However, at the highest doses, resveratrol antagonized the serum cholesterol-lowering activity of E\(_2\). In another study with immature rats, resveratrol tested by oral and subcutaneous routes, at concentrations ranging from 0.03 to 120 mg/kg of body weight/day, showed no effect on uterus weight (Ashby et al., 1999). In a recent study with Wistar rats, both compounds, resveratrol and E\(_2\), increased uterine weight, enlarged the uterine lumen, and induced hypertrophy of epithelial, stromal, and myometrial cells (Freyberger et al., 2001). Another experiment in which resveratrol showed estrogenic properties was carried out in stroke-prone spontaneously hypertensive rats (Mizutani et al., 2000). In our studies with ovariectomized female Sprague-Dawley rats, resveratrol was administered in the diet (3 g/kg diet) and E\(_2\) was injected subcutaneously (50 \( \mu g/kg \) of body weight) for 30 days. As expected, E\(_2\) increased (approximately threefold) uterine weight compared to the control group. On the other hand, resveratrol consumption caused no changes in uterine weight; hence, the compound was considered neither estrogenic nor antiestrogenic (Bhat and Pezzuto, 2002).

It was shown that resveratrol binds to both ER-\( \alpha \) and ER-\( \beta \) with comparable affinity, but with a 7000-fold lower affinity than E\(_2\) (Bowers et al., 2000). Consistent with this report, we have observed that resveratrol mediates antiestrogenic effects in endometrial cancer (Ishikawa) cells by a novel mechanism that involves selective downregulation of ER-\( \alpha \) but not ER-\( \beta \) (Bhat and Pezzuto, 2001).

While more data are accumulating on the estrogenic effects of resveratrol, controversy still persists with regard to its ability to serve as a chemopreventive
agent in breast cancer. It has been demonstrated that resveratrol is an agonist for the cAMP/kinase-A system, a documented proapoptotic and cell-cycle suppressor in breast cancer cells (El-Mowafy and Alkhala, 2003). This study highlights a new cellular mechanism for the anti-breast cancer effects of resveratrol, i.e., activation of the adenylyl-cyclase/protein kinase A cascade. This pathway appears to disrupt necessary signaling mechanisms in breast cancer cells, thereby augmenting the cytotoxicity of resveratrol. Such effects of resveratrol were not mediated by interaction with ER, thus further indicating the versatile properties of the resveratrol molecule. This research may substantiate the clinical utility of resveratrol against breast cancer.

We have observed that resveratrol exhibits mixed estrogenic/antiestrogenic properties in some ER-positive mammary cancer cell lines, or acts as a pure antiestrogen in other mammary cell lines and in rodent models, or inhibits the formation of carcinogen-induced preneoplastic mammary lesions and tumors (Bhat et al., 2001). These studies have led us to speculate that resveratrol could function as a novel selective estrogen receptor modulator (SERM).

There are a variety of ER forms in the human prostate: ER-α, ER-β, ERβ2, ERα (A, B, C, D, and E). Data obtained from experiments with transgenic mice indicate that estrogen may affect the adult prostate through ER-α, and the epithelial/mesenchymal ER-α-mediated effects of estrogen are recognized in the prostate (Risbridger et al., 2001). Resveratrol binds with low affinity to ER-α and ER-β and exerts either estrogenic or antiestrogenic effects (Bhat and Pezzuto, 2001; Bhat et al., 2001). The in vitro expression of ER-α and ER-β in prostate epithelial cells is also controversial (Lau et al., 2000; Sasaki et al., 2002). The discrepancies in ER mRNA expression might depend on the primer pairs used to amplify the ER mRNA, as there are different ER isoforms present in prostate epithelial cells. Another explanation may lie in the methylation status of the ER gene. Even though the origins of the cell lines are the same, methylation may occur with progressive passaging of the cells, altering methylation status and gene expression in the same cell line (Morrissey and Watson, 2003). A molecular modeling study was conducted to clarify these ambiguities. Some residues participate in the binding of resveratrol to the ER and switch its action from agonist to antagonist (El-Mowafy et al., 2002).

The estrogenic and antiestrogenic properties of resveratrol broaden the spectrum of its potential medical applications. A subset of patients at high risk for cardiovascular complications when treated with traditional estrogen therapy have been identified (age, body weight, preexisting cardiovascular disease, and other qualifying factors) (Henriksson et al., 1989). Resveratrol, reported to inhibit platelet aggregation and mediate other positive cardiovascular activities, may be a candidate for testing in a population of this type. Resveratrol has also been shown to affect the growth and tumorigenic potential of several cancer cell lines, as evidenced by inhibition of the expression and function of the androgen receptor (AR) in LNCaP prostate cancer cells. The compound down-regulated expression of androgen-induced genes such as p21, in addition to mediating several other effects (Mitchell et al., 1999). In the same cell line, however, it was found that resveratrol neither altered the expression of nor bound to the AR, but mediated antiandrogenic effects, such as decreased intracellular and secreted prostate-specific antigen (PSA) levels. In a related study,
it was found that resveratrol mediated growth inhibition and apoptosis in LNCaP cells. The authors extended these observations to some androgen-nonresponsive cell lines, wherein resveratrol caused growth inhibition and disrupted the G1/S phase transition of the cell cycle without causing apoptosis (Hsieh and Wu, 1999).

12.3 CONCLUSIONS

The desirable characteristics of a chemopreventive agent include the following (Aziz et al., 2003):

1. Little or no toxic effect in normal, healthy cells
2. High efficacy against multiple tumor sites
3. Suitable form for oral consumption
4. Known mechanism of action
5. Low cost to the consumer
6. Acceptance by human population

Resveratrol, representing a relatively new class of chemopreventive compounds, appears to fulfill the above criteria. Resveratrol, amply present in grapes, red wine, and several kinds of nuts and berries, has shown remarkable promise against various types of cancer including breast, prostate, liver, colorectal, lung, blood, and thyroid cancers (Aziz et al., 2003). In several in vitro and in vivo models, resveratrol has proven capable of retarding or preventing different stages of carcinogenesis. The precise mechanisms through which resveratrol exerts antitumor effects are not fully understood, although it is clear that several biochemical and molecular pathways are affected. The ability of resveratrol to trigger apoptosis pathways preferentially in tumor cells may account for much of the chemopreventive activity of the compound. Continued efforts are needed, especially well-designed preclinical studies in animal models, to establish the usefulness of resveratrol as cancer chemopreventive agent.

Resveratrol can be considered a versatile agent that is able to act via both estrogen-dependent and estrogen-independent mechanisms, occasionally even in the same biological system. Through binding to estrogen receptors, resveratrol might modulate estrogen levels. Additional studies on the estrogenic and antiestrogenic activities of resveratrol need to be conducted with various hormone-responsive prostate and breast cancer cell lines. Resveratrol has promise as a phytoestrogen in prostate cancer prevention and treatment.

It is noteworthy that toxicity of resveratrol in animals has not been observed. In our experience, it has proven to be nontoxic, even at high doses (3 g/kg diet) for 120 days. Since resveratrol is an active ingredient of several traditional medicines used for centuries in India, China, and Japan, the general medicinal value and safety of this compound is suggested. These findings are encouraging for the potential clinical application of resveratrol. However, it is not clear whether doses as high as those used in animals can be given to humans to achieve a pharmacological effect.

Resveratrol may also sensitize drug-resistant tumor cells to become sensitive to drug-mediated effects. This suggests that nontoxic doses of resveratrol as a sensi-
tizing agent may be clinically beneficial in combination with other drugs. Long-term epidemiological studies will determine the true preventive and therapeutic efficiency of dietary or supplemental resveratrol on tumor risk and development. In the future, resveratrol derivatives with specific affinity for appropriate receptors are worth exploring.

Recently, resveratrol was established as a potent agent in age-related studies. The research was designed to find and develop drugs to lengthen life and prevent or treat age-related diseases such as cancer. Yeast cells treated with low doses of resveratrol survived for an average of 38 generations, compared to 19 for untreated yeast. This effect was similar to that of a reduced-calorie diet; resveratrol served as an agent that mimicked fasting. In these experiments, resveratrol stimulated sirtuin (Sir2), an enzyme that regulates life span, increases DNA stability, and negatively regulates the tumor suppressor gene p53 (Howitz et al., 2003). Another study demonstrated that resveratrol was capable of protecting hippocampal cells against sodium nitroprusside (SNP)-induced toxicity. These data may be of particular interest given to deleterious role of ROS accumulation during normal brain aging and with some neurodegenerative diseases (Bastianetto and Quirion, 2002). Thus, new avenues of research have been opened, expanding the potential utilities of resveratrol.

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Cancer Chemoprevention by Wine Polyphenols and Resveratrol


Cancer Chemoprevention by Wine Polyphenols and Resveratrol


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RTECS (Registry of Toxic Effects of Chemical Substances, National Institute for Occupational Safety and Health), Records Nos. 28397, 30627, 44221, 53642, 58483, 110677, 1999.


13 Flavonoids: Common Constituents of Edible Fruits and Vegetables

Nicole Monfilliette-Cotelle

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13.1 INTRODUCTION

Flavonoids and their glycosides are the chief constituents of polyphenols that are present in edible plants such as fruits, vegetables, nuts, seeds, tea, olive oil, and red wine. These natural products were known for their beneficial effects on health a long time before flavonoids were isolated as effective compounds. Certain plants and spices containing flavonoids have been widely used for thousands of years in traditional folk medicine. Flavonoids are diphenylpropane derivatives that exert a wide range of biochemical and pharmacological effects (Middleton et al., 2000). Their antioxidant properties, cytostatic effects in tumorigenesis, and ability to inhibit a broad spectrum of enzymes, such as protein kinase C, tyrosine protein kinase, and topoisomerase II, have led researchers to regard these compounds as potential anticancer agents. These findings have also led some health-conscientious individuals to increase the consumption and use of dietary supplements containing high concentrations of plant flavonoids. These data have also prompted medicinal chemists
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Carcinogenic and Anticarcinogenic Food Components

use the molecular backbone of flavonoids as a lead compound in the design of drugs, e.g., flavopiridol (the first cyclin-dependent kinase inhibitor to be tested in Phase III clinical trials).

13.2 CHEMICAL STRUCTURES, FACTORS AFFECTING THE FOOD CONTENT OF NATURAL FLAVONOIDS, AND DAILY INTAKE

The flavonoids are the most common polyphenols present in plant food. Their common structure is that of a benzo-γ-pyrene derivative, depicted in Figure 13.1. Flavonoids comprise more than 5000 compounds (Harborne and Williams, 2000) and can be categorized into several groups according to their chemical structures (Figure 13.2). Condensed tannins or proanthocyanidins are polymers of flavans linked through carbon–carbon and ether linkages. Although 15 subclasses of proanthocyanidins have been identified (Porter, 1993), only three — procyanidins ([epi]catechin polymers), prodelphinidins ([epi]gallocatechin polymers) and propelargonidins ([epi]afselechin polymers) — or their mixtures appear to be prominent in human foods of plant origin. In these tannins, the monomeric units are primarily linked through single 4-6 or 4-8 carbon–carbon bonds (B linkages), or through 4-8 carbon–carbon linkages and 2-7 ether bonds (A linkages). Other linkages also have been identified but were isolated from nonfood plants or constituted minor compounds in foods such as cocoa (Beecher, 2003; Bohm, 1998).

The chemical nature of flavonoids depends on structural class, degree of hydroxylation, other substitutions and conjugations, and degree of polymerization. Flavonoids are usually associated with a sugar moiety, though occasionally they occur in plants as aglycones. The associated sugar can be present as a monosaccharide, disaccharide, or oligosaccharide. Glucose is the most common sugar residue, although galactose, rhamnose, xylose, and arabinose are also found, as well as glucuronic and galacturonic acids and many others. During metabolism, flavonoids can undergo modifications such as addition of hydroxyl groups, methylation, sulfation, or glucuronidation.

Numerous studies have evaluated the effects of flavonoids on health. Furthermore, various epidemiological studies investigated the relationships between polyphenol consumption and cancer or cardiovascular diseases. It was found that food compounds provide health benefits (in addition to their nutritional value) or have a role in disease risk prevention. Hence they have been termed functional foods.
or bioactive compounds. However, the levels of bioactive compounds are available only recently in a few databases but are restricted to a certain number of flavonoids (e.g., the U.S. Department of Agriculture [USDA] database is restricted to 19 flavonoids) and thus, cannot be considered as representing the complete flavonoid content of a food. Table 13.1 presents some examples of sources of food flavonoids.

The flavonoid content in plant foods is influenced by various factors, such as growth, season, geographic location, climate, weather, soil conditions, degree of

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**FIGURE 13.2** Chemical structures of flavonols, flavones, flavanones, and anthocyanidins.
ripeness, processing (cooking, pasteurization, fermentation) (Peterson and Dwyer, 1998) and storage (Aherne and O’Brien, 2002). The distribution pattern and concentration of flavonoids may also depend on light exposure (e.g., the highest concentrations of flavonones, 4-oxoflavonoids, and flavonols and their glycosides are found generally in leaves and outer parts of plants with only trace amounts in their subterranean parts) (Hermann, 1998; Harborne, 1986).

The flavonoid daily intake reported in the literature is highly variable (Table 13.2). It depends on various factors including cultural dietary habits, country, and factors affecting flavonoid content in foods. The calculations are based on three or four classes of flavonoids: flavanones, flavones, flavonols, flavan-3-ols, and sometimes isoflavones, and few compounds in these classes. Consumption of total flavonoids expressed as aglycones ranges from 20 to 170 mg/day (Kühnau, 1976). However, Scalbert and Williamson (2000) estimate total polyphenol intake to be approximately 1 g/day, of which two-thirds are flavonoids.

13.3 FLAVONOIDS DATABASE AND EPIDEMIOLOGICAL STUDIES

The association between ingestion of a class or classes of micronutrients and health promotion is often demonstrated through epidemiological studies. Such studies require databases on the food content of each member of the classes of phytonutrients.

**TABLE 13.1**

**Sources of Food Flavonoids**

<table>
<thead>
<tr>
<th>Flavonoids</th>
<th>Rich Food Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chalcones</td>
<td>Apple, cantaloupe, melon</td>
</tr>
<tr>
<td>Flavones&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Grapefruit, lemon, orange, carrot, celery, parsley, red sweet pepper</td>
</tr>
<tr>
<td>Flavonols&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Orange, broccoli, Brussels sprouts, cauliflower, onion, turnip greens, red wine, black/green tea</td>
</tr>
<tr>
<td>Flavanones&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Grapefruit, lemon, orange, tomato, honey</td>
</tr>
<tr>
<td>Flavans, flavan-3-ols&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Apple, apricot, nectarine, peach, pear, red grapes, strawberries, green beans, red wine, black/green tea</td>
</tr>
<tr>
<td>Anthocyanidins&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Apple, blackberries, black currants, blueberries, cherries, chokecherries, cranberries, elderberries, nectarines, peaches, raspberries, red grapes, red/green pears, strawberries, carrot, red cabbage, red onion, red beans, red wine, cocoa</td>
</tr>
<tr>
<td>Isoflavonoids&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Green beans, legumes, soybeans, soy products</td>
</tr>
<tr>
<td>Biflavanoids</td>
<td>Fruits and cereals, apples, blackberries, blackcurrants, cranberries, grapes, peaches, strawberries (Peterson and Dwyer, 1998)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Structures of representative compounds are in Figure 13.2.

<sup>b</sup> Includes catechins, epicatechins, and their gallates.

<sup>c</sup> Includes genistein and daidzein.

For flavonoids and isoflavonoids, contents in selected foods have been assembled in databases that are available on the Web (Flavonoids: USDA database for the flavonoids content of selected foods: http://www.nal.usda.gov/fnic/foodcomp; Isoflavonoids: USDA–Iowa State University Database on the Isoflavone Content of Foods — 1999: http://www.nal.usda.gov/fnic/foodcomp/data/isoflav/isoflav.html). These U.S. Department of Agriculture (USDA) databases were realized from the scientific literature containing data on the flavonoid or isoflavonoid content of foods and the analysis of about 60 fresh fruits, nuts, vegetables, and soy-containing foods at the Food Composition Laboratory (FCL) and Nutrient Data Laboratory (NDL). The methodology for evaluating each set of analytic data used five criteria established by these laboratories:

1. Analytical method
2. Analytical quality control
3. Number of samples
4. Sample handling
5. Sampling plan

Most of the compounds in food are present in glycosylated form, except for the flavan-3-ols (catechins and theaflavins), which are present either in free forms or as gallic acid esters. Most analytical procedures converted the glycosides into aglycones, and results were reported as aglycones and expressed as mg per 100 g of fresh weight of edible portion of food. Values for tea were given as mg per 100 ml (100 g weight) of tea infusions (as consumed).

For the flavonoid database, a total of 19 compounds in the five subclasses of dietary flavonoids were evaluated using new procedures developed at the NDL.

### TABLE 13.2
**Estimated Flavonoid Consumption in Several Countries**

<table>
<thead>
<tr>
<th>Country</th>
<th>Flavonoids Evaluated (Intake in mg/day)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denmark</td>
<td>Flavanones (7 to 14); flavone (1 to 2); flavonols (15 to 30); total (23 to 46)</td>
<td>Dragsted et al., 1997</td>
</tr>
<tr>
<td>Finland</td>
<td>Flavanones; flavones; flavonols; total (24)</td>
<td>Knekt et al., 2002</td>
</tr>
<tr>
<td>France</td>
<td>Flavonols; flavones; total (14)</td>
<td>Commenges et al., 2000</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>Flavanones (23); flavones; flavan-3-ols (50); isoflavones (&lt;1); total (73)</td>
<td>Hertog et al., 1993</td>
</tr>
<tr>
<td>Japan</td>
<td>Flavone (&lt;1); flavonols (16); isoflavones (47); total (63)</td>
<td>Arai et al., 2000</td>
</tr>
<tr>
<td>Spain</td>
<td>Flavonols; flavones; total (9)</td>
<td>Garcia-Closas et al., 1999</td>
</tr>
<tr>
<td>United States</td>
<td>Flavonols; flavones; flavanones; total (100)</td>
<td>Kühnau, 1976</td>
</tr>
<tr>
<td></td>
<td>Flavones (&lt;1); flavonols (20 to 22); Isoflavones (&lt;1); Isoflavones (12); total (20 to 34)</td>
<td>DeKleijn et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sampson et al., 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wu et al., 2002</td>
</tr>
</tbody>
</table>

For flavonoids and isoflavonoids, contents in selected foods have been assembled in databases that are available on the Web (Flavonoids: USDA database for the flavonoids content of selected foods: http://www.nal.usda.gov/fnic/foodcomp; Isoflavonoids: USDA–Iowa State University Database on the Isoflavone Content of Foods — 1999: http://www.nal.usda.gov/fnic/foodcomp/data/isoflav/isoflav.html). These U.S. Department of Agriculture (USDA) databases were realized from the scientific literature containing data on the flavonoid or isoflavonoid content of foods and the analysis of about 60 fresh fruits, nuts, vegetables, and soy-containing foods at the Food Composition Laboratory (FCL) and Nutrient Data Laboratory (NDL). The methodology for evaluating each set of analytic data used five criteria established by these laboratories:
These procedures were based on criteria described earlier by Holden et al. (1987) and Mangels et al. (1993).

The USDA–Iowa State University database on the isoflavones was obtained from 38 references. Only the data for the most prominent isoflavones (daidzein, genistein, glycitein, and their glycosides) were evaluated using the expert system described by Mangels et al. (1993) and Chug-Ahuja et al. (1993) for five general categories. The analytical method described by Murphy et al. (1997) was used as the reference method for evaluating analytical methodologies in the published articles, but criteria for inclusion in this database were relaxed so as to include as many foods as possible.

Proanthocyanidins are not included in the flavonoid database because a separate database for these compounds is being developed.

Diets rich in fruits and vegetables and low in fat have been recommended for preventing cancer. Nevertheless, epidemiological evidence supporting the biological effects of the main flavonoids and their potential cancer-protective properties is still limited and conflicting. Recent epidemiological studies have mainly concentrated on whole foods (fruits and vegetables) and not specifically on food rich in flavonoids. Several studies have attempted to assess the relation between consumption of food rich in flavonoids and prevention of cancer: the Zutphen Elderly study found no association between flavonol and flavone intake and total cancer mortality; specific forms of cancer, such as lung cancer, were also not associated with flavonol and flavone intake (Hertog et al., 1994); the Netherlands cohort study found no association between flavonol and flavone intake and stomach cancer, colon cancer, or lung cancer during 4.3 years of follow-up (Goldbohm et al., 1995); a prospective study in Finland showed a reduction in lung cancer risk of about 50% in the quartile of highest flavonol intake and no reduction in risk of cancer at other sites after 24 years of follow-up (Knekt et al., 1996). For whole foods, some protective associations have emerged from meta-analytic approaches (Gandini et al., 2000; Steinmetz and Potter, 1996; Riboli and Norat, 2003), such as a possible inverse association between soy product intake and premenopausal breast cancer (Lee et al., 1991; Hirose et al., 1995), green tea consumption and stomach cancer (Yu et al., 1995), and both fruit and vegetable intakes and lung, esophageal, and colorectal cancer (Dorant et al., 1994; Steinmetz et al., 1993).

Nevertheless, a great wealth of experimental results concerning the action of flavonoids on multiple cancer-related biological pathways such as cell signaling, cell cycle regulation, and angiogenesis shows that flavonoid compounds are probably not solely responsible for these anticarcinogenic effects. Numerous other substances present in fruits and vegetables are also known for their anticarcinogenic properties, e.g., dithiolthiones and isocyanates, folic acid, saponins, and monoterpenes (Kris-Etherton et al., 2002).

Studies on the association of fruit and vegetable consumption with reduced cancer risk provide weaker evidence in cohort studies than in case-control studies. There are several possible explanations for these discrepant findings: recall bias, selection bias, and dietary changes that result from cancer (Temple and Gladwin, 2003).
13.4 SELECTED BIOLOGICAL ACTIVITIES

13.4.1 Anti- and Prooxidant Activities

Humans possess antioxidant systems to protect against free reactive oxygen species (ROS). These systems include enzymatic defenses, such as superoxide dismutase, catalase, and glutathione peroxidase, and nonenzymatic defenses, such as glutathione (GSH), albumin, and uric acid. In cases of ROS overproduction or endogenous defense impairment, supplementation with exogenous antioxidants is needed to diminish the cumulative effects of oxidative damage.

Antioxidants may act by several mechanisms:

1. Suppressing ROS formation either by inhibition of enzymatic systems responsible for free radical generation (such as cyclooxygenase, lipoxygenase, or xanthine oxidase) or by chelating metal ions, which can be initiators of hydroxyl radical production by Fenton reaction
2. Scavenging ROS (Cotelle, 2001)
3. Inducing antioxidant enzymes (e.g., SOD), Phase II metabolizing enzymes (e.g., glutathione transferase) (Breinhold et al., 1999) that enhance the excretion of oxidizing species, and metallothionein, a metal-binding protein with antioxidant properties (Kuo and Leavitt, 1999)

Protection against oxidative damage is one property attributed to flavonoids. The antioxidant efficacy of flavonoids has been studied most extensively in cell-free systems in which ROS are produced either enzymatically, e.g., by the xanthine/xanthine oxidase system, or chemically by a transition metal alone or in combination with a reducing agent such as ascorbate, or by radiolysis. The elimination of ROS by flavonoids is monitored directly or by measuring lipid peroxidation levels. Most controversies encountered in describing structure/activity relationships (SARs) for flavonoid antioxidant activity result from the diversities in radical generation in the antioxidant assays. The medium (aqueous or lipophilic) and the free radical–scavenging SARs are often mixed with SAR for chelation or SAR for the inhibition of enzymes involved in the initiation of reaction. Nevertheless, SAR research has established several consistent structural features required for chelation and radical scavenging in vitro.

The proposed binding sites for trace metals to flavonoids are hydroxyl groups at C3′ and C4′ or hydroxyl groups at C3 or C5 along with o xo substitution at C4. Chelating complexes with divalent cations may be formed between the C5-OH and C4-oxo group or between the C3-OH and C4-oxo (Cheng and Breen, 2000). However, the most important structural requirement for metal chelation is the catechol structure of the B-ring. For radical scavenging, the proposed major structural features are the presence of a catechol group at the B-ring, which has the better electron donating properties, and a 2,3-double bond conjugated with the C4-oxo group, which is responsible for electron delocalization. Moreover, the presence of a free C3-hydroxyl group has been suggested to strongly increase the scavenging properties of flavonoids (Figure 13.3). The free C3-OH substituent is thought to increase the
stability of the flavonoid radical because the planarity induced by the C3-OH permits conjugation, electron dislocation, and a corresponding increase in flavonoid phenoxyl radical stability (flavanols and flavonols with a 3-OH are planar). The torsion angle of the B-ring with respect to the rest of the molecule strongly influences free radical scavenging ability (Van Acker et al., 1996).

Glycosylation has been suggested to decrease the activity (Burda and Oleszek, 2001; Williamson et al., 1999), and methoxyl groups introduce unfavorable steric effects that perturb planarity and increase lipophilicity and membrane partitioning (Ollila et al., 2002). However, O-methylation enhances antioxidant activity in some microsomal systems because microsomal peroxidation assays are a complex model that permits multiple metabolic mechanisms.

Inhibition of reactive nitrogen species (RNS) generation by flavonoids involves the same basic SARs as the suppression of ROS: 3',4'-catechol, 3-OH, polyhydroxyl groups on the B-ring, and O-methylation and/or glycosylation (Haenen et al., 1997; Lai and Yen, 2002).

It is generally accepted that the main structural requirement for the antioxidant activity of flavonoids is the presence of multiple hydroxyl groups. The same structural features were required for prooxidant activity (Cao et al., 1997), and this activity is thought to be linked to a catechol or pyrogallol pattern (Galey, 1997; Hodnick et al., 1986). Thus, at higher doses or under certain conditions, flavonoids can generate free radicals such as superoxide anion and the hydroxyl radical or induce oxidative DNA cleavage either alone or in the presence of transition metal ions, such as iron or copper (Rahaman et al., 1989); the rate of DNA cleavage may depend on the number of hydroxyl groups in the flavonoid molecule. (–)-Epigallocatechin-3-gallate (EGCG) has been shown to be a more efficient producer of hydroxyl radicals (·OH) in the presence of Cu(II), leading to a greater rate of DNA cleavage, than (–)-epicatechin (EC) is. Copper is an essential constituent of chromatin, and it is well known that levels of copper ion levels are elevated in many malignancies (Azam et al., 2004).

Galati et al. (2002) observed that phenol rings were metabolized by peroxidase/H$_2$O$_2$, to form prooxidant phenoxy radical that, in some cases, were sufficiently

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**FIGURE 13.3** Sites of interaction for possible metal chelation and radical scavenging.

Catechol moiety and bonds in **bold** represent the proposed major structural features for radical scavenging

вяз

$=$ Site of metal chelation

---
reactive to cooxidize GSH or NADH, accompanied by extensive oxygen uptake and ROS formation. The order of catalytic effectiveness found for oxygen activation when polyphenols were metabolized by peroxidase in presence of GSH was: phloretin > phloridzin > 4,2'-dihydroxy chalcone > naringenin > apigenin > isoliquiritigenin > kaempferol. Polyphenolics with catechol rings also cooxidized ascorbate, likely mediated by semiquinone radicals. The order of catalytic effectiveness found for ascorbate cooxidation was fisetin, luteolin, quercetin > eriodictyol > catechin > taxifolin. GSH was not cooxidized, but GSH conjugates were formed, likely mediated by the o-quinone metabolites. After experiments with hepatocytes, the same authors concluded that polyphenols containing a phenol ring are generally more prooxidant than polyphenols with a catechol ring.

Oxidation of catechols to quinones and their isomeric quinone methides generates potent electrophiles that could alkylate DNA. Therefore, these effects might explain the mutagenic and cocarcinogenic effects observed for quercetin (Brown, 1980; MacGregor and Jurd, 1978; Silva et al., 2000). In order to prevent these problems, we have synthesized a series of flavonoids where a di-tert-butylhydroxyphenyl group replaced the catechol or pyrogallol moiety at C2 of the flavonoid core molecule. The theoretical design of these new antioxidants was aimed at achieving a combination structure that might overcome the drawbacks of both butyl-hydroxyanisole (BHT) and quercetin with the idea that the formation of such molecules are synergetically more active. By measuring their inhibition of low-density lipoprotein (LDL) oxidation, free radical scavenging capacity and copper-chelating and reducing ability, we have shown that some of them were potent antioxidant compounds without prooxidant effects (Lebeau et al., 2000).

Before considering the flavonoids as therapeutic agents, it is important to take into account their potential toxicity. This is particularly important in the case of polyhydroxylated flavonoids since they have been shown anti or prooxidant, under certain conditions, such as pH, concentration (flavonoids at low and high doses may act as antioxidant and a prooxidant, respectively) (Laughton et al., 1989), and thus can act as antimutagens or mutagens. Caution should be taken in ingesting flavonoids at levels above that which would be obtained for typical vegetarian diet, because higher flavonoids levels obtained by supplementation may lead to the formation of ROS and ultimately DNA damage (Skibola and Smith, 2000).

### 13.4.2 Cell Cycle Arrest

Cell cycle progression is orchestrated by cyclin-dependent kinases (CDKs), whose activity is dependent on association with cyclin subunits (Figure 13.4). According to the literature, flavonoids are able to modulate CDK activity using several strategies, based either on direct effects on the catalytic CDK subunit, on specific interactions with the ATP-binding site of CDKs or indirect effects by targeting the regulatory upstream pathways that modulate the CDK activity. Four main mechanisms have been to date suggested for these indirect modulation routes:

1. Alteration of the expression and synthesis of CDK/cyclin subunits or CKIs (Casagrande and Darbon, 2001), e.g., baicalein inhibits the proliferation
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of rat heart endothelial cells via G₁ and G₂ arrest in association with the
down-regulation of the expression and function of CDK1, CDK2, cyclin
D₂, and cyclin A proteins, and up-regulation of cyclin E, p15 INK4b, p53,
and p21CIP1/WAF1 (Hsu et al., 2004).

3. Targeting CAK, cdc25 (Aligiannis et al., 2001), and wee1/myt1.
4. Manipulating the proteolytic machinery that regulates the catabolism
of CDK/cyclin complexes or their regulators (Senderowicz, 2003).

Several flavonoids have proven to be effective inhibitors of CDKs, e.g., quercetin,
flavopiridol, and 2-thioflavopiridol. Over a range of concentrations from 10 nM to
10 μM, quercetin has shown a dose-dependent and reversible inhibition of cancer
cell proliferation. Cell cycle analysis has shown that cells are blocked at G₁/G₂
interphase, a result consistent with CDK inhibitors. Quercetin is a relatively weak
CDK inhibitor that does, however, present the flavone core as a viable
structure template. Flavopiridol (NSC-649890, L86-8275) (Figure 13.5) utilizes the
flavone core while introducing additional interaction sites through the incorporation
of piperidine at the 8-position and by modification of the 2-aryl substitution pattern;
this flavone is the most advanced CDK inhibitor, currently in Phase II clinical trials
for refractory myeloma, advanced gastric carcinoma, and high-grade non-Hodgkin’s
and mantle cell lymphoma. This compound is a nonselective kinase inhibitor show-

FIGURE 13.4 Targets of some flavonoids towards cell cycle phases.
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ing in vitro activity against CDK4, CDK2, CAK, and CDK1 (Sielecki et al., 2000) and CDK9 (De Azevedo et al., 2002).

Generally, the flavonoid activity is related to a multifaceted attack on multiple target molecules that are critically involved in growth inhibition.

Casagrande and Darbon (2001) tried to establish the possible relationships between the structure of flavonoids and their effect on cell cycle progression in human melanoma cells OCM-1. They have demonstrated that a ring C with oxo function at position 4 and a C2-C3 double bond was required for maximal antiproliferative activity. The authors established that flavonoids that arrest cells in G1 inhibited CDK2 (quercetin, luteolin, and daidzein), while those that blocked cells in G2 inhibited CDK1 (kaempferol, apigenin, and genistein) (Table 13.3). Flavonoids are able to interrupt cell cycle progression at either G1 and/or G2/M checkpoints depending on the specificity of cell type or their concentrations (Lepley and Pelling, 1997; Gupta et al., 2001), e.g., apigenin induced a G2 arrest in mouse keratinocyte cell lines (Lepley et al., 1996), and in human carcinoma cell lines (Wang et al., 2000), it induced a G1 arrest in diploid fibroblasts (Lepley and Pelling, 1997) (Table 13.3). The reasons for these differences are presently unknown but may reflect the relative importance of different signaling pathways in different cell types. Moreover, flavonoids may suppress tumor cell growth, not only in tumor cells that produce a wild-type p53 but also in tumor cells that produce a mutated p53. Even if the activity of p53 is lost in cancer cells, flavonoids are suggested to be able to restore a part of the function of p53 to arrest the cell cycle. Therefore, supplementation of the existing cell-cycle machinery with extrinsic reversible regulators such as flavonoids may possibly block initiation or progression of cancer.

13.4.3 APOPTOSIS

Apoptosis is a form of physiological cell death characterized by cell shrinkage, blebbing of the plasma membrane, and chromatin condensation associated with DNA cleavage into approximately 200–base pair fragments. Many compounds obtained from natural resources, including flavonoids, have been reported to exert anticancer effects that are mediated by apoptotic cell death.

Numerous dietary flavonoids have been able to induce apoptosis in different cells including human leukemia HL-60 cells (Wang et al., 1999; Lee et al., 2002;
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Chen et al., 2003; Shen et al., 2003), colorectal carcinoma cells (COLO205) (Lee et al., 2002), and hepatocellular carcinoma cells (SK-Hep) (Lee et al., 2002). Their capacity to induce apoptosis is generally linked to the number of hydroxyl groups. Nevertheless, the 3-hydroxyl group may play a negative effect in inducing apoptosis, which can be counteracted by a greater number of hydroxyl groups in the C-ring. The flavones and flavonols apigenin, myricetin, quercetin, kaempferol, wogonin, fisetin, naringenin, and hesperetin induced apoptosis in HL-60 cells whereas the

<table>
<thead>
<tr>
<th>Flavonoid</th>
<th>Human Cancer Cells</th>
<th>Phase Arrest</th>
<th>Molecular Targetsa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genistein</td>
<td>Breast MCF-10Fb</td>
<td>G2/M (reversible)</td>
<td>(+) Cyclin B1, p34cdc2</td>
</tr>
<tr>
<td></td>
<td>Prostate LNCaPc</td>
<td>G2/M</td>
<td>(–) Cyclin B</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(+) p21, p53-independent</td>
</tr>
<tr>
<td>Silibinin</td>
<td>Prostate DU145d</td>
<td>G1</td>
<td>Rb-related protein</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(+) p21CIP1, p27KIP1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(–) CDK4, CDK2</td>
</tr>
<tr>
<td>Tangeretin</td>
<td>Colon COLO205e</td>
<td>G1</td>
<td>(–) CDK4, CDK2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(+) p21, p53, p27</td>
</tr>
<tr>
<td>Morin</td>
<td>Oral (squamous)f</td>
<td>G2/M</td>
<td>(–) GADD45, (–) cdc2</td>
</tr>
<tr>
<td>Acacetin</td>
<td>Liver Hep G2g</td>
<td>G1</td>
<td>(+) p21WAFI</td>
</tr>
<tr>
<td>Isoliquiritigenin</td>
<td>Lung AS49h</td>
<td>G2/M</td>
<td>(+) p21CIP1/WAFI</td>
</tr>
<tr>
<td></td>
<td>Prostate DU 145, LNCaP</td>
<td>S, G2/M</td>
<td>(–) GADD153</td>
</tr>
<tr>
<td>Apigenin</td>
<td>Prostate LNCaPc</td>
<td>G2/M</td>
<td>(+) p21, p53-dependent</td>
</tr>
<tr>
<td></td>
<td>Prostate LNCaPb</td>
<td>G1</td>
<td>(–) Cyclin D1, D2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(+) p21WAFI, p27KIP1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p53-dependent</td>
</tr>
<tr>
<td>Luteolin</td>
<td>Prostate LNCaPb</td>
<td>G2/M</td>
<td>(–) cdk2, (+)p21WAFI</td>
</tr>
<tr>
<td>Quercetin</td>
<td>Breast MCF7l</td>
<td>G2/M</td>
<td>(+) p21, p53-independent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(+) p21CIP1/WAFI</td>
</tr>
</tbody>
</table>

a (+) denotes an activation or an expression, (–) denotes an inactivation or a repression.
f Brown, J., O’Prey, J., and Harrison, P.R., Carcinogenesis, 21, 137, 2002.

Chen et al., 2003; Shen et al., 2003), colorectal carcinoma cells (COLO205) (Lee et al., 2002), and hepatocellular carcinoma cells (SK-Hep) (Lee et al., 2002). Their capacity to induce apoptosis is generally linked to the number of hydroxyl groups. Nevertheless, the 3-hydroxyl group may play a negative effect in inducing apoptosis, which can be counteracted by a greater number of hydroxyl groups in the C-ring. The flavones and flavonols apigenin, myricetin, quercetin, kaempferol, wogonin, fisetin, naringenin, and hesperetin induced apoptosis in HL-60 cells whereas the
glycosides naringin and hesperidin (7-rutinose derivatives) did not affect the viability of cells, suggesting that rutinoside at C7 counteracts the induction of apoptosis by flavonoids (Chen et al., 2003). Similar observations were made with rutin and quercitrin in HL-60 cells, in that addition of a sugar at C3 attenuated the apoptosis-inducing activity of quercetin (Shen et al., 2003).

Flavonoids have also proven to induce apoptosis in numerous cancer cell lines (prostate, pancreas, liver, lung, colon, bladder) by various mechanisms (Tyagi et al., 2003; Chan et al., 2000; Kumi-Diaka et al., 2000; Gupta et al., 2001; Hastak et al., 2003; Chung et al., 2001; Buchler et al., 2003; Hsu et al., 2004; Kuo and Lin, 2003; Nguyen et al., 2003b; 2003c; Tyagi et al., 2003; Iwashita et al., 2000). Subclasses of flavonoids found in the above-mentioned studies are flavones and flavonols bearing numerous free hydroxyl groups on A- and B-rings. Nevertheless, other studies have reported antiapoptotic activities of catechins (EGCG, EGC, and ECG) on prostate and liver cancer cells (Chung et al., 2001; Kuo and Lin, 2003) and of tri- and tetrahydroxylated chalcones on mouse melanoma cells (Iwashita et al., 2000).

13.4.4 Inhibition of Angiogenesis

More than 30 years ago, the critical role of tumor angiogenesis in cancer progression was hypothesized by Folkman et al. (1971). It was only after the discovery of the first compounds with specific angiostatic effects in the early 1990s (Ingber et al., 1990; O’Reilly et al., 1994) that the research field of angiogenesis rapidly expanded and provided an increasing body of evidence that inhibition of angiogenesis could attenuate tumor growth.

Preclinical studies show that most of the inhibitors of angiogenesis block tumor growth without causing toxicity. Angiogenesis processes seem to be a promising target for the control of tumor growth progression, invasion, and metastasis.

Silymarin (SM), the active extract of milk thistle, has been used as a liver detoxicant for many years. The inhibitory effects of silymarin concern several angiogenic responses, including matrix metalloproteinase-2 (MMP-2) expression and vascular endothelium growth factor (VEGF) secretion by prostate and breast cancer cell lines (Jiang et al., 2000), and a colon cell line (Yang et al., 2003).

Delphinidin, a polyphenol belonging to the anthocyanins, overcomes in vitro and in vivo angiogenesis (Favot et al., 2003), apparently via endothelial cell migration and proliferation.

Quercetin was found to inhibit several important steps of angiogenesis including proliferation, migration, and tube formation of human microvascular dermal endothelial cells in a dose-dependent manner and displayed an antiangiogenic effect in vivo in a model of the chicken chorioallantoic membrane (Tan et al., 2003).

Genistein was shown to inhibit invasion in vitro of MCF-7 and MDA-MB-231 breast carcinoma cells by down-regulating MMP-9 and up-regulating the tissue inhibitor of matrix metalloproteinase-1 (TIMP-1), and to decrease vessel density and levels of VEGF (Tosetti et al., 2002).

Luteolin, apigenin, and fisetin have been shown to inhibit endothelial cell proliferation and migration, and luteolin also inhibited the secretion of tumor cell MMP-2 and MMP-9 (Cao et al., 2002).
Sartor et al. (2002) have evaluated the potential of 27 flavonoids in blocking gelatinase (MMP) activity. The structural features characterizing the most effective molecules against MMP-2 and MMP-9 found by the authors were:

1. The presence of at least three hydroxyl groups at the A or B ring.
2. The “planarity” of the molecule, thus while three hydroxyl groups present at the A or B ring of planar compounds (such as baicalein) confer a low IC$_{50}$ for gelatinases, they are not sufficient for nonplanar compounds (EGC), which also need a galloyl moiety in position C3 to be effective (EGCG).
3. That glycosidic compounds were ineffective against gelatinase (rutin, gossypin); this inhibition does not seem to be linked to antioxidant properties of these compounds.

Fotsis et al. (1997) studied 14 flavonoids for inhibition of angiogenesis in vitro. They assessed the ability of these compounds to inhibit b-FGF–induced proliferation of endothelial cells in vitro (Fotsis et al., 1997). Three flavonoids — 3’,4’-dihydroxyflavone, luteolin, and 3-hydroxyflavone — were more potent inhibitors than was the isoflavonoid genistein. Endothelial cells from aorta, brain, adrenal cortex, and umbilical vein were all inhibited by these flavonoids in a similar fashion. SARs showed that a nonhydroxylated ring C with an oxo function at position 4 and a C2-C3 double bond are required for maximal biological activity. Catechin, which lacks the oxo function at position 4 and the C2-C3 double bond, is completely devoid of antiproliferative activity.

### 13.5 HUMAN CLINICAL TRIALS

Results of numerous epidemiological studies suggest protection by nonnutritive dietary compounds such as natural flavonoids against some forms of cancer. Likewise, a large number of in vitro and in vivo animal studies have shown that flavonoids are involved in regulation of cell differentiation, proliferation, and death, i.e., the cellular processes relevant to cancer. Certain natural and synthetic flavonoids have emerged as anticancer drug candidates, e.g., flavopiridol [5,7-dihydroxy-8-(4-N-methyl-2-hydroxy pyridyl)-6’-chloroflavone (Figure 13.5), which is a semisynthetic flavone, an analogue of the alkaloid rohitukine (Harmon et al., 1979), extracted from an Indian tree. Flavopiridol is a selective inhibitor of cyclin-dependent kinases (CDKs). This compound also decreases expression of proteins that regulate the cell cycle, such as cyclin D1 (Carlson et al., 1999), cyclin D3, the antiapoptotic protein Mcl-1 (Ma et al., 2003), and the angiogenic VEGF (Melillo et al., 1999). This compound can also induce cell-cycle arrest by preventing the phosphorylation of CDKs at Thr 160/161 by inhibition of CDK7/cyclin H (Carlson et al., 1999; Worland et al., 1993).

The efficacy of flavopiridol administered with standard chemotherapeutic agents such as paclitaxel, cytarabine, topotecan, doxorubicin, mitomycin-C, etoposide, 5-fluorouracil (Bible and Kaufmann, 1997; Schwartz et al., 1997) was evaluated in in vitro models, and synergistic effects in enhancing apoptosis were reported (Mot-
wani et al., 1999). Phase III trials for flavopiridol in combination with chemotherapy agents have been initiated targeting lung cancer and colon cancer.

A few other flavonoids have reached Phase I trials, such as:

2. Genistein, which is a protein tyrosine kinase inhibitor, has been recently attached to two antibodies leading to two antibody-genistein conjugates, B43(anti-CD19)-genistein (Ek et al., 1998; Chen et al., 1999; Uckun et al., 1999) and EGF-genistein (Myers et al., 1998), for treatment of B-lineage leukemia and lymphoma and breast cancer, respectively.
3. Quercetin and more precisely QC12. Quercetin is a naturally occurring flavonoid with many biological activities including inhibition of a number of tyrosine kinases. A phase I, dose escalation trial of quercetin defined the maximum tolerated dose (MTD) as 1700 mg/m² three times per week, but the vehicle, dimethyl sulfoxide (DMSO) was unsuitable for further clinical assessment of quercetin. A water-soluble, prodrug of quercetin, 3’(N-carboxymethyl)carbomyl-3,4’,5,7-tetrahydroxyflavone, named QC12, has been synthesised. Quercetin was found in all patients following i.v. infusion of QC12 with peak levels of quercetin 19.9 +/-11.8 μM. The relative bioavailability of quercetin was estimated to be 20 to 25% quercetin released from QC12, but this compound is not orally bioavailable (Mulholland et al., 2001).

13.6 MULTIDRUG RESISTANCE

A major problem in the treatment of cancer patients with chemotherapeutics is the occurrence of drug resistance. At least two proteins are well known for causing multidrug resistance (MDR): Pgp (P-glycoprotein) and MRP-1 (multidrug resistance related-protein 1).

Naturally occurring flavonoids were evaluated in vitro by measuring their binding affinity toward the C-terminal nucleotide-binding domain of Pgp (Boumendjel et al., 2002). The results showed that flavonols, chalcones, and flavones were the most active compounds. These authors concluded that higher affinity was obtained with molecules containing an A/C ring hydroxylated on the 3 and 5 positions, possessing a 4-carbonyl group and a C2-C3 double bond and finally a hydrophobic motif on either the A- or B-ring, and that flavonoids can act bifunctionally by partly overlapping the ATP-binding site and a vicinal hydrophobic region interacting with steroids, within a cytosolic domain of Pgp. Others studies, using quercetin, morin, and their pentaetherified derivatives on Pgp function of human myelogenous leukemia (K562) cells and K562/ADM cells, indicated that the size of the alkyloxy (or alkenyloxy) moiety may play a critical role in the inhibition of Pgp (Ikegawa et al., 2002). The chemosensitizing effect of sinensetin (5,6,7,3’,4’-pentamethoxyflavone) was studied and found equipotent to verapamil and cyclosporin-A and at least 10-
to 100-fold superior to those of other flavonoids such as hesperidine, flavone, and quercetin (Choi et al., 2002). These results suggest that the ability of sinensetin to reverse Pgp-mediated MDR is due to its inhibition of Pgp, thus resulting in an increase in the intracellular accumulation of anticancer drugs.

Inhibition of MRP-mediated transport of anticancer drugs by genistein, kaempferol, and flavopiridol seems to be due to a direct interaction with the substrate-binding site of MRP (Hooijberg et al., 1997). Not only the inhibition of the ATPase activity but also the competitive inhibition by genistein of daunorubicin efflux supports the idea of MRP-mediated transport of certain (iso)flavonoids. Interestingly, the glucose moiety may play a role because genistin has no influence on the ATPase activity.

Effects of dietary flavonoids on the transport of daunomycin and vinblastine were investigated in Panc-1 cells, a human pancreatic adenocarcinoma cell line that expresses MRP-1 (Nguyen et al., 2003a). Morin, chalcone (diphenylpropenone), silymarin, phloretin, genistein, quercetin, biochanin A, and kaempferol all significantly increased the accumulation of both these anticancer drugs in Panc-1 cells suggesting that they can inhibit MRP-1–mediated drug transport. Similar studies were realized to inhibit the efflux of 2’,7’-bis-(carboxypropyl)-5(6)-carboxyfluorescein from human erythrocytes, and the strongest inhibitors were found among flavanones bearing a hydrophobic prenyl, geranyl, or lavandulyl group at position 8 (and hydroxyl groups at 5 and 7) in ring A (Bobrowska-Hagerstrand et al., 2003).

Flavonoids were found to reverse multidrug resistance by binding to the ATP-binding site of Pgp and/or MRP-1 because of their structural similarity to the adenine moiety of ATP. In addition, some of these compounds may display bifunctional interactions at the ATP-binding site and a vicinal steroid-interacting hydrophobic sequence of the latter proteins. Other authors (Walle and Walle, 2003; Vaidyanathan and Walle, 2003) have demonstrated that various flavonoids are efficiently effluxed by MRP-1 or Pgp, thus furnishing another way, though transient, of increasing drug concentration in tumor cells.

13.7 CONCLUSIONS

The results of epidemiological studies (both cohort and case-control) attempting to correlate the consumption of food flavonoids and incidence of cancer are inconsistent and have had conflicting results. Nevertheless, flavonoids have been reported to inhibit various events related to the cancer process including cellular oxidant stress, the cell cycle, angiogenesis, reversal of multidrug resistance, and apoptosis.

The structural heterogeneity of (natural, synthetic, or metabolized) flavonoids and the diverse experimental methods (analytical techniques or cell lines used) pose challenges in assembling a collective hierarchy of SAR for each action field.

In general, it was found that the effectiveness of flavonoids for inhibiting the cancer process was linked to certain structural features, such as the presence of hydroxyl group(s) on the B-ring, planarity of the molecule, and conjugation between the B-ring and the C-ring. These requirements are generally linked to the redox potentials of compounds that interfered with the redox status of tumor cells (many molecular mechanisms of cells are governed by redox status).
Most studies reported in this article have been realized in vitro or in vivo at pharmacological doses (i.e., the doses tested are significantly higher than those that would exist in human plasma after consumption of whole foods). Moreover, little is known about flavonoid dietary intakes, absorption, metabolism, or interaction with other nutrients at the usual levels of dietary intake. The available knowledge is confined to several selected dietary flavonoids (e.g., quercetin) or synthetic flavonoids (e.g., flavopiridol) studied in the clinical phase.

At present, it appears difficult to evaluate the benefits of flavonoid derivatives found in food and beverages in the prevention of cancer due to a lack of precise content of flavonoids in food and an insufficient number of cohort studies and in vivo studies. Nevertheless, if flavonoids would be used as therapeutic agents, combinations of various flavonoids or flavonoids with known anticancer drugs should be used for treatment of human cancers without excluding combined therapy with immune therapy or radiotherapy.

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14 Carotenoids in Cancer Prevention

Cristina Fortes

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14.1 INTRODUCTION

Carotenoids are ubiquitous components of edible fruits and vegetables and represent an important group of potential chemopreventive agents. In this regard, the most extensively studied carotenoid has been β-carotene, which has provitamin A activity. However, other carotenoids, such as lycopene, do not have provitamin A activity but show a higher potential to suppress experimental carcinogenesis. Carotenoids are thought to reduce the damage caused by free radicals to cell membranes and associated receptors, modulate cell immune responses, and inhibit initiated tumor cells. This chapter gives an account of the major natural carotenoids and reviews studies on their cancer-preventive activities.
14.2 DIETARY SOURCES OF CAROTENOIDS

Carotenoids are the most abundant class of pigments in nature. They are yellow, orange, and red pigments present in many commonly eaten fruits and vegetables, though a particular fruit or vegetable may contain a dominant quantity of a specific carotenoid. Approximately 600 carotenoids are found in nature; some exist as pure hydrocarbons, i.e., compounds containing only hydrogen and carbon (e.g., β-carotene, α-carotene, lycopene), while others contain oxygenated functional groups (e.g., lutein, β-cryptoxanthin, zeaxanthin) (IARC, 1998).

The carotenoids commonly found in foods are all-trans (E) forms of polytenes formed from eight 5-carbon isoprenoid units; however, a nonnegligible proportion of cis-isomers can be produced during food processing (Borel, 2003).

The biosynthesis of carotenoids in plants is presented in Figure 14.1. Each enzymatic step from phytoene to lycopene adds one double bond to the molecule, resulting in lycopene, which is a symmetrical molecule containing 13 double bonds. The biosynthetic step after lycopene involves enzymatic cyclization of the end groups, which results in γ-carotene (one beta ring) and β-carotene (two beta rings). The concentration of each carotenoid in a fruit or vegetable suggests which enzyme or enzymes may be rate limiting in the biosynthetic cascade. For example, the very high concentration of lycopene in red tomatoes suggests a lack of sufficient enzyme activity such as cyclase activity to convert lycopene to γ-carotene (Beecher, 1998). The natural functions and biological actions of carotenoids are determined by their molecular structures.

The principal provitamin A carotenoids include mainly β-carotene, γ-carotene, and β-cryptoxanthin, while the main nonprovitamin A carotenoids are lycopene and the xanthophylls lutein and zeaxanthin (Borel, 2003). Six carotenoids are present in significant amounts in the human diet and tissues: β-carotene, lycopene, lutein, β-cryptoxanthin, γ-carotene, and zeaxanthin (El-Soehmy et al., 2002; Borel, 2003). Plasma levels of carotenoids reflect the intake of vegetables and fruits. The concentration of cryptoxanthin in plasma is usually lower than that of β-carotene, lycopene, and lutein (Gregory et al., 1990; Irwig et al., 2002; Palli et al., 2003; Briefel et al., 1996). Zeaxanthin is a relatively minor component of plasma carotenoids, except in people for whom yellow maize is a staple food (Irwig et al., 2002).

Dietary preferences, cooking methods, and many other factors have a major influence on the real contribution of specific foods to the carotenoid composition of plasma. Greater concentrations of carotenoids are found in the peel than in the pulp, and concentrations increase considerably during ripening. Temperature and the selection of cultivars influence the concentration of carotenoids in edible plants, with higher concentrations found in foods grown in hotter environments (Rodriguez-Amaya, 1999).

The average concentrations of serum carotenoids in different populations are shown in Table 14.1. Differences in the concentrations of serum carotenoids among
populations reflect variations in the consumption of vegetables and fruits. Not surprisingly, serum concentrations of lycopene and β-carotene are higher in Costa Rica and Italy than in Great Britain or the United States. The main sources of specific carotenoids in food depend on geographic location and/or food availability and seasonality. For example, papaya and watermelon are the main sources of β-cryptoxanthin intake in Latin America (Irwig et al., 2002) but not in Europe or the United States. In Western countries, β-cryptoxanthin comes from oranges, orange juice, and tangerines (Pelz et al., 1998; Manzi et al., 2002; Granado et al., 1996). The use of extra virgin olive oil contributes to the high plasma concentrations of lutein and β-carotene found in Mediterranean populations (Su et al., 2002), while green vegetables and carrots, respectively, account for similar observations in Germany (Pelz et al., 1998). Pumpkin is one of the main sources of β-carotene intake in Australia and Brazil (Manzi et al., 2002; Rodriguez-Amaya, 1999).

Regarding seasonality, two studies illustrate that dietary intake and serum concentration of carotenoids change throughout the year. Generally, greater quantities of carotenoids are consumed in the summer, and this is reflected by their increased concentrations in serum (Rautalahti et al., 1993; Granado et al., 1996). Per capita consumption of total carotenoids seems to vary from 3 to 15 mg/day (Pelz et al., 1998; Granado et al., 1996; Irwig et al., 2002), and no Recommended Dietary Allowance (RDA) for carotenoids has been established.

β-Carotene is the best-known carotenoid; it is found in the majority of orange vegetables and fruits and in dark-green leafy vegetables. α-carotene is generally found in the same sources as β-carotene, where it accounts for around 40% of the

### TABLE 14.1
Average Concentrations of Carotenoids in Various Populations (μmol/L of Serum)

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>Great Britaina</th>
<th>Costa Ricab</th>
<th>United Statesc</th>
<th>Italyd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
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<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.25</td>
<td>0.25</td>
<td>0.61</td>
<td>0.59</td>
</tr>
<tr>
<td>Lutein/Zeaxanthin</td>
<td>0.29</td>
<td>0.29</td>
<td>0.24</td>
<td>0.26</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>0.13</td>
<td>0.16</td>
<td>0.15</td>
<td>0.19</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>0.24</td>
<td>0.32</td>
<td>0.47</td>
<td>0.60</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>0.06</td>
<td>0.07</td>
<td>0.12</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Note: The data from Costa Rica are for adolescents.


Carcinogenic and Anticarcinogenic Food Components

total carotenoid content. Major sources of both carotenoids are carrots, pumpkin, spinach, kale, and fruits such as peaches and apricots. The content of carotenoids varies from 18.2 μg/100 g in carrots to 97 μg/100 g in peaches. In some diets (e.g., in Latin American and African countries), papaya, mangoes, sweet potato, and palm oil are the main food sources of these carotenoids (Rodriguez-Amaya, 1999; Holden et al., 1999). It has been estimated that the mean daily intakes of α-carotene and β-carotene in these countries are 0.40 and 2.15 mg, respectively (Borel, 2003).

Green vegetables have the highest concentration of lutein (L) and zeaxanthin (Z); the ratio of these carotenoids (L/Z) varies from 12 to 63 (Humphries and Khachik, 2003). Lutein and zeaxanthin are most prominent in kale, collards, spinach, broccoli, lettuce, zucchini, and yellow corn (Holden et al., 1999). The content of lutein and zeaxanthin varies from 1.8 mg/100 g in yellow corn to 39.5 mg/100 g in kale. About 2 mg/day of lutein are reported to be consumed in Europe and America (Irwig et al., 2002; Granado et al., 1996; Borel, 2003).

Taraxanthin, violaxanthin, and neoxanthin are epoxy analogs of lutein and zeaxanthin; they occur widely in spinach, broccoli, lettuce, cabbage, green beans, mustard leaves, and green peas and in fruits such as mango and papaya. However, the xanthophyll epoxides seem not to be absorbed by humans and are not detected in human plasma or breast milk (Barua and Olson, 2001). Cryptoxanthins include α-cryptoxanthin, β-cryptoxanthin, and zeinoxanthin. Only β-cryptoxanthin is usually measured in blood plasma. Good alimentary sources of β-cryptoxanthin are Japanese persimmons, papaya, and red peppers. Citrus fruits such as tangerines, oranges, and pummelos are also good sources of β-cryptoxanthin in Western countries (Holden et al., 1999; Irwig et al., 2002). The average concentrations of β-cryptoxanthin are 122 μg/100 g in oranges and 2.2 mg/100 g in red sweet peppers. Mean daily intake of β-cryptoxanthin varies from 0.12 to 0.14 mg (Borel, 2003).

Lycopene is the pigment principally responsible for the characteristic deep-red color of ripe tomato fruits. Tomatoes and related tomato products are the major source of lycopene in the human diet. Lycopene is also found in watermelon, papaya, guava (523 μg/100 g), and pink grapefruit. The concentration of lycopene depends on variety, maturity (lycopene content increases as tomatoes ripen), and the environmental conditions under which the fruit is cultivated (tomato fruits grown in a greenhouse are lower in lycopene). Tomatoes usually contain about 0.3 to 0.5 mg of lycopene per 100 g, but its concentration may range from 0.05 mg/100 g in yellow to 15 mg/100 g in deep-red varieties. Tomato sauce contains around 16 mg/100 g (Holden et al., 1999; Humphries and Khachick, 2003). The mean daily intake of lycopene ranges from 6.6 to 12.7 mg (Borel et al., 2003; Irwig et al., 2002; Manzi et al., 2002).

14.3 EFFECTS OF FOOD PROCESSING ON CAROTENOID CONTENT

Carotenoids are relatively stable within the food matrix; however, because they are highly unsaturated, they are susceptible to isomerization and oxidative degradation. The major cause of carotenoid loss is enzymatic and nonenzymatic oxidation, which depends on the availability of oxygen and the carotenoid chemical structure. Caro-
Carotenoid oxidation is stimulated by light, heat, some metals, enzymes, and peroxides, and it is inhibited by antioxidants. Many factors in food processing affect bioavailability of carotenoids, such as the destruction of the food cellular structure, changes of surface area or porosity, length and severity of the processing conditions, storage time and temperature, transmission of light, and permeability of the packaging to O₂.

Bioavailability of carotenoids increases upon thermal or mechanical processing of food but decreases upon dehydration, freezing, and storage. Both mechanical homogenization and heat treatment enhance the bioavailability of carotenoids from vegetables (up to a sixfold increase) (van Het Hof et al., 2000) by breaking down cell walls. This weakens the bonding forces between carotenoids and the plant tissue matrix, making them more accessible and enhancing cis-isomerization. For example, α-carotene and lutein appear to be more available from juice than from raw or cooked vegetables (McEligot et al., 1999). Daily consumption of cooked carrots and spinach over a 4-week period seems to increase the mean plasma content of β-carotene three times more than the consumption of the same amount of raw vegetables does (Rock et al., 1998). Thermal processing can induce carotenoid trans-to cis-isomerization. Cis-isomers generally have a lesser tendency to crystallize than all-trans-isomers do, so the cis-isomers may be more readily solubilized, absorbed, and transported. Cooking seems to increase the percentage of cis-isomers of lutein and zeaxanthin up to 22 and 17%, respectively (Updike and Schwartz, 2003).

Percentage loss of specific carotenoids varies with storage of food (Lee and Coates, 2002; Fish and Davis, 2003; Negi and Roy, 2001). For example, the loss rate of lycopene in watermelon during freezing varies from 4 to 6% during an initial freeze-thaw cycle to a loss of approximately 30 to 40% over a year’s storage at −20°C and a loss of approximately 5 to 10% over the same period at −80°C. Moreover, lycopene seems to be slightly more stable in pureed compared to diced watermelon tissue at −20°C, but not at −80°C (Fish and Davis, 2003). In red grapefruit juice, the losses of lycopene and β-carotene during freezing (−23°C) for 12 months are about 20 and 7%, respectively (Lee and Coates, 2002). Dehydration, double packaging, and cold storage seem to decrease β-carotene content by about 40% (Negi and Roy, 2001).

### 14.4 METABOLISM AND BIOAVAILABILITY OF CAROTENOIDS

#### 14.4.1 DIGESTION: GASTRIC AND LUMEN EVENTS

Transformation of carotenoids begins in the stomach, where they are partially released from food matrices by the action of pepsin. Gastric lipase may also play a role in carotenoid hydrolysis. Carotenoids are hydrophobic, so they need to be dissolved in lipids in order to be absorbed at the enterocyte brush border. Pancreatic lipase, which hydrolyzes triglycerides, can facilitate the release of some carotenoids from lipid droplets. In the duodenum, digestive enzymes such as proteases, amylases, and lipases continue to release carotenoids from the food matrix (Tys-sandier et al., 2001; Borel, 2003).
14.4.2 Absorption

It is assumed that absorption of carotenoids is mediated by the same intraluminal processes as those described for major lipids (triacylglycerols, cholesterol, and phospholipids). These include emulsification, solubilization in mixed micelles, diffusion across the unstirred water layer, and permeation through the enterocyte membranes (Borel, 2003). The mass transfer, therefore, involves both the dietary lipid and its hydrolysis products (free fatty acids and mono- and diacyl glycerols), phospholipids, and bile salts, which emulsify the lipid to form micelles. It has been suggested that carotenoids are passively absorbed in the micellar phase with no involvement of special epithelial transporters (IARC, 1998). This mass transfer from the bulk aqueous food to lipid structures is a complex process, which is hindered by the presence of food structure and also depends upon whether the carotenoid is present in chloroplasts as lipoprotein complexes or whether it is crystalline in chromoplasts.

Most of the data on carotenoid absorption from foods and isolates are based on either acute or chronic fecal mass balance methods and show great variability. Carotenoid absorption from foods is low (approximately 3 to 5 mg per meal) and depends on the physicochemical characteristics of the carotenoids ingested and the fat content of the diet (Southon and Faulks, 2001; van Het Hof et al., 2000; Borel, 2003). Free carotenoids given orally as supplements, whether in solution or in suspension in oil, are much better absorbed than those from a food matrix (Faulks et al., 1997). β-carotene absorption from oil solutions, aqueous dispersion, or antioxidant-protected commercial beadlets can be quite high, perhaps up to 50% in comparison to 1 to 2% from raw carrot (Erdman et al., 1993).

Dietary fat stimulates bile flow from the gall bladder, which facilitates the emulsification of fat and fat-soluble vitamins into lipid micelles within the small intestine. Without micelle formation, carotenoids are poorly absorbed. Therefore, the absence of dietary fat or very low levels of fat in the diet might reduce carotenoid absorption. In fact, greater bioavailability is found from heat-treated vegetable foods that have been coprocessed with oils (Southon and Faulks, 2001). Polar carotenoids are absorbed better than nonpolar ones are. Xanthophylls, which are polar carotenoids, distribute preferentially at the droplet surface of dietary lipids, while more apolar carotenoids such as carotenes distribute preferentially in the core (Borel et al., 1996). This has implications for the absolute amount and type of carotenoid that can be carried by emulsified lipid droplets and in the much more highly structured micelles. Cis-isomers of carotenoids seem to be better absorbed than the all-trans form. The short length of the cis-isomers and their low tendency to aggregate allow greater solubility in mixed micelles (Boileau et al., 2002). On the other hand, factors that increase the thickness of the unstirred layer on the surface of the gut, such as dietary fiber, may inhibit the absorption of carotenoids (Southon and Faulks, 2001).

14.4.3 Transport

Absorbed carotenoids, retinyl ester, and small amounts of retinol are transported on chylomicrons from the intestinal mucosa via the lymphatic system into the circulatory system. The chylomicrons are acted upon by endothelial lipoprotein lipase in
the extrahepatic capillary bed. At this point, some carotenoids may be absorbed by adipocytes. The chylomicron remnants, including residual carotenoids, are then cleared from the circulation by passage through the liver. Carotenoids are transported in plasma exclusively by lipoproteins, with the distribution among lipoprotein classes determined in large part by the physical properties of the carotenoid. Carotenoids are carried mainly by very-low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) (Furr and Clark, 1997). A specific cellular carotenoid-binding protein (CCBP) with a high degree of specificity for binding β-carotene, α-carotene, and cryptoxanthin, analogous to the retinoid-binding protein, has been characterized in mammalian liver. Thus, CCBP might also play a role in the storage and transport of these carotenoids (Rao et al., 1997).

14.4.4 Tissue Distribution

Due to their hydrophobic character, carotenoids are associated with lipid portions of human tissues, cells, and membranes. Adipose tissue is the main site of storage for carotenoids in the body and reflects long-term carotenoid status (El-Sohemy et al., 2002). Carotenoids are also present in smaller amounts in liver, muscle, skin, adrenal glands, reproductive organs, mammary gland, and plasma. Less than 10% of total carotenoids in the body are present in plasma, but plasma concentration reflects carotenoid concentrations in other body tissues, although correlations for individual carotenoids vary substantially (IARC, 1998). Human breast milk also reflects dietary intake of carotenoids and is an important dietary source of carotenoids and vitamin A for infants. To date, 35 dietary carotenoids have been identified and characterized in extracts from human serum and milk (Khachik et al., 1997). The major ones found in breast milk are the provitamin A carotenoids, e.g., α-carotene, β-carotene, and β-cryptoxanthin (Canfield et al., 2003).

14.4.5 Bioavailability

The bioavailability of carotenoids is the fraction of the ingested and metabolized dose that reaches the target sites. The bioavailability of carotenoids varies since it is affected by many factors including the fat content ingested with the carotenoids, the amount and types of carotenoids present in the diet, the dietary matrix, the crystalline structure of the carotenoid, and food processing. Host-related factors such as nutritional status, age, and disease states have also been implicated as possible factors interfering with the bioavailability of carotenoids (IARC, 1998; van Het Hof et al., 2000). The type and amount of fat in the diet influence the bioavailability of carotenoids in that β-carotene absorption seems to be enhanced more by long-chain triacylglycerols than by medium-chain triacylglycerols (Borel et al., 1998). For the optimal uptake of β- and α-carotene, 3 g of fat per meal is required, and for lutein as much as 36 g is needed (Roodenburg et al., 2000).

Bioavailability might depend additionally on the interactions among carotenoids. Much of the evidence suggests an interaction between β-carotene and oxycarotenoids such as canthaxanthin and lutein, and between the hydrocarbon carotenoids β-carotene and lycopene. Studies on the antioxidant capacities of carotenoids reveal inter-
actions between specific carotenoids, particularly lutein and lycopene, and suggest that carotenoids do not act individually, but rather cooperatively with each other and with other antioxidants (Lasheras et al., 2002). For example, α-tocopherol, vitamin C, and β-carotene operate synergistically, providing an effective barrier against oxidation (Young and Lowe, 2001). However, the synergistic protection afforded by carotenoids and other antioxidants depends upon a balance among all these components, e.g., an increase in the concentration of one might disturb the balance and reduce the overall antioxidant effectiveness. Supplementation with a single purified carotenoid may antagonize the bioavailability of other carotenoids. One study showed that purified β-carotene and lutein supplements (6 and 9 mg daily, respectively) administered over 4 weeks significantly increased β-carotene and lutein concentrations in plasma but decreased that of lycopene (van Het Hof et al., 1999). The most likely mechanisms of carotenoid interaction appear to be competition for incorporation into micelles, carotenoid exchange between lipoproteins in the postprandial state, and inhibition of provitamin A (β-carotene) cleavage (van den Berg, 1999).

Disease conditions that impair lipid absorption (e.g., cystic fibrosis, malnutrition) may lead to impaired carotenoid absorption, resulting in low bioavailability. Malnourished children have lower mean serum retinol levels than well-nourished children do, even when they consume large quantities of carotenoid-containing foods (Oso et al., 2003). Infection also seems to interfere with bioavailability of carotenoids. Acute response to infection involves changes in hepatic protein synthesis including increased synthesis of C-reactive protein (CRP) and reduced synthesis of retinol binding protein and several other nutrient-transport proteins (Ross, 2000). Elevated CRP concentration has been suggested to decrease concentrations of various plasma carotenoids such as β-carotene, α-carotene, β-cryptoxanthin, lycopene, and lutein (Ford et al., 2002). The bioavailability of lycopene is apparently impaired in elderly people, but there is no major difference in the bioavailability of β-carotene, α-carotene, and probably lutein associated with aging (Cardinault et al., 2003). Alcohol consumption may also affect carotenoid metabolism and bioavailability (Tanumihardjo, 2002).

14.4.6 BIOTRANSFORMATION OF CAROTENOIDS INTO VITAMIN A

Although over 600 carotenoids have been identified, only about 50 are provitamin A compounds. Carotenoids with at least one unsubstituted β-ionone ring, such as α-carotene, β-carotene, and β-cryptoxanthin, are vitamin A precursors (Figure 14.1). In case of regular dietary intake of carotenoids, the intestinal mucosa is the main site of conversion to vitamin A, but other organs, particularly the liver, convert significant amounts when greater-than-normal quantities of carotenoids are ingested. When dietary intake of β-carotene is approximately 2 mg/day, the absorbed β-carotene is mostly converted to retinol (IARC, 1998). In the past, β-carotene was assigned one-sixth of potential vitamin A activity, while other provitamin A carotenoids were assigned one-twelfth. However, the traditional conversion factors of 1 to 6 μg for β-carotene and 1 to 12 μg for other provitamin A carotenoids have recently been revised to 1 to 12 μg and 1 to 24 μg, respectively, by the U.S. National Research Council (Tanumihardjo et al., 2002).
14.5 ANTIOXIDATIVE AND OTHER ACTIVITIES OF CAROTENOIDS IN CANCER PREVENTION

Several mechanisms of action have been proposed to contribute to the cancer-preventive effects of carotenoids including antioxidant properties, effects on cell-to-cell communication, modulation of immune function, cell transformation, and induction of differentiation.

The process by which normal cells become progressively transformed to malignancy is now known to require the sequential acquisition of mutations that arise as a consequence of damage to the genome. This damage can be the result of endogenous processes such as errors in DNA replication, the intrinsic chemical instability of certain DNA bases, or attack by free radicals. Carotenoids prevent cancer probably due to their antioxidative potential, as they break the propagation of free radical chain reactions and reduce the amount of reactive oxygen species (ROS) prone to attack DNA molecules. Carotenoids may also increase the activity of enzymes such as catalase and glutathione peroxidase, which decompose peroxides and intercept or scavenge free radicals. In fact, dietary intervention studies show that carotenoids, in particular lutein and zeaxanthin, increase erythrocyte glutathione reductase activity (Castenmiller et al., 1999).

The ability of dietary carotenoids to act as antioxidants in biological systems seems to be dependent upon a number of factors such as the nature of carotenoid structure and the oxidizing species, as well as interaction with other antioxidants. The properties and therefore functions of a carotenoid molecule are primarily dependent upon its structure and hence its chemistry. In particular, the conjugated C=C double bond system is considered to be the single most important factor in the quenching activity of carotenoids. The ability to quench ROS is directly related to the position of the excited-state energy levels, which is primarily governed by the length of the conjugated C=C chain. Carotenoids with nine or more conjugated double bonds are potent quenchers of singlet oxygen. For example, lycopene (11 conjugated and 2 unconjugated double bonds) is among the most efficient singlet oxygen quenchers among the natural carotenoids (Paiva and Russell, 1999; IARC, 1998).

Differences in the antioxidant behavior of different carotenoids can be attributed to differences in their location and orientation in the lipid bilayer. Carotenoids are incorporated into membranes according to their solubility, which is affected by the presence and the nature of polar substituent groups and cis/trans isomerization. Differences in the structure of two xanthophylls, namely, violaxanthin and zeaxanthin, underlie different interactions with thylakoid membrane lipids, bringing about changes in membrane fluidity and thermostability. For example, the incorporation of zeaxanthin in place of violaxanthin into thylakoid membranes results in a lower susceptibility to lipid peroxidation (Young and Lowe, 2001).

However, the antioxidant activity of carotenoids, in particular β-carotene, can also shift into prooxidant activity at high oxygen pressure and at high concentrations (blocking the absorption of other carotenoids with better antioxidant profile). For example, the relatively high partial pressure in the lung combined with reactive oxygen species from tobacco smoke may be conducive for both β-carotene oxidation
and the formation of oxidative metabolites that can act as propagators of free-radical formation (Palozza et al., 1997).

The antioxidant actions of carotenoids, particularly lycopene, have also been investigated in human intervention studies (Riso et al., 1999; Porrini et al., 2002). A dietary intervention study of carotenoid-rich foods (40 mg lycopene, 22.3 mg β-carotene, 15.7 α-carotene, 11.3 mg lutein) conducted on 23 nonsmoking males showed a decrease of DNA oxidative damage in peripheral blood lymphocytes (Pool-Zobel et al., 1997). Another intervention study conducted on 72 healthy participants showed that consumption of spinach, rich in lutein and zeaxanthin, resulted in greater erythrocyte glutathione reductase activity (Castenmiller et al., 1999).

An intervention trial conducted on 23 healthy men showed that tomato juice consumption for 2 weeks reduced plasma thiobarbituric acid–reactive substances (a marker of lipid peroxidation) and increased the lipoprotein oxidation lag period, while carrot juice and spinach powder had no effect (Bub et al., 2000). Another dietary intervention trial conducted on 60 healthy adults showed that 15 days of tomato product consumption (23 to 35 mg lycopene) increased the lipoprotein oxidation lag period (Hadley et al., 2003). In addition to the above antioxidant effects of carotenoids, their cancer prevention potential includes improvement of immune response and modulation of gene expression related to cell proliferation (Goode-nough and Paul, 2003).

Immune cells rely on cell-mediated communication, particularly via membrane-bound receptors. Lipid peroxidation appears to decrease membrane fluidity, leading to alterations in intracellular signaling and cell function, which then adversely affect immune response. Carotenoids, in particular β-carotene and canthaxanths, seem to increase the production of tumor necrosis factor (TNF), interleukins (e.g., IL-4), cytotoxic T-cells, macrophages, and natural killer cells. The influence of carotenoids on immune response is as yet unclear since some studies showed a positive effect (Fuller et al., 1992; Murata et al., 1994; Watzl et al., 2000), while others did not (Corridan et al., 2001; Gossage et al., 2000).

β-Carotene supplements, 30 mg administered daily for 1 month, seem to protect against photosuppression of immune function (Fuller et al., 1992). A single-blind trial, conducted on 20 participants for 9 months, to test the effects of 60 mg of β-carotene taken daily on lymphocyte subsets, showed an increase in the ratio of CD4 (“helper” T-cells, which stimulate cell-mediated immune responses) to CD8 cells (“suppressor” T-cells, which inhibit responses) (Murata et al., 1994). However, other studies have not confirmed an increase in cell-mediated immunity after carotenoid supplementation (Watzl et al., 2000; Gossage et al., 2000; Corridan et al., 2001). In a 2-month intervention study conducted on 53 healthy, well-nourished elderly persons, daily tomato juice consumption (47.1 mg of lycopene daily for 2 months) did not affect cell-mediated immunity (Watzl et al., 2000). A 12-week double-blind randomized placebo-controlled trial of the effects of daily lycopene (13.3 mg) or β-carotene (8.2 mg) supplements on cell-mediated immunity showed no effect on immune response in 52 participants (Corridan et al., 2001). An intervention study conducted in lactating women showed that β-carotene supplementation (30 mg/day) for 28 days did not affect T-lymphocyte proliferative response to phytohemagglutinin (Gossage et al., 2000). The results of the latest intervention
studies suggested that, in well-nourished, healthy individuals, a moderate level of carotenoid supplementation is neither beneficial nor harmful for immune response. However, supplementation might be recommended for individuals who have low plasma levels of carotenoids or a low immune response.

β-carotene, α-carotene, canthaxanthin, lutein, and lycopene have been also shown to enhance gap-junction intercellular communication and to up-regulate the expression of connexin 43, a gene that encodes a major gap-junction protein (IARC, 1998). Gap junction communication (GJC) relies on the exchange of small molecules through intercellular channels that directly connect the cytoplasm of adjacent cells (Goodenough and Paul, 2003). GJC is impaired in many human tumors, and its restoration or up-regulation is associated with decreased proliferation (Bertram, 1999). Carotenoids such as β-carotene, canthaxanthin, and in particular lycopene have been shown to enhance connexin 43 expression, which allows direct intercellular GJC and inhibits proliferation of various cell lines (Livny et al., 2002; Stahl and Sies, 1998; IARC, 1998; Levy et al., 1995; Paiva and Russell, 1999; Livny et al., 2002).

β-carotene and lycopene have been also shown to induce apoptosis in human cells such as prostate adenocarcinoma cells, human cervical dysplasia-derived cell lines, and melanoma and T-lymphoblast cell lines (Muller et al., 2002). The biological activity of the provitamin A carotenoids in cancer prevention may also be explained by the enhanced expression of the nuclear retinoic acid receptors (RARs) that directly control gene transcription. RARs are key molecules in differentiation and the prevention of epithelial carcinogenesis (Hansen et al., 2000; IARC, 1998).

14.6 EPIDEMIOLOGICAL STUDIES AND SAFETY ISSUES

Epidemiological studies have consistently shown that individuals who consume large amounts of fruits and vegetables have a reduced risk of many cancers, including those of the esophagus, lung, stomach, colorectum, bladder, and breast (World Cancer Research Fund and American Institute for Cancer Research, 1997; Riboli and Norat, 2003). Intake of tomatoes and tomato products has recently been shown to protect against prostate cancer (Giovannucci et al., 2002).

There have not been many studies investigating the role of specific carotenoids in cancer prevention. Two meta-analyses showed that dietary β-carotene intake protects against both breast (relative risk [RR], 0.82; 95% confidence interval [CI], 0.76 to 0.91) and ovarian cancer (RR, 0.84; 95% CI, 0.75 to 0.94) (Gandini et al., 2000; Huncharek and Kupelnick, 2001). Case-control studies support a protective role for lycopene, β-carotene, and β-cryptoxanthin against lung, pharyngeal, esophageal, and oral cancers (Chen et al., 2002; Negri et al., 2000; La Vecchia, 2002; Wright et al., 2003). Lycopene also appears to be associated with a protective effect against gastric cancer (De Stefani et al., 2000).

The results of large prospective studies conducted in the United States and the Netherlands on dietary carotenoids and cancer risk are presented in Table 14.2.
Whereas lycopene showed a protective effect against both lung and prostate cancers, \( \beta \)-cryptoxanthin seems to be associated with a reduction in bladder cancer risk and strongly associated with a reduction in lung cancer risk (Mannisto et al., 2004). However, other prospective studies do not confirm many of these findings (Michaud et al., 2002; Rohan et al., 2002; Vorrips et al., 2000).

Since observational studies suggest that people who consume relatively large amounts of fruits and vegetables containing carotenoids have somewhat lower risk of cancer, large randomized trials of long duration were designed to test this hypothesis directly. Only \( \beta \)-carotene has been tested for cancer-preventive activity in intervention trials to date. The six trials that studied the effect of \( \beta \)-carotene supplementation alone or in association with other agents are (Ommen et al., 1996; The ATBC Cancer Prevention Study Group, 1994; Hennekens et al., 1996; Blot et al., 1993; Greenberg et al., 1996):

1. The Linxian trials (2)
2. The \( \alpha \)-Tocopherol \( \beta \)-Carotene Cancer Prevention Study (ATBC)
3. The \( \beta \)-Carotene and Retinol Efficiency Trial (CARET)
4. The Physicians’ Health Study (PHS)
5. The Skin Cancer Prevention Study (SCPS)

With the exception of the protective effect of β-carotene at 15 mg/day in combination with selenium and α-tocopherol against gastric and lung cancers in the Linxian trial, none of the chemoprevention trials conducted so far have provided evidence for a protective effect of supplementation with β-carotene alone or in combination with α-tocopherol or retinol. The reduction in gastric and lung cancer risk shown in the Linxian trial was not confirmed in other trials. By contrast, a statistically significant increase in lung cancer risk was observed in the ATBC and CARET studies. The CARET study, conducted on 18,000 high-risk smokers in the United States, found that a combination of β-carotene (30 mg) and retinyl palmitate (25,000 IU) led to a 28% increase in the incidence of lung cancer. A similar result was seen in the ATBC trial carried out in Finland among 29,133 smokers, with α-tocopherol (50 mg) and β-carotene (20 mg). In the Linxian study conducted in north-central China, the baseline levels of β-carotene and other antioxidants were much lower than those found in the ATBC, CARET, and PHS studies.

The ATBC and CARET trials have forced a reevaluation of the use of isolated natural compounds or supplements as chemopreventive agents. It was hypothesized that the deleterious effects of β-carotene in smokers observed in both the ATBC and CARET studies were related to the generation of β-carotene radical cations and the presence of low levels of vitamin C found in the serum of heavy smokers. The attack of free radicals from smoke or asbestos on β-carotene results in the formation of high levels of cleavage products with prooxidant activities towards subcellular organelles such as mitochondria. Low levels of vitamin C in plasma, combined with high levels of β-carotene that cause an imbalance in other antioxidant levels, would therefore be unable to quench the relatively long-lived β-carotene radical cation and therefore to preclude the damage caused by this compound (Mortensen et al., 2001).

A case-control study conducted by Nyberg and colleagues on 158 lung cancer cases and 154 controls showed that high and low levels of total carotenoids were associated with higher hypoxanthine-guanine phosphoribosyl transferase (HPRT) mutant frequency (Nyberg et al., 2003).

Since the antioxidant effectiveness of carotenoids is dependent on oxygen pressure (pO₂), and different tissues and organs vary greatly in pO₂, carotenoids may be expected to function differently in distinct parts of the body and may therefore offer less antioxidative protection in the lungs than in other tissues.

Evaluation of toxicity demonstrated that neither β-carotene nor lycopene is genotoxic or teratogenic. No signs of organ toxicity have been found in subacute, subchronic, or chronic oral treatment in experimental animals receiving doses of β-carotene or lycopene up to 1000 mg/day per kg body weight in the diet. It seems that exposure to β-carotene resulting in mean plasma concentrations of no more than 2.2 μM (1.2 μg/ml) is safe for the general population (Woutersen et al., 1999).

In animal studies, a wide safety margin has been established based on repeated dose safety and reproductive/teratology studies in rodents. In humans, there is no indication of any significant adverse effects related to very high exposures to lycopene from dietary sources (McClain and Bausch, 2003).
At present, there are no quantifiable biochemical or physiological markers of carotenoid “status.” Neither carotenoid “deficiency” nor “toxicity” can be defined. However, low plasma carotenoid concentration is used as an indicator of people “at risk” of cancer based on the inverse association between the intake of carotenoid-containing vegetables and fruits, plasma and tissue concentrations of carotenoids, and the development of cancer (Southon and Faulks, 2001).

14.7 CONCLUDING REMARKS

Carotenoids are pigments present in fruits and vegetables and are thought to reduce the damage caused by free radicals to cell membranes and associated receptors, modulate cell immune responses, and prevent the initiated tumor cells’ development. The principal carotenoids present in the human diet and tissues are the provitamin A carotenoids, which include β-carotene, γ-carotene, and β-cryptoxanthin, and the nonprovitamin A carotenoids, such as lycopene and xanthophylls. Many epidemiological studies have shown strong associations between diets rich in carotenoids and a reduced incidence of some cancers. These findings led to the suggestion that the antioxidant properties of those compounds might help to protect against cancer. However, the negative outcomes of several large β-carotene supplementation trials and failure to identify carotenoids as the primary beneficial components of fruits and vegetables provide arguments against supplementation with individual carotenoids.

The overall body of evidence regarding β-carotene, α-carotene, β-cryptoxanthin, lutein, and zeaxanthin is still insufficient to conclude that increasing levels of those carotenoids by supplementation will confer important cancer-preventive benefits. The surprising results of the chemoprevention trials described above are a “red flag” signaling the need for further research.

However, evidence supports a possible protective role for the intake of lycopene and β-cryptoxanthin present in tomato and citrus fruit, respectively. For now it seems wiser to encourage consumption of the fruits and vegetables themselves and pay more attention to studies defining optimal levels of intake that can be achieved within a well-balanced diet. Recent attempts to improve carotenoid synthesis in food groups otherwise deficient in such elements by genetic engineering could also be seen as a good health promotion strategy.

ACKNOWLEDGMENTS

The author thanks Dr. Sancia Gaetani, INRAN, Rome, Italy, for her useful comments on the manuscript and Maya Nicolosi for her editorial assistance.

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15 Chemopreventive Potential of Compounds in Cruciferous Vegetables

Ole Vang

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15.1 INTRODUCTION

Since the pioneer experiments of Wattenberg and co-workers in 1978 showing tumor inhibitory effects of various indoles (Wattenberg and Loub, 1978), great interest emerged in the anticarcinogenic properties of cruciferous vegetables and compounds therein. Evaluation of the available evidence on anticarcinogenic properties of cruciferous vegetables, isothiocyanates, and indoles by IARC revealed that in humans (IARC, 2004):

There is limited evidence that eating cruciferous vegetables reduces risk for cancers of the stomach and lung. There is inadequate evidence that eating cruciferous vegetables reduces risk for cancers for all other sites.

and

There is inadequate evidence to assess the independent effects on human cancer risk of isothiocyanates and indoles, as opposed to their combined effects with other compounds in cruciferous vegetables.

According to the same source,

There is “sufficient evidence that intake of cruciferous vegetables reduces the occurrence of cancer” in experimental animals, particularly of colon, mammary gland, and liver cancers.

15.2 COMPOUNDS IN CRUCIFEROUS VEGETABLES WITH POTENTIAL HEALTH-PROMOTING ACTIVITIES

The family Brassicaceae, also known as Cruciferae, is a large family that includes a few types of edible plants. The commonly consumed cruciferous vegetables are varieties of the species *Brassica oleracea*, including broccoli, Brussels sprouts, cabbage, and cauliflower, or *B. napus*, including Chinese cabbage and rape. Radish and cress also belong to the Cruciferae family.

Sulfur-containing glucosidic compounds or glucosinolates (GSLs) are found exclusively in plants of order Capparales, including the Cruciferae family. Both isothiocyanates (ITCs) and indoles are generated during the degradation of GSLs, which contain a common glycone moiety and a variable side chain derived from amino acids. ITCs are formed during degradation of GSLs with aliphatic or aromatic side chains, whereas indoles are formed from GSLs with indolyl side chains (Table 15.1). In addition to the GSLs, polyphenols, flavonoids, vitamins, and other sulfur-containing compounds that are present in cruciferous vegetables may have health-promoting activities. This chapter focuses on the GSLs and their products.
### TABLE 15.1
Glucosinolates and their Myrosinase-Catalyzed Degradation Products

<table>
<thead>
<tr>
<th>Glucosinolate</th>
<th>Chemical Structure</th>
<th>Degradation Product</th>
<th>Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucoiberin</td>
<td>H₃C ─E ─R</td>
<td>3-Methylthiopropyl-ITC</td>
<td>Broccoli, cabbage, Brussels sprouts</td>
</tr>
<tr>
<td>Glucoerucin</td>
<td>H₃C ─E ─R</td>
<td>4-Methylthiobutyl-ITC</td>
<td></td>
</tr>
<tr>
<td>Glucoiberin</td>
<td><a href="#">Chemical Structure</a></td>
<td>3-Methylsulphinylpropyl-ITC</td>
<td></td>
</tr>
<tr>
<td>Glucoraphanin</td>
<td>H₃C ─O ─R</td>
<td>4-Methylsulfynylbutyl-ITC</td>
<td>Broccoli, Brussels sprouts</td>
</tr>
<tr>
<td>Glucoerysolin</td>
<td>H₃C ─O ─R</td>
<td>4-Methylsulfynylbutyl-ITC</td>
<td></td>
</tr>
<tr>
<td>Glucocheirolin</td>
<td>H₃C ─O ─R</td>
<td>3-Methylsulfonylpropyl-ITC</td>
<td></td>
</tr>
<tr>
<td>Sinigrin</td>
<td>H₃C ─C =R</td>
<td>2-Propenyl-ITC</td>
<td>Cabbage, Brussels sprouts</td>
</tr>
<tr>
<td>Gluconapin</td>
<td>H₃C ─C =R</td>
<td>Allyl-ITC (AITC)</td>
<td></td>
</tr>
<tr>
<td>Glucotropaeolin</td>
<td><a href="#">Chemical Structure</a></td>
<td>Benzyl-ITC (BITC)</td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 15.1 (Continued)
**Glucosinolates and their Myrosinase-Catalyzed Degradation Products**

<table>
<thead>
<tr>
<th>Glucosinolate</th>
<th>Chemical Structure</th>
<th>Degradation Product</th>
<th>Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gluconasturtin</td>
<td><img src="" alt="Gluconasturtin" /></td>
<td>Phenylethyl-ITC (PEITC)</td>
<td>Watercress, radishes, turnips</td>
</tr>
<tr>
<td>Glucobrassicin</td>
<td><img src="" alt="Glucobrassicin" /></td>
<td>Indole-3-ylcarbinol (I3C)</td>
<td>Broccoli, cabbage</td>
</tr>
<tr>
<td>Neoglucobrassicin</td>
<td><img src="" alt="Neoglucobrassicin" /></td>
<td>N-Methoxy-indole-3-ylcarbinol (NI3C)</td>
<td>Broccoli</td>
</tr>
<tr>
<td>4-Methoxy-glucobrassicin</td>
<td><img src="" alt="4-Methoxy-glucobrassicin" /></td>
<td>4-Methoxy-indole-3-ylcarbinol</td>
<td>Cabbage</td>
</tr>
<tr>
<td>4-Hydroxy-glucobrassicin</td>
<td><img src="" alt="4-Hydroxy-glucobrassicin" /></td>
<td>4-Hydroxy-indole-3-ylcarbinol</td>
<td>Cauliflower</td>
</tr>
</tbody>
</table>
When plant tissue is disrupted, an endogenous plant thioglucosidase (myrosinase) catalyzes the degradation of GSLs by cleaving the thio-glucose bond, which gives rise to the unstable thiohydoximate-S-sulfonate (Figure 15.1). The latter rearranges spontaneously to form nitriles, isothiocyanates, or the corresponding alcohol (indole) depending on the structure of the GSL (Table 15.1) and the reaction conditions. Indole-3-ylcarbinol (I3C) is not stable; under acid conditions, as in the stomach, the dimeric 3,3′-diindolylmethane (DIM) and other oligomeric condensation products are formed. In the presence of ascorbic acid, various ascorbigens are formed from the indoles (Figure 15.2). Formation of the numerous indole products has been reviewed previously (Vang and Dragsted, 1996), but in contrast to I3C, no data are available on the products formed from the other indoles. The indoles formed from degradation of indolyl-GSLs other than glucobrassicin (GB) are assumed to be more stable than I3C. The levels of the GSLs and other potential health-promoting compounds in cruciferous vegetables depend on the varieties (Vang et al., 2001a), growing conditions (Mithen et al., 2003), and season. Moreover, insect or pathogen infections induce GSL accumulation in plant tissues and induce the formation of brassinins (Figure 15.2). The conditions of postharvest storage have been shown to affect the total GSL content of broccoli. After 1 week of cold storage and 3 days at
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˚C, an ~80% reduction (Vallejo et al., 2003), and after a week in air of low O$_2$, a 20% increase in the total GSL content were observed (Hansen et al., 1995). Storage of chopped cabbage reduced aliphatic GSL, but enhanced indolylic GSL (Verkerk et al., 2001). Processing before consumption may highly affect the level and type of products formed. Maceration of raw cruciferous vegetables caused the conversion of ~80% of the aliphatic GSLs to nitriles, and the ratio of nitrile to ITC depended on the variety (Mithen et al., 2003). Mild cooking leaves the endogenous myrosinase intact, which enhances the production of ITCs, whereas longer cooking (>2 minutes) denatures the myrosinase. The GSLs are chemically and thermally stable as found for GB (Chevolleau et al., 1997), but are degraded by intestinal microflora when the myrosinase is denatured by cooking.

More than 90 aliphatic or aromatic GSLs have been described, but only a few of them occur frequently in the diet, e.g., 4-methylsulphinylbutyl-, 2-propenyl-, 3-butenyl-, and 3-methylsulphinyl-propyl-GSLs are formed as a result of digestion of certain cultivars of _B. oleracea_. Other derivatives, e.g., 4-pentenyl-, propenyl-, and phenylethyl-GSLs, may be consumed with Chinese cabbage, mustard, and watercress, respectively (Table 15.1).

In total, six indolyl-GSLs have been identified, and two are dominant in the human diet, GB and neoGB (Table 15.1). GB is the main indolyl-GSL in nearly all cruciferous vegetables. NeoGB is found at high levels in broccoli (Vang et al., 2001a), 4-hydroxy-3-indolylmethyl-GSL is found at the same level as GB in cauliflower (Kushad et al., 1999), and 4-methoxy-3-indolylmethyl-GSL is present at half the level of GB in white cabbage (Ciska et al., 2000).

**FIGURE 15.2** Structures of indole compounds formed during ingestion of cruciferous vegetables. 3,3’-diindolylmethane (DIM) and indolo[3,2-b]carbazole (ICZ) are formed from indole-3-carbinol (I3C) in the acid environment of the stomach. Brassinin is formed during degradation of glucobrassicin (GB) and ascorbigen (ASG) is formed during degradation of GB when ascorbic acid is present.

15˚C, an ~80% reduction (Vallejo et al., 2003), and after a week in air of low O$_2$, a 20% increase in the total GSL content were observed (Hansen et al., 1995). Storage of chopped cabbage reduced aliphatic GSL, but enhanced indolylic GSL (Verkerk et al., 2001). Processing before consumption may highly affect the level and type of products formed. Maceration of raw cruciferous vegetables caused the conversion of ~80% of the aliphatic GSLs to nitriles, and the ratio of nitrile to ITC depended on the variety (Mithen et al., 2003). Mild cooking leaves the endogenous myrosinase intact, which enhances the production of ITCs, whereas longer cooking (>2 minutes) denatures the myrosinase. The GSLs are chemically and thermally stable as found for GB (Chevolleau et al., 1997), but are degraded by intestinal microflora when the myrosinase is denatured by cooking.

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Various biological effects of cruciferous vegetables, ITCs, and indoles have been identified; those related to chemoprevention of carcinogenesis are reviewed below.

15.3 MODES OF CHEMOPREVENTIVE ACTION OF COMPOUNDS IN CRUCIFEROUS VEGETABLES

15.3.1 MODULATION OF METABOLISM OF EXOGENOUS AND ENDOGENOUS CARCINOGENS

15.3.1.1 Cruciferous Vegetables

One of the most important mechanisms of cancer-chemopreventive activities of cruciferous vegetables and their components is the modulation of Phase I and Phase II enzymes resulting in reduced activation and/or increased detoxification of carcinogens. Indoles are known as strong inducers of Phase I, e.g., cytochrome P450 (CYP) enzymes, whereas ITCs are generally strong inhibitors of CYP enzymes. Recent data indicate that indoles may also inhibit Phase I enzyme activities. Both ITCs and indoles induce Phase II enzymes, but it is difficult to quantify their individual impact since various other compounds in the vegetables may contribute to the observed effects. Dietary cruciferous vegetables increase CYP-mediated metabolism in humans; for example, CYP1A2 activity is consistently induced, as shown by the increase in metabolism of caffeine (Kall et al., 1996), antipyrine, and phenacetine, as well as the 2-hydroxylation of estradiol (E$_2$) (Fowke et al., 2000; Kall et al., 1996). Consumption of broccoli also increased the CYP2E1-mediated metabolism of 6-hydroxylation of chlorzoxazone (Kall et al., 1996). Excretion of heterocyclic amines, e.g., 2-amino-1-methyl-6-phenylimidazo[4,5- b]pyridine (PhIP), in urine at the end of a 12-day period of cruciferous vegetable consumption was reduced by about 20%. This was probably due to an increase in the amine metabolism catalyzed by CYP1A2 whose activity was found to be increased (Muray et al., 2001). In smokers who consumed watercress, an increased excretion of the smoke mutagen 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronide in urine was observed. This may be due to the PEITC-mediated inhibition of 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) metabolism.

Similar effects are observed in animal models. Broccoli administered for 7 days to rats induced the colonic and hepatic CYP1A1 and 2B measured at the mRNA or protein level. The modulation of Phase I enzyme activity depended on the specific content of GLSs (Vang et al., 2001a), and also other active components of broccoli (Vang et al., 2001b). Dietary cabbage induced the hepatic and intestinal Phase I enzymes in rats, and reduced aflatoxin B$_1$ (AFB$_1$) binding to hepatic DNA. Brussels sprouts administered for 4 days did not affect Phase I enzymes but increased the hepatic glutathione-S-transferase (GST) and NAD(P)H:quinine reductase (QR) activities (Phase II enzymes). Hepatic GST activity was increased in rats receiving either GSL-rich or GSL-free Brussels sprouts, indicating that other compounds are also responsible for the GST induction. Renal QR activity was not affected by broccoli feeding (Sørensen et al., 2001). Both CYP1A2 and UDP-glucuronosyltransferase 2 (UDPGT-2) activities were induced by red cabbage and especially
Brussels sprouts, which contain higher levels of GSLs than red cabbage does (Kassie et al., 2003).

The changes in hepatic xenobiotic metabolism due to the consumption of cruciferous vegetables are affected by intestinal microflora. A GSL-rich diet (rapeseed meal) decreased (by 34%) the total CYP protein level in conventional rats but not in germ-free rats. The bacterial status did not modify the levels of CYP1A2 and CYP2B1/2, whereas a GSL-rich diet induced CYP3A and reduced the CYP2E1 protein level in germ-free rats (Nugon-Baudon et al., 1998).

Together with the modulation of xenobiotic metabolism that affects the carcinogenic response, modulation of the antioxidant defense system may decrease the cellular damage by reactive compounds. The antioxidative defense system includes catalase, glutathione reductase (GSSG-Red), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and glutathione (GSH). The effect of dietary broccoli on these enzymes has been investigated in various rat tissues. Broccoli grown with varying amounts of fertilizer or different varieties of broccoli induced hepatic and renal GSSG-Red, renal and colonic GSH-Px, and hepatic SOD activities to a variable extent (Vang et al., 1997). The activities of colonic GSH-Px and GSSG-Red correlated with the GSL composition in the broccoli samples (Vang et al., 2001b).

15.3.1.2 Isothiocyanates

ITCs induce Phase II enzymes, particularly GSTs, QR, and UDPGT. GSTs catalyze the conjugation reaction of GSH with electrophiles, including ITCs. Thus, ITC as an inducer of GST could facilitate its own excretion by increasing its rate of conjugation with GSH. The GSL glucosiberin increased the intestinal GST and QR activities in rats but only at 10 µmol/kg/day, compared with the normal human intake of glucosiberin at approximately 1 µmol/kg/day. Sulforaphane (SUL) is a potent inducer of various Phase II enzymes in primary cultures of rat and human hepatocytes and in murine hepatoma cells (Basten et al., 2002; Maheo et al., 1997). SUL induced GSTA1 in human colon cells (Petri et al., 2003) and the expression of QR, GST, γ-glutamyl-transpeptidase, and the intracellular GSH (Brooks et al., 2001). In other human cells, PEITC, BITC, and SUL induced NQO1, an isoform of QR (Bonnesen et al., 2001). In contrast to this, it was recently shown that glucoraphanin, from which SUL is generated, powerfully induced the activities of CYP1A1, 1A2, 2B1/2, 2C11, and 3A1/2 in rat lung. Concomitant with Phase I enzyme induction, an increased formation of the reactive oxygen species was observed. The GST activity was only slightly induced by glucoraphanin. This suggests that glucoraphanin may increase cancer risk in combination with exposure to environmental mutagens (Paolini et al., 2004). Moreover, it was recently shown that glucoraphanin reduced QR activity in Hepa 1c1c7 cells, and addition of myrosinase enhanced the QR activity (Zhu and Loft, 2003).

The induction of Phase II enzymes by ITC seems to occur through the antioxidant response element. This process is mediated by Nrf2, a transcription factor, and probably more than 50 genes are induced by the ITCs via Nrf2. In the mouse small intestine, SUL induced not only QR and GST known to be controlled by Nrf2, but also other xenobiotic-metabolizing enzymes and proteins of the antioxidant defense system (Thimmulappa et al., 2002). Moreover, SUL induced GST and QR activities
in the small intestine of wild-type but not in Nrf2-knockout mice (McMahon et al., 2001). At low concentrations of ITCs, activation of Nrf2 with subsequent induction of Phase II and other defensive genes is the effect of MAPKs activation. At higher concentrations, these agents may activate the caspase pathways leading to apoptosis (Kong et al., 2001).

Generally, ITCs inhibit CYP-mediated activities. BITC and PEITC inactivated CYP2B1 in a time- and concentration-dependent manner. BITC has been shown to inactivate the activity of CYP2B1 primarily through irreversible chemical modification of the enzyme (Goosen et al., 2000) and CYP2E1 enzyme activity due to a chemical modification of the apoprotein. On the other hand, allyl isothiocyanate (AITC) and SUL were weak inhibitors of CYP2E1 compared to BITC (Jiao et al., 1996). Further, in primary human hepatocyte cultures, SUL inhibited the expression and activity of CYP3A4 enzyme (Maheo et al., 1997). Likewise, various ITCs are modulators of tobacco-specific N-nitrosamine metabolism, reducing the levels of their activation and increasing the formation of inactive metabolites (Staretz et al., 1997).

15.3.1.3 Indoles

Among the indoles, only the commercially available I3C has been the subject of extensive studies. I3C affects carcinogen metabolism, likely by changing the profile of CYP-related activities. I3C increased the metabolism of the food mutagen PhIP (He et al., 2000), probably through induction of CYP1A1 and 1A2. The overall metabolism of PhIP, including the formation of the proximate genotoxic metabolite N-OH-PhIP, was increased in rat liver microsomes in vitro by I3C condensation products (Vang et al., 1999). This may indicate an increased mutation risk following exposure to I3C. When rats were treated with I3C before exposure to another food mutagen, 2-amino-3-methyl-imidazo[4,5-f]quinoline (IQ), they excreted reduced amounts of IQ and other promutagens in the urine and feces (Xu et al., 1996). Dietary treatment of rats with I3C caused an overall increase in the metabolism of AFB1, which has been explained by the concomitant increase in the hepatic CYP1A1, 1A2, 2B1/2, and 3A enzymes (Manson et al., 1998).

Induction of CYP1 is prompted by activation of the Ah receptor (AhR). Among the numerous I3C-condensation products identified, it is still unclear which product is responsible for the CYP1 induction. DIM is formed at high levels, but comparison of DIM and I3C indicates that DIM is a markedly less efficacious inducer of CYPs in the rat at doses relevant to human supplementation (Leibelt et al., 2003). In contrast, indolo[3,2-b]carbazole (ICZ) (Figure 15.2) is formed at extremely low levels but is a very strong inducer via activation of the AhR.

Numerous experiments have clearly shown induction by indoles of CYP1 mRNA, protein, and the related activities in various species and tissues, including liver and intestine (Vang and Dragsted, 1996). The indole-GSLs, GB and NeoGB, induce both rat hepatic CYP1A1 protein and activity, but a mixture of GB, NeoGB, and 4-methoxyGB was the most powerful inducer (Bonnessen et al., 1999). Furthermore, intact indolyl-GSLs were more powerful inducers than the in vitro myrosinase-degraded indolyl GSLs, indicating a more efficient formation of the active compounds in situ in the gastrointestinal tract (Bonnessen et al., 1999).
Oral administration of N-methoxyindole-3-carbinol (NI3C) to rats increased the hepatic CYP1A1 and 1A2 protein levels and the related activities to a lesser extent than I3C did (Stephensen et al., 2000). Furthermore, NI3C did not induce hepatic CYP2B-related activity as I3C did. Ascorbigen (ASG) only weakly induced hepatic CYP1A1 and 1A2 but not CYP2B1/2 protein.

Indoles also induce different CYP mRNA, protein isoforms and their activities in vitro. In cultured human liver slices, DIM induced CYP1A-related activities consistent with an increase of CYP1A2 protein (Lake et al., 1998). Both I3C and various I3C-products, including DIM, NI3C, and ASG, were found to induce CYP1A1 in hepatoma, colonic, and mammary cells by activation of the aryl hydrocarbon receptor (AhR) (Bonnesen et al., 2001; Stephensen et al., 1999; 2000). DIM is a weak agonist for the AhR, but neither I3C nor ASG bind to the receptor, which may explain the conversion and local high concentration of the active I3C-derived compound in the in vitro experiments (Staub et al., 2002). NI3C can activate AhR and thus induce the expression of CYP1A1 (Stephensen et al., 2000). Other proteins, e.g., QR, are also regulated via the AhR, and the QR activity is induced by I3C, DIM, and 2,3-bis(indol-3-ylmethyl)indole in Hepa 1c1c7 cells (Zhu and Loft, 2003). On the other hand, Chen et al. (2002) found that only ICZ but not I3C enhanced the expression of QR mRNA and activity and in hepatoma cells.

In some systems, CYP1A1-related activities may be inhibited by indoles. For example, both I3C and DIM reduced the CYP1A1 activity in human T47-D cells, and ASG and NI3C inhibited CYP1A1 activity in Hepa 1c1c7 cells (Stephensen et al., 1999; 2000).

Various Phase II activities were enhanced by indoles but to a lesser extent than Phase I activities. High acute or chronic doses of I3C induce GST and QR activities (Nho and Jeffery, 2001), and the hepatic GST Yc isoform (subunit rGSTA) (Manson et al., 1998), but the purified indolyl glucosinolates GB, NeoGB, or a mixture of these and 4-methoxyGB administered at low dose levels do not induce the hepatic GST subunits, rGSTA1/2, A3, or M3 (Bonnesen et al., 1999), nor does I3C induce the activities of QR and GST (Vang et al., 1999). The mechanism of I3C-induced Phase II activities has been studied using a mouse Nrf2-knockout model, and the data indicate that I3C regulates the intestinal GST expression through a Nrf2-protein dependent mechanism whereas the I3C regulation of QR activity involves other mechanisms (McMahon et al., 2001).

Hydroxylation of endogenous 17β-estradiol (E2) is modulated by I3C. This modified metabolism may explain the observed reduction of hormone-related carcinogenesis by I3C. The indole-induced change of E2 metabolism has been investigated in several model systems. Both I3C and brassinin induce the C2-hydroxylation of E2 in human breast cancer cells (Bradlow et al., 1997), which increases the 2-/16α-hydroxylation ratio of E2. A similar increase of 2-/16α-hydroxylation E2-ratio was observed in vivo in mice (Wong et al., 1998). Since AhR knock-out mice did not show any I3C-mediated induction of 2-hydroxylation of E2, and several CYP1A inducers did not induce E2 2-hydroxylation, it was concluded that regulation of 2-hydroxylation of E2 depends on functional AhR but not on the CYP1A activity (Wong et al., 1998). In men, a 1-week treatment with I3C at 500 mg per day increased the urinary excretion of 2-OH-E2 by about twofold, whereas the other metabolites
were decreased (Michnovicz et al., 1997). A similar result was obtained in women treated with 400 mg I3C per day for 4 weeks (Wong et al., 1997) or 2 months (Michnovicz et al., 1997). In contrast, I3C after a 12-week treatment at 200 or 400 mg per day did not significantly induce the 2-OH/16αOH ratio in patients with cervical intraepithelial neoplasia (Bell et al., 2000). The conflicting results may partly be explained by the observation that I3C-mediated increase in the 2-OH/16αOH ratio is dependent on the genotype, as no induction is observed in women having Msp1 polymorphism of CYP1A1 (Taioli et al., 1999).

15.3.2 Modulation of DNA Damage

15.3.2.1 Cruciferous Vegetables

In general, DNA damage is reduced by cruciferous vegetables. Dietary Chinese cabbage administered for 10 days to rats diminished the level of PhIP-DNA adducts in colon, heart, lung, and liver, which may be explained by the simultaneous up-regulation of CYP1A1 and CYP1A2 activities (Tan et al., 1999). In V79 cells transfected with rat CYP1A2 and sulfotransferase 1C1 isoenzymes, the genotoxic activity of PhIP was strongly reduced in a dose-dependent manner by broccoli extracts (Edenharder et al., 2002). Treatment of rats with various Brussels sprout extracts and purified GSLs resulted in diminished urinary excretion of 8-oxoGua only after administration of cooked Brussels sprout homogenates. Raw Brussels sprouts or purified indole GSLs did not affect the excretion (Deng et al., 1998). The extracts of cooked and autolyzed Brussels sprouts and some GSLs inhibited hydrogen peroxide–induced DNA strand breaks (Zhu and Loft, 2003). However, Brussels sprouts increased the oxidative DNA damage in the liver (Sørensen et al., 2001).

15.3.2.2 Isothiocyanates

In accordance with the change of carcinogen metabolism by inhibiting CYP enzymes, ITCs generally reduced the formation of DNA-adducts in various organs. Both BITC and PEITC inhibited the metabolism of benzo[a]pyrene (B[a]P) in mouse lung and liver microsomes and the formation of B[a]P-diol-epoxide-DNA adducts (Sticha et al., 2000). The N-nitrosodimethylamine- and the NNK-induced DNA adduct formation were reduced by PEITC and sinigrin in rat lung, and PEITC was found to inhibit the NNK-induced formation of O6-methylguanine adducts. Decreased mutagenicity is clearly a marker for reduced cancer risk.

15.3.2.3 Indoles

Several experiments have shown the reduced carcinogen-DNA adduct levels in livers of rats exposed to I3C, including the adducts derived from B[a]P and AFB1, as well as those formed as a result of exposure to cigarette smoke in the lung (Arif et al., 2000). I3C also decreased DNA adduct formation from carcinogenic heterocyclic amines, IQ and PhIP (He et al., 2000; He and Schut, 1999; Xu et al., 1996). The reduced DNA-adduct levels were found only when I3C was given to rats before exposure to PhIP (He et al., 1997), which indicates decreased levels of activation
of these carcinogens. An initial increase in the formation of IQ- and PhIP-derived DNA adducts (8 to 24 h) but a clear reduction beyond 24 h was seen in rats treated with I3C after exposure to these food mutagens (Xu et al., 1996). The reason for the observed shift in the levels of DNA-adducts is unclear, as I3C did not affect the rate of adduct removal (He and Schut, 1999). Other experiments suggested, however, the possible enhancement of DNA repair by I3C (Blum et al., 2001).

15.3.3 Effects on Cell Proliferation and Apoptosis

The effect of cruciferous vegetable components on cell proliferation and/or apoptosis was observed basically as result of treatment with isolated compounds. One exception is the study of Smith et al. (2003) showing that in dimethylhydrazine (DMH)-treated rats, raw Brussels sprout extract increased apoptosis and had no effect on colon cells in control animals.

15.3.3.1 Isothiocyanates

Certain ITCs can inhibit the proliferation of cultured cancer cells by causing cell cycle arrest and/or induction of apoptosis. Inhibition of the cell cycle with accumulation in the G2/M phase by isothiocyanates (AITC and PEITC) has been shown in several cell lines including human colon cancer cells (Gamet-Payrastre et al., 2000) and leukemic cells (Fimognari et al., 2002). SUL and PEITC caused human prostate cancer cell arrest at G0/G1 phase and induced the expression of cyclin D1 and cell cycle regulator protein p21WAF1 (Chiao et al., 2000). The cell cycle arrest in HT-29 cells by SUL correlated with an increased expression of cyclin A and B1 (Gamet-Payrastre et al., 2000).

Apoptosis was enhanced by ITC in the respiratory tract of rats exposed to cigarette smoke (D’Agostini et al., 2001) and in mice implanted with human prostate cancer cells (Srivastava et al., 2003). Apoptosis was stimulated in human colon adenocarcinoma cells by SUL and BITC (Bonnnesen et al., 2001), and at the molecular level, SUL induced the proapoptotic protein Bax, along with apoptosis (Gamet-Payrastre et al., 2000). AITC treatment reduced the levels of antiapoptotic protein Bcl-2 in the tumor lysate, and AITC exhibited cleavage of BID protein, which is known to promote apoptosis (Srivastava et al., 2003). Moreover, SUL increased p53 and Bax protein expression and slightly affected bcl-2 expression (Fimognari et al., 2002).

15.3.3.2 Indoles

Numerous reports have shown that I3C inhibits cell proliferation and increases apoptosis. The effect of indoles on cell proliferation is different in the E2-dependent and -independent cell. I3C inhibits the E2-dependent proliferation of breast cancer cells (Ashok et al., 2001), and DIM is up to 500-fold more potent (Chen et al., 1998). Indole-induced inhibition of E2-independent cell proliferation is also observed in various breast cancer cell lines. The 50% inhibitory concentration (IC50) for the inhibition by I3C is in the range of 30 to 180 μM (Cover et al., 1998; Ge et al., 1999) and overexpression of the oncogene Her-2/neu in MDA-MB-435 cells does
not change their sensitivity to the I3C (Rahman et al., 2000). Human prostate cells are sensitive to I3C inhibition, with an IC_{50} in the range of 55 to 200 \mu M (Chinni et al., 2001; Frydoonfar et al., 2003), whereas human colon cells seems to be less sensitive (IC_{50} around 200 to 400 \mu M)(Gamet-Payrastre et al., 1998). In colon cells, N13C was found to be more potent compared with I3C (IC_{50} around 30 \mu M). I3C inhibited DNA synthesis and cell division of human breast cells through a G_1 cell cycle arrest via a specific down-regulation of cdk6 (Cover et al., 1999). A simultaneous increase of the cdk-inhibitor p21^{WAF1} was observed, together with a dephosphorylation of retinoblastoma protein (Cover et al., 1999), alterations related to G_1 arrest. A similar effect was observed in prostate cells (Chinni et al., 2001). In contrast to the G_0/G_1 phase accumulation observed for I3C, N13C induced a G_2/M phase accumulation in human colon cells (Neave et al., 2005). The constitutive activation of signal transducer and activator of transcription 3 (STAT3) was inhibited by I3C at the levels above 10 \mu M (Lian et al., 2004). Modulation of STAT3 activation may be a crucial step in the chemopreventive process of I3C.

Apoptosis in human breast cancer cells was induced in a dose-dependent manner by DIM, and by DIM and ICZ in colon cells (Bonnesen et al., 2001). DIM-mediated apoptosis in breast cancer cells was independent of the p53 pathway (Ge et al., 1996) and did not affect the Bax gene (Ge et al., 1999), whereas I3C-induced apoptosis in B[a]P-transformed human mammary epithelial cells was dependent on p53 (Katdare et al., 1998). Increase of the ratio of Bax/Bcl-2 and free Bax by DIM may explain its action in breast cancer cells (Hong et al., 2002); an additional mechanism is the increase of the ratio of Bax to Bcl-2 favoring apoptosis (Rahman et al., 2000). In I3C-treated cells, translocation of Bax to mitochondria causes mitochondrial depolarization and activation of caspases (Rahman et al., 2000). In breast epithelial cells, I3C induced translocation of Bax to the mitochondria in both tumorigenic and nontumorigenic cells, and induction of apoptosis was observed only in cancer cells (Sarkar et al., 2003). The I3C-induced apoptosis in human breast and prostate cells is partly regulated via the Akt signal cascade (Chinni and Sarkar, 2002), and an I3C-dependent increase in the PTEN protein is also a part of the Akt signal cascade (Meng et al., 2000).

15.4 INHIBITION OF CARCINOGENESIS IN ANIMAL MODELS

Inhibition of carcinogenesis by cruciferous vegetables, ITCs, and indoles has been tested in several animal models, but this chapter focuses on tumors of intestinal tract, breast, and liver.

15.4.1 CRUCIFEROUS VEGETABLES

The incidence of DMH-induced colon tumors has been reduced by 25 to 80% in mice upon feeding various cruciferous seeds (Barrett et al., 1998). Of most interest was the effect of high-GSL rapeseed meal, as crambe or canola are of importance in the human diet. Red color extracts of red cabbage were found to decrease the incidence of colorectal adenomas and carcinomas induced by DMH and PhIP in rats (Hagiwara et al., 2002).
Brussels sprouts reduced the number of azoxymethane (AOM)-induced aberrant crypt foci (ACF, a marker of colon carcinogenesis), but broccoli did not (Rijken et al., 1999). In 2-amino-3-methyl-imidazo[4,5-f]quinoline (IQ)-induced ACF, Brussels sprouts decreased the ACF frequency (Kassie et al., 2003), without any difference between cooked or uncooked vegetables. On the other hand, Smith et al. (2003) found that only uncooked Brussels sprouts reduced ACFs in colon of DMH-induced rats, which indicates that GSL breakdown products rather than the intact compounds have the cancer-preventive activity. Garden cress was found to decrease both the number of total ACFs and the multiplicity of crypts (Kassie et al., 2002).

Mammary carcinogenesis induced by 7,12-dimethylbenz[a]anthracene (DMBA) or methylnitrosourea (MNU) was found to be inhibited by cruciferous vegetables. For example, cabbage and cauliflower reduced both tumor incidence and multiplicity, and Brussels sprouts reduced the incidence of mammary tumors. Broccoli sprouts, which contain a different composition of GSLs compared to mature broccoli, was highly effective in reducing the incidence of DMBA-induced mammary tumors (Fahey et al., 1997).

Liver tumorigenesis induced by AFB1, IQ, or DMBA was inhibited by cruciferous vegetables (Stoner et al., 2002). Further, Brussels sprouts and red cabbage diminished the frequency and size of IQ-induced liver GST-Pγ foci (Kassie et al., 2003).

### 15.4.2 Isothiocyanates

Several ITCs reduced the occurrence of cancers in different organs, including esophagus, lung, and stomach (reviewed in Hecht, 2000). PEITC and BITC reduced the number of tumors in rat esophagus. The formation of N-nitrosomethylbenzylamine-induced esophageal tumors was inhibited by 1.5 µmol PEITC/g diet, which corresponds to that reducing the levels of N7- or O6-MeGua adducts. PEITC seems to be active only during tumor initiation, as no chemopreventive effect was observed when it was administered after carcinogen exposure.

NNK-induced tumors in the mouse lung were reduced by PEITC but not BITC, which correlated with a decrease in DNA adduct level. Recent data showed that BITC inhibits the incidence of B[a]P-induced lung tumors in mice (Hecht et al., 2002). Postinitiation treatments with ITCs led to a reduced incidence of NNK-induced lung tumors in mice by BITC (Stoner and Morse, 1997) and in rat lung by PEITC (Hecht et al., 1996).

Thus far, only BITC was found to be effective in reducing the occurrence of colon tumors, whereas sinigrin (Smith et al., 1998), which is degraded to AITC, as well as PEITC and SUL (Chung et al., 2000) reduced the number of ACF in rat colon. Both ITCs were active when administered after the carcinogen treatment, indicating that the ITCs do not reduce colon ACF by modulating carcinogen metabolism.

### 15.4.3 Indoles

The first animal experiments showing a cancer-inhibitory effect of indoles were performed by Wattenberg and Loub (1978) in the mouse and by Bailey et al. (1982) in rainbow trout. Among the indoles only I3C and DIM have been investigated for
their anticarcinogenic properties; the early experiments are described in several reviews (e.g., Brignall, 2001; Vang and Dragsted, 1996). The anticarcinogenic effects of indoles differ depending on whether I3C is given before, during, or after the carcinogen treatment and on the type and dose of the carcinogen used. In general, I3C treatment before and during the carcinogen exposure showed a protective effect in various species and organs, especially for the hormone-dependent cancers of the mammary gland, endometrium, and cervix. Dietary I3C or a single dose given to rats before the carcinogen DMBA reduced mammary tumor incidence and multiplicity (Wattenberg and Loub, 1978). In the same study, DIM was found to reduce tumor incidence but not multiplicity. In mice receiving dietary I3C, DIM, or indole-3-acetonitrile before exposure to B[a]P, all three indole compounds reduced the mammary tumor incidence (Wattenberg and Loub, 1978). In the mouse strains, which spontaneously develop mammary tumors, dietary I3C reduced the incidence and multiplicity and delayed the time of tumor development (Bradlow et al., 1991). On the other hand, PhIP-induced mammary tumors in rats were not reduced by dietary I3C given during and after the carcinogen treatment (Mori et al., 1999). Postinitiation treatment with I3C did not reduce the DMBA-induced mammary tumor incidence or multiplicity (Malejka-Giganti et al., 2000) and did not affect the incidence of mammary tumors following combined exposure to AOM, AFB1, and DMBA (Stoner et al., 2002). Exposure of mammary tumor-bearing rats to DIM suppressed mammary tumor growth (Chen et al., 1998). Dietary I3C decreased the incidence of spontaneous development of endometrial, uterine, and cervical cancers in rats, which was attributed to the reduction of PCNA (component of the δ DNA polymerase) expression by I3C (Jin et al., 1999). Moreover, both I3C and DIM induced apoptosis in the cervical epithelium of estrogen-treated animals (Chen et al., 2001).

Several studies have shown an inhibitory effect of I3C on hepatocarcinogenesis. The incidence of AFB1-induced hepatocarcinomas in rats was reduced by high doses of I3C (Manson et al., 1998), and that of diethylnitrosamine (DEN)-induced tumors was reduced by dietary I3C administered for 8 months after initiation (Oganesian et al., 1997). Induction of hepatic foci by treatment with a combination of DEN, methylNitrosourea, and DMH was reduced after 20 weeks of treatment with I3C, whereas a slight increase was observed when the animals were exposed to I3C for up to 48 weeks (Kim et al., 1997). Postinitiation I3C did not affect the incidence of IQ-induced tumors in rat liver (Xu et al., 2001).

The effect of indoles on the tumor formation in colon has been analyzed by means of intermediate markers such as ACF. Dietary I3C given either before and during PhIP treatment, or after PhIP treatment, or continuously showed a reduced number of colon ACF. Likewise, the number of IQ-induced ACF was reduced by dietary I3C (Xu et al., 1996), and the incidence and multiplicity of colon tumors was reduced by a postinitiation treatment with I3C (Xu et al., 2001). However, I3C did not affect DMH-induced colon tumors. The spontaneous development of intestinal polyps in Min mice was unaffected, whereas AOM-enhanced polyp formation (Kim et al., 2003) and the numbers of AOM-induced ACF (Stoner et al., 2002) were decreased by I3C.
15.5 CARCINOGENIC AND OTHER ADVERSE EFFECTS OF CRUCIFEROUS VEGETABLES

One major toxic effect of various cruciferous vegetables is the goitrogenicity described in cattle and humans caused by goitrin found in *Brassica* seeds. Another potentially adverse effect of cruciferous vegetables and/or compounds therein is their interaction with drug(s). In general, an increased clearance rate of various drugs has been observed during consumption of cruciferous vegetables. No adverse effects on thyroid, liver, or kidney function has been observed in volunteers after consumption of 300 g/day cooked Brussels sprouts for 3 weeks (Cashman et al., 1999), and only minor side effects (gastrointestinal discomfort) were reported during a 4-week ingestion of cruciferous vegetables (52 to 372 g/day) (Fowke et al., 2000).

*In vitro* studies have indicated that cruciferous vegetables have cytotoxic and genotoxic activity. Nitrite-treated Chinese cabbage extracts were found to be mutagenic in bacterial tests. The mutagenic effect was related to the content of indole GSLs, although the overall effect was marginal. Cruciferous vegetables, especially Brussels sprouts, also showed mutagenic effects without nitrite treatment (Kassie et al., 1996). The direct mutagenic effect was associated with the ITC fraction.

### 15.5.1 Isothiocyanates

In rats, no toxic effect has been observed when fed various GSLs, sinigrin, glucoraphanin, glucosinalpin, or glucotropaeolin (60 mg GSL/kg bw per day) for 4 weeks. Toxic effects were observed when mice were exposed chronically to more than 40 mg ITCs/kg bw (AITC, PEITC, BITC). Mice treated with BITC daily at 7.5 mg/kg bw for 53 weeks had decreased weight gain. The acute toxicity of several ITCs has been tested in rats, and the LD$_{50}$ for iberin and AITC was ~90 mg/kg bw. PEITC showed a higher LD$_{50}$, i.e., 150 and 700 mg/kg bw for subcutaneous and oral administration, respectively.

Some mutagenic effects have been observed for AITC, BITC, and PEITC in bacterial systems (Kassie et al., 1999) and mammalian cells (Kassie et al., 2003), but high doses of ITC (90 to 270 mg/kg bw) were required to induce moderate genotoxic effects in mice (Kassie et al., 1999). Both the cytotoxic and the genotoxic effects were likely caused by the ITC-induced production of reactive oxygen species. Several tumor-promoting activities have been observed with ITCs. AITC induced cell transformation of Chinese hamster cells, whereas a recent study did not show cell transformation by PEITC itself, but it enhanced B[a]P-induced cell transformation (Perocco et al., 2002).

### 15.5.2 Indoles

The LD$_{50}$ value has been estimated for I3C in various animal systems and using different administration routes. In mouse, rats, and rabbits, the LD$_{50}$ for oral administration of I3C was found to be 1400 to 1800 mg/kg bw, whereas the LD$_{50}$ was much lower after intraperitoneal injections (~400 mg/kg bw). A few experiments have indicated a marginal toxicity in rats following exposure to dietary I3C (150 to 300 mg/kg bw) for up to 20 weeks (Kim et al., 1997; Manson et al., 1998). In the
mouse, no toxic effects have been observed with up to 180 mg I3C/kg bw for 8 months (Oganesian et al., 1997). Neither DIM (Chen et al., 1998) nor ICZ (Pohjanvirta et al., 2002) has induced toxic responses in rats. In humans, no toxic effects have been observed with 400 mg/day for 4 or 12 weeks (Bell et al., 2000; Wong et al., 1997). In a prospective trial, I3C was given daily at 7.5 to 10 mg/kg bw for 8 to 18 months. In three patients who took excessive doses, disequilibrium and light tremor were observed, but the symptoms disappeared when the dose was reduced to 400 mg/day (Rosen et al., 1998). Several animal studies have indicated tumor-promoting activity of I3C. Inhibition of gap junctional intercellular communication (GJIC) is a valuable marker for tumor-promoting effects. ICZ, which has some structural and functional properties in common with the known tumor promoter 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD), inhibited the GJIC in primary rat hepatocyte cultures (Herrmann et al., 2002), induced the expression of COX-2, and potentiated the IL-1β induction of COX-2, as well as the formation of prostaglandin E2 (Sherratt et al., 2003), which is believed to contribute to tumor formation by increasing cell proliferation, preventing apoptosis, and facilitating angiogenesis. On the other hand, I3C may inhibit tumor promotion, as I3C counteracted the stearic acid–induced down-regulation of GJIC in V79 cells (Rijnkels et al., 1998). Postinitiation treatment of rats with I3C increased the number of mammary tumors as well as tumor multiplicity (Kang et al., 2001), and in a rat multi-organ study, the AOM/AFB1/DMBA-induced hepatic GST-P+ foci formation was enhanced by postinitiation treatment with I3C (Stoner et al., 2002). In rainbow trout, promotion of AFB1-induced hepatocarcinogenesis by postinitiation treatment with I3C was shown in several experiments (Oganesian et al., 1999).

15.6 EPIDEMIOLOGICAL STUDIES WITH CRUCIFEROUS VEGETABLES

The possible association between the intake of fruit and vegetables and cancer incidence has been evaluated in several reviews (IARC, 2004; Steinmetz and Potter, 1996). It is generally accepted that fruits and vegetables protect against cancers of the stomach, esophagus, lung, oral cavity and pharynx, endometrium, pancreas, and colon. Depending on the type of vegetable, the potency of a particular effect varies; the most protective are raw vegetables, followed by allium vegetables, carrots, green vegetables, cruciferous vegetables, and tomatoes (Steinmetz and Potter, 1996). A protective effect of cruciferous vegetables for colon cancers has been observed in 67% of case-control studies and for rectal cancers in all studies. Some case-control studies (40%) showed a protective effect of cruciferous vegetables for oral cavity and pharynx cancers (Steinmetz and Potter, 1996), and a meta-analysis only identified 8% of case-control studies as showing an increased risk of colon cancer associated with cruciferous vegetables. A protective effect of cruciferous vegetables against cancers in other organs has been observed in single studies. Risk for renal cell carcinoma was reduced with consumption of cruciferous vegetables even after adjustment for carotenoids (Yuan et al., 1998), and the risk of stomach cancers was reduced by cruciferous vegetables (Hara et al., 2003).
Thus far, only one study has investigated the therapeutic effect of I3C. Thirty patients with cervical intraepithelial neoplasia were treated with 200 or 400 mg per day for 12 weeks, and four of eight or four of nine patients, respectively, showed a complete regression of tumors, whereas in the placebo group no regression was observed (Bell et al., 2000).

15.7 CONCLUSIONS

The available data on the biological effects of cruciferous vegetables and their components clearly indicate their anticarcinogenic activity. There is some doubt, however, concerning the specific active compounds and the exact mode of action. It is not possible to point out a single ITC or indole or a group of ITCs or indoles as the active anticarcinogenic compound(s). The anticarcinogenic effects rely likely on the complex responses of multiple substances (known and yet unknown) acting in a concerted action. Therefore the data obtained with single substances (ITCs or indoles) or even their mixtures may not reflect the true effect of the entire complement contained in cruciferous vegetables. Few experiments have been done with a combination of several active substances, and the effects were not necessarily additive, e.g., only a mixture of four substances (PEITC, I3C, 1,4-phenylene-nebis(methylene)-selenocyanat, and d-limonene) inhibited NNK-induced pulmonary tumorigenesis, though none of the single substances did (Hecht, 2000). In another study, the combined treatment of human colon cells with ICZ and SUL, but not the single compounds, reduced the number of B[a]P-induced DNA strand-breaks (Bonnesen et al., 2001). Thus combined treatment effects need to be studied more thoroughly both in vitro and in vivo, and the biological effects of complex mixtures, such as those present in the “natural” environment of vegetables, need to be determined.

The two groups of active compounds in cruciferous vegetables have different mechanisms of action. Both modulate carcinogen metabolism, in different ways, and both modulate cell proliferation, likely by different mechanisms. The underlying mechanism for the antiproliferative effects observed for both groups of compounds needs to be elucidated.

The data also indicate that the ITCs and especially indole-derived products may have tumor-promoting effects. This should be taken into account in recommendations for the use of high amounts of these substances as dietary supplements.

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Carcinogenic and Anticarcinogenic Food Components


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16 Phytoestrogens and Their Effects on Cancer

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16.1 INTRODUCTION

Phytoestrogens (PE) are nonsteroidal compounds of plant origin that can bind to estrogen receptors (ER) as agonists and consequently mimic biological actions of endogeneous estrogens. The majority of plant metabolites that exhibit affinity for ER belong to a definite structural category known as phenylpropanoids (phytochemicals combining aromatic C₆ and aliphatic C₃ fragments), divided into classes of flavonoids and lignans. These compounds, phenolic in character, represent a very old evolutionary trait and function in plants as optical signals for pollinating insects, stimulators of nodulation, phytoalexins, radiation screens, herbivore deterrents, etc., while estrogenic activity does not appear to grant to the host any particular environmental advantage. Conversely, animals that feed on plants containing such constituents can suffer from an acquired hormonal imbalance, including infertility.

Although the estrogentic activity of certain plants (e.g., Genista tinctoria L.) was discovered in around 1930, the finding only came to public attention many years later in connection with “clover disease,” a term that referred to breeding problems
of Australian sheep grazing on subterranean clover (Pope and Wright, 1954). The phytoestrogenic activity of agricultural raw materials and food products is a relatively new observation. Interestingly, isoflavones, a subclass of flavonoids endowed with distinctly phytoestrogenic properties, are constituents of soy, one of the principal agricultural crops and the source of great variety of processed food. Although soybeans have been cultivated for millennia, and consequently their safety is taken for granted, this assumption is not necessarily valid for each of their constituents, particularly when ingested in a concentrated form. In the Western world, soy has suffered from a poor image as food until recently, despite the excellent nutritional characteristics of soy oil and soy protein.

In recent years, the attention of consumers as well as scientists has concentrated on certain nonnutritional constituents of soy, primarily phytoestrogenic isoflavones (soy isoflavones; SIF), which are believed to exert a beneficial influence on human health, with particular reference to the skeletal, cardiovascular, and reproductive systems (Giliani and Anderson, 2004). This interest was evoked by an abundance of epidemiological data, which revealed marked differences in mortality and morbidity from cardiovascular diseases (CVD) and cancers among Westerners and Asian populations living on a soy-rich diet (Adlercreutz and Mazur, 1997; Fournier et al., 1998). The message concerning possible chemopreventive action of PE in general, and SIF in particular, coincides in time with continuing action by environmental activists, who perceive xenoestrogens, particularly of industrial origin, as endocrine-disrupting chemicals and a threat to the future of wildlife and mankind (reviewed in Metzler, 2001; 2002). It has been demonstrated that endogenous steroidal estrogens not only perform a variety of functions in the female reproductive tract but also exert a protective action over the cardiovascular (CV), skeletal, and central nervous (CNS) systems. On the other hand, they are also known to promote cell proliferation, which may elevate risk of cancer (Colditz, 1998; Clemons and Goss, 2001).

16.2 ESTROGEN RECEPTORS AND THEIR LIGANDS

The estrogens necessary for the development and maintenance of reproductive functions in females exert their action through nuclear receptors (Ruenitz, 1997; Gruber et al., 2002). There are three main natural estrogens: estradiol (E2), estrone (E), and estriol (E3) (Figure 16.1), which are synthesized from cholesterol through a well-known biochemical pathway (Gruber et al., 2002; Ackerman and Carr, 2002).

Estrogen receptors are ligand-activated transcription factors controlling or mediating the growth, development, and function of various tissues. There are two distinct subtypes of ER in mammals: ERα and ERβ, which are coded by different genes located on separate chromosomes.

The ligand binding domain (LBD) forms a spacious and elastic cavity, which is located at the extended part of the C-terminal region of this protein. Its primary amino acid sequence and topology are similar for both ER types that, however, does not preclude ligand binding selectivity. Thus, while endogenous E2 binds to ERα and ERβ with the same affinity, phytoestrogen genistein (GST) is an Erβ-selective ligand by a factor of 30 (Nilsson et al., 2001).
Ligand binding initiates a cascade of events involving phosphorylation and recruitment of coactivators, coregulators, and corepressors, leading to transcriptional initiation (Glass and Rosenfeld, 2000; Klinge, 2000). Activated ER complexes bind to the estrogen response element (ERE) on the promoter DNA sequence of an ERE-containing gene (Yi et al., 2002).

Due to the differential biodistribution, the tolerance for structural diversity of ligands, the multistep activation process, and its multifactorial regulation, ER can elicit a variety of tissue-specific effects (Moggs and Orphanides, 2001; Nilsson et al., 2001). It is now evident that binding of various ligands to ER can result in different topologies of a complex, distinguished as agonistic and antagonistic (Pike et al., 1999). Many current ER activity tests based on genetic engineering are available for *in vitro* (Mueller, 2002) and *in vivo* (Diel et al., 2002) screening, in which environmental estrogens can be compared to endogenous steroids in terms of potency and efficiency.

The isoflavones genistein (GST), daidzein (DAI), biochanin A (BIA), foronotein (FOR), and glycine (GLN) (Figure 16.2), which occur in many nonedible plants of the Leguminaceae family, were recognized early as relatively potent PE and remain among the most studied nonnutritional food constituents (Setchell, 1998; Middleton et al., 2000). Recently, some of their metabolites, particularly the isoflavane equol (EQU), came to attention as a likely cause of estrogenic effects of PE-containing food. The ability of these compounds to bind to ER is believed to result from overall structural similarity between estrogenic steroids and isoflavones, with particular reference to the distance (ca. 11 Å) of the two oxygen atoms located at C-7 and C-4′ (corresponding to 3-OH and 17-OH in E2), thought to be responsible for molecular recognition.

Although the binding affinity of isoflavones to ER is rather low (an estimated 1/100 to 1/10,000 that of E2, depending on the experimental model, with only
genistein reaching 0.1 to 0.87 of relative binding affinity), their ability to induce endocrine effects in vivo is undisputable. It should be remembered that PE concentrations resulting from dietary intake can easily exceed physiological levels of E2 by three orders of magnitude (Setchell et al., 2001). Moreover, the relative transactivation capabilities of PE and E2 at high (1000 nM) concentrations are of the same order of magnitude for both ER subtypes.

Apart from isoflavones and structurally related coumestans, many other polyphenols, belonging to diversified classes of natural products, have been recognized as PE, including recently discovered phytoalexins of soy, named glyceollins, which belong to a novel category of pterocarpan-related endocrine-active compounds (Burow et al., 2001). Also, sapogenol constituents of soy have been recognized as hormonally active (soyasapogenol A is estrogenic) and growth inhibitory (soyasapogenol B), which adds a new dimension to an already-complicated picture (Rowlands et al., 2002).

Although lignans have been known for a century as a principal class of higher plant metabolites, their influence on the estrogenic status of humans exposed to certain types of diets was established only very recently. Around 1980, it turned out that lignans, occurring in grains as the dimeric phenylpropanoids syringaresinol, pinoresinol, lariciresinol, secoisolariciresinol (SEC), and matairesinol (MAT), are converted in the mammalian intestinal tract into enterolacton (ENT) and enterodiol.

**FIGURE 16.2** Representative phytoestrogens.
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(ND), which are weakly estrogenic compounds but which can attain significant concentrations in body fluids (Raffaelli et al., 2002; Saarinen et al., 2002; Wang et al., 2002). It is postulated that chemoprotective effects of lignans on an epidemiological level are as important for inhabitants of the Western world as soy and other legume isoflavones are for Asian populations.

Estrogenic stilbenes are a well-known group of synthetic hormonal drugs. However, this structural category is represented also among plant metabolites, as exemplified by resveratrol.

Some macrocyclic polyphenols produced by fungi, such as β-zearalenone, exhibit phytoestrogenic properties, too. Since they are classified as mycotoxins and therefore are undesirable in food, they will not be included in further discussions of dietary PE.

It is very important to realize that physiological consequences of PE intake with food can vary, depending on many factors pertinent to both the sample and the organism. The influences of the food matrix, its composition, and overall nutritional status are rather obvious, though not easy to specify. Since PE are endowed with pleiotropic biological activity, it has to be understood that their effects on endocrine physiology are not limited to ER binding-dependent mechanisms. By inhibiting certain enzymes (e.g., protein kinases, topoisomerases, steroid aromatases and dehydrogenases, cytochromes P450, conjugating enzymes) and complexing with functional proteins such as sex hormone–binding globulins, they can alter distribution and/or metabolism of endogenous steroid hormones. Both the transcriptional (ER-regulated) and ER-independent actions of PE may produce remarkably different effects in individual subjects. PE exhibit tissue-dependent and tissue-specific hormonal effects and therefore constitute hormone equilibrium modulators (which can be ER agonistic in one tissue and antagonistic in another), rather than purely estrogenic agents, as suggested by their generally accepted group name — phytoestrogens (Bryant, 2002; Vollmer et al., 2002; Giliani and Anderson, 2004).

16.3 PHYTOESTROGENS IN PLANTS, FOODSTUFFS, AND DIETARY SUPPLEMENTS

Based on a traditional test, measuring the induced increase in weight of a rodent uterus, many hundreds of plants and herbal preparations were classified as having estrogenic activity. Corresponding data for food sources (Reinli and Block, 1996) differ in reliability, depending on the date of the experiment and quality of the analytical procedure applied. In the last decade, chromatographic methods of separation, detection, and quantification of PE have brought down the limits of detection for individual PE to a fraction of a picomole (Franke et al., 2002; Wang et al., 2002; Wilkinson et al., 2002). In plants, the phenolic PE are usually modified by glycosylation during last steps of their biosynthesis, with monosaccharides (α-glucose, α-galactose, L-rhamnose, apiose) or oligosaccharides. Glycosides, in turn, are frequently esterified with a variety of carboxylic acids. All these compounds, usually nonestrogenic as such in the original biological matrix, have limited enzymatic and chemical stability and are likely to revert to corresponding aglycones upon process-
Carcinogenic and Anticarcinogenic Food Components

ing or storage. Thus, for example, soy- and red clover–derived nutraceutical products should preferably be analyzed for up to five isoflavone aglycons (genistein, daidzein, biochanin A, formononetin, and glycitein), coumestan, corresponding isoflavone 7-O-β-D-glucopyranosides (genistin, daidzin, oonin, sissotrin, glycitin), and their 6”-O-acetates and 6”-O-malonates (over 20 individual analytes in all), to account for known chemical entities containing estrogenic components (Murphy et al., 1999; Nurmi et al., 2002). Since expensive equipment is a limiting factor of such analyses, only two main constituents, GST and DAI, are usually determined.

Soy (various cultivars of Glycine max, which replaced wild G. soja previously grown in Asia) is the major source of dietary PE, but other plants, including red clover (Trifolium partense L.), kudzu root (Pueraria lobata L.), alfalfa sprout (Medicago sativa L.), black cohosh (Cimicifuga racemosa L.), mung bean sprout (Vigna radiata L.), green bean (Phaseolus vulgaris L.) dong quai (Angelica sinensis L.), chast tree (Vitex agnus-castus), licorice (liquorice, Glycyrrhiza glabra L.), and hops (Humulus lupulus L.), also contain substantial amounts of these components (Eagon et al., 1999). In contrast to isoflavones, which are found in many Leguminaceae plants but only in significant amounts in very few, precursors of enterolignans are much more widespread and occur in many foods. Flaxseed (Linum sativum L.) is by far the richest source of secoisolarciresinol (SEC; precursor of enterodiol; END, Figure 16.3), containing as much as 370 mg per 100 g of dry weight. Crushed and defatted material may contain as much as 600 to 700 mg per 100 g. A great variety of grains, brans, vegetables, fruits, nuts, berries, and teas contain hundreds of micrograms of lignans per 100 g sample (Mazur and Adlercreutz, 1998). Matairesinol,

![Diagram](image)

FIGURE 16.3 Conversion of plant precursors into mammalian estrogenic lignans.
which is a precursor of enterolactone (ENT), is usually present in plant material as a much smaller fraction of total lignans than SEC is.

Coumestrol (COU, which has higher binding affinity to ER than genistein does) is also widespread in plant materials, e.g., mung bean sprouts contain approximately 1 mg per 100 g, while alfalfa sprouts have only approximately 45 µg per 100 g.

Kudzu root and red clover surpass soybeans in SIF content, but the soy-derived products are by far the most significant source of dietary PE for an average consumer. In Table 16.1, typical levels of the main SIF (GST and DAI) are presented. Glycitein and its corresponding glycosides constitute of only 5 to 10% of total isoflavones in most soy foods, but in soy germs GLN can account for as much as 40% of SIF. Red

<table>
<thead>
<tr>
<th>Material/Product</th>
<th>Genistein</th>
<th>Daidzein</th>
<th>Total Isoflavones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybeans, raw (U.S., various genotypes, locations, years)</td>
<td>13.0 to 138.2</td>
<td>9.9 to 124.2</td>
<td>121.2 to 244.4</td>
</tr>
<tr>
<td>Soybeans, raw (Kenwood 94, Iowa locations, U.S., 1995–6)</td>
<td>60 to 160</td>
<td>40 to 125</td>
<td>124 to 307</td>
</tr>
<tr>
<td>Soybeans, mature seeds (U.S., commodity grade)</td>
<td>91.7</td>
<td>52.2</td>
<td>153.4</td>
</tr>
<tr>
<td>Soybeans, mature seeds (U.S., food quality)</td>
<td>73.7</td>
<td>46.4</td>
<td>128.4</td>
</tr>
<tr>
<td>Soybeans, raw (Brazil)</td>
<td>67.5</td>
<td>20.2</td>
<td>87.6</td>
</tr>
<tr>
<td>Soybeans, raw (Japan)</td>
<td>64.8</td>
<td>34.5</td>
<td>118.5</td>
</tr>
<tr>
<td>Soybeans, raw (Korea)</td>
<td>72.3</td>
<td>72.7</td>
<td>145.0</td>
</tr>
<tr>
<td>Soybeans, raw (Taiwan)</td>
<td>31.5</td>
<td>28.2</td>
<td>59.8</td>
</tr>
<tr>
<td>Soy flour, raw; full-fat</td>
<td>96.8</td>
<td>71.2</td>
<td>177.9</td>
</tr>
<tr>
<td>Soy flour, defatted</td>
<td>71.2</td>
<td>57.5</td>
<td>131.2</td>
</tr>
<tr>
<td>Soy flour, textured</td>
<td>78.9</td>
<td>59.6</td>
<td>148.6</td>
</tr>
<tr>
<td>Soy milk, fluid</td>
<td>6.1</td>
<td>4.5</td>
<td>10.9</td>
</tr>
<tr>
<td>Soy protein concentrate (aqueous wash)</td>
<td>55.6</td>
<td>43.0</td>
<td>102.1</td>
</tr>
<tr>
<td>Soy protein concentrate (alcohol extracted)</td>
<td>5.3</td>
<td>6.8</td>
<td>12.5</td>
</tr>
<tr>
<td>Soy protein isolate</td>
<td>59.6</td>
<td>33.6</td>
<td>97.4</td>
</tr>
<tr>
<td>Soybeans, flakes; full-fat</td>
<td>80.0</td>
<td>48.23</td>
<td>129.0</td>
</tr>
<tr>
<td>Soybeans, flakes; defatted</td>
<td>85.7</td>
<td>37.0</td>
<td>125.8</td>
</tr>
<tr>
<td>Tofu, raw, regular</td>
<td>13.6</td>
<td>9.0</td>
<td>23.6</td>
</tr>
<tr>
<td>Tempeh</td>
<td>24.8</td>
<td>17.6</td>
<td>43.5</td>
</tr>
<tr>
<td>Soybean curd, fermented</td>
<td>22.4</td>
<td>14.3</td>
<td>39.0</td>
</tr>
<tr>
<td>Soybean chips</td>
<td>27.5</td>
<td>26.7</td>
<td>54.2</td>
</tr>
<tr>
<td>Soy protein isolate Supro 675 (PTI, St. Louis, MO, U.S.)</td>
<td>84.9</td>
<td>44.1</td>
<td>140</td>
</tr>
<tr>
<td>Soy based infant formulas (U.S., U.K.)</td>
<td>0.9 to 2.3</td>
<td>0.5 to 1.9</td>
<td>1.4 to 4.2</td>
</tr>
</tbody>
</table>

a Mean values (or range) obtained from various literature sources. Soy isoflavones are present in plant material mainly as D-glucosides (ca. 20 mol%) and O-acylated D-glucosides (mainly 6-O-malonates; ca 77 mol%). Fermentation and/or thermal processes, characteristic for food manufacturing, tend to increase proportion of aglycones in a product. Contents in the table are calculated for unconjugated aglycones.
clover leaves, in which PE can amount to 5% of dry mass, contain FOR and BIA predominantly.

The place of SIF in human nutrition is exceptional, at least regionally. World production of soy is over 150 million metric tons, approximately half of the total oil seed crops. SIF are not removed during the initial industrial process, oil extraction, and are carried over to the protein fraction. However, the content of total isoflavones in soy flour and soy protein is similar to the whole bean values (0.1 to 0.3%, Table 16.1).

In Asia, many fermented soy products (natto, tofu, koji, tempeh, miso, soy sauce, soy milk, and soy paste), which typically provide 0.2 to 0.4 mg of isoflavones per gram of wet weight, constitute important elements of the traditional diet, dating back to ancient times. Since the mid-1950s, almost half of the global soybean crop has been harvested in America, and the United States has become the largest manufacturer of soy products. Despite that, only some 15 years ago, average per capita daily consumption of soy products in the United States was ca. 5 g, as compared to 32 to 35 g in Japan. But the U.S. market for soy food is very dynamic and is expected to reach $6 billion in 2005, a nearly threefold increase since 1999 (Messina and Loprinzi, 2001).

Refined industrial intermediates, rich in isoflavones, include soy flour, soy protein concentrates, and soy isolates (varying in isoflavone content from 0.5 to 2.0 mg per gram of the material), the latter used in infant formulas and nutritional drinks. The fact that for infants fed on soy formula (total isoflavone content 32 to 47 μg/ml; ca. 20% of the entire formula market in the United States), the isoflavone challenge is greater than for any other group, has raised considerable concern. While amounts typically consumed by adults in Japan are estimated as 0.08 to 0.13 mg per kg of body weight (bw) of total genistein per day, the calculated intake for infants fed solely soy formula amounts to 5.4 mg/kg bw of genistein and 2.3 mg/kg bw of daidzein per day. Recent studies (Klein, 1998) have assessed potential endocrine problems concerning an estimated 750,000 U.S. infants fed on soy formulas every year. In a retrospective cohort study among 248 adults aged 20 to 34 years, who as infants participated in controlled feeding studies taking soy formula and therefore were exposed to large doses of SIF, no statistically significant differences were observed for both genders, in comparison to a cow milk–fed group (Strom et al., 2001).

As seen from the above short account, massive amounts of plant-derived endocrine-active compounds, such as SIF, are consumed with food. Ingestion of 100 g soy protein can carry SIF equivalent to 200 mg of genistein, approximately ten times more than the daily intake from a traditional soy-rich Asian diet. However, PE intake estimates become rather hazy when nutraceuticals and food supplements are taken into account. A variety of PE-containing products (mostly with isoflavones as active principles) are present on the U.S. and European food and OTC drug markets, in accordance with the Dietary Supplement Health and Education Act (DSHEA, 1994), Nutrition Labeling and Education Act (NLEA, 1990), and similar European regulations (which do not allow health claims but are fairly liberal concerning specifications), with declared contents of isoflavones ranging from 10 to 100 mg per dose. Importantly, a health claim concerning cardiovascular protection has been granted by the U.S. Food and Drug Administration (FDA, 1999) to soy protein (containing ca. 2 mg/g SIF), with a recommended daily dose of ca. 25 g (Endres, 2004).
16.4 BIOAVAILABILITY, PHARMACOKINETICS, AND METABOLISM OF PHYTOESTROGENS

In epidemiological studies that attempted to correlate frequency of defined pathologies for different human populations with geographic locations and dietary habits, questionnaires were at first applied to assess habitual intake of certain types of food containing estrogenic compounds. Analysis of urine for excreted PE and their metabolites was later introduced for verification of such assessment. Presently, in clinical experiments, food is analyzed for PE prior to administration of a defined dose, and then various biomarkers of consumption (by analysis of body fluids) are followed in order to confirm compliance with the study protocol and to establish individual differences in metabolism (Persky et al., 2002; Lampe, 2003).

Soy isoflavones are among the best-studied natural products in terms of their biological activity, but the results of these studies are often considered controversial, and molecular mechanisms of these effects are still poorly understood. In order to establish a link between PE and cancer, strict pharmacological criteria have to be applied to studies covering bioavailability, metabolism, and pharmacokinetics. Initially, SIF glycosides (and their esters), which are primary constituents of plant tissues but inactive as PE, release phytoestrogenic aglycones in an enzymatic process catalyzed by β-glycosidases. It was assumed until recently that such a process is carried out exclusively by the gut bacterial flora. The question whether SIF glycosides can be passively absorbed or actively transported into cells is still a matter of some dispute, but participation of colonic bacteria in the bioavailability of isoflavone and lignane aglycons and their further metabolism is undeniable (Bowey et al., 2003). Nevertheless, it has been demonstrated that hydrolysis of genistin and release of genistein can start in the mouth and can be effectively completed in the small intestine by the action of lactase phlorizin hydrolase (Heinonen et al., 2003). Nevertheless, it has been demonstrated that hydrolysis of genistin and release of genistein can start in the mouth and can be effectively completed in the small intestine by the action of lactase phlorizin hydrolase (Heinonen et al., 2003).

SIF aglycones can undergo a variety of biotransformations: oxidation, reduction, conjugation, and demethylation (Roberts-Kirchhoff et al., 1999; Heinonen et al., 2003). BIA and FOR undergo 4'-O-demethylation, which transforms them into GST and DAI, respectively. The most important metabolic pathway, reductive in character and leading among other products to distinctly estrogenic isoflavon S(−) equol (EQU), is depicted in Figure 16.4.

Remarkably, the ability to produce EQU is an individual trait, present in only approximately 35% of the population. It seems likely that EQU producers enjoy more effective protection from metabolic disorders connected with estrogen deficiency.

All the above-mentioned compounds, as well as their precursors, are subject to in vivo transformations known as Phase II metabolism via conjugation. SIF are easily converted into two types of conjugates: glucuronides and sulfates, both characteristic of the phenol detoxification process. The following distribution pattern of excreted conjugates was observed for DAI: 7-glucuronide (54%); 4'-glucuronide (25%); monosulfates (13%); unconjugated daidzein (7%); mixed sulfoglucuronides (0.9%); diglucuronide (0.4%); and disulfate (ca. 0.1%) (Clarke et al., 2002).

Little is known about the estrogenic activity of SIF metabolites, with the notable exception of EQU. Glucuronides of SIF aglycones are believed to be very weakly estrogenic, while primary glycosides are apparently devoid of PE activity. It has
been suggested that GST, the principal PE from soy, is metabolically converted to a more active derivative, 6-methoxyoxygenistein (or 5-hydroxyglycitein), and therefore can be considered a prodrug (Peterson et al., 1998).

Recently, the human plasma kinetics of individual SIF, administered orally to healthy premenopausal women as a standardized single bolus dose, was examined (Setchell et al., 2001). Although all isoflavones were efficiently absorbed from the intestinal tract, there were striking differences in the pharmacokinetics of aglycones and their β-glycosides. The mean time to attain peak plasma concentrations for the aglycones GST and DAI was 5.2 and 6.6 h, respectively, whereas for the corresponding β-glycosides, it was delayed to 9.3 and 9.0 h, respectively, consistent with the retention time needed for hydrolytic cleavage of the glycoside moiety for bioavailability. Moreover, GST concentrations were consistently higher than DAI when equal amounts of the two isoflavones were administered, and this was accounted for by the more extensive distribution of DAI.

In a randomized controlled trial, healthy men ingested SIF preparations as a single dose of genistein (1 to 16 mg/kg bw) exceeding the doses previously administered in humans. Minimal clinical toxicity was reported: hypophosphatemia and elevated blood lipase activity. SIF in free and conjugated forms were rapidly cleared from plasma, and after repeated doses no signs of accumulation were found (Busby et al., 2002). However, investigation of hormone replacement therapy (HRT) safety suggests that isoflavones consumed orally at a dose below 2 mg/kg bw per day should be considered safe for most populations (Barnes, 2003). For a 50 mg dose of GST (ca. 0.7 mg/kg bw), which would likely be assumed acceptable by advocates of HRT nutraceutical preparations as a daily intake for a healthy human subject, the expected plasma concentration would be ca. 1 μM. Most of this amount would circulate as conjugates, with only 2 to 5% of the aglycone available for absorption.
by tissues. However, it has to be pointed out that great individual differences (up to tenfold) in blood concentration of SIF have been observed.

Although it is well known that PE exert tissue-specific effects, very few data are available concerning their selective accumulation in various organs. In rats fed on GST (5 and 100 µg/g of feed), considerable differences between genders were found in GST elimination from serum (Chang et al., 2000). Moreover, it has been established that endocrine-responsive tissues (such as brain, liver, mammary, ovary, prostate, testis, thyroid, and uterus) exhibit dose-dependent increases in GST aglycone concentrations, up to 100% of the total isoflavone determined, while in blood the aglycone fraction did not exceed 5%. The highest amount of GST was found in female liver (7.3 pmol/mg tissue). A very low accumulation in the brain (0.04 pmol/mg for male rats) most probably reflects poor penetration of the blood–brain barrier by isoflavones, which nevertheless show some influence on the CNS, as proven in numerous experiments.

It has been suggested that effects in rats fed a control diet (SIF plasma concentration <0.1 µmol/l) are similar to those in human adults consuming a Western diet, while the effects on rats fed 100 µg/g genistein diet are similar to those in humans consuming an Asian diet or using nutritional supplements (approx. 1 µmol/l). Rats fed 500 µg/g genistein in the diet have blood levels similar to those of infants fed soy-based formulas (2 to 7 µmol/l of the total SIF in serum). It is possible that this analogy can be extended also to the tissue distribution.

Dramatic differences in the Cmax plasma values for GST were observed following intravenous (6921 and 4392 ng/ml) and oral (21 and 22 ng/ml) administration to male and female rats, respectively (Coldham et al., 2002). Generally, a fairly consistent picture concerning PE supply with food, intake biomarkers, and pharmacokinetic parameters in human subjects exists, and analytical methods fit to support any design of clinical trials are available. It is evident that the overall availability of orally administered SIF, which are the most important group of PE thus far, does not critically depend on the state of conjugation, although some pharmacokinetic parameters differ between nonestrogenic primary glycosides and free aglycones, which act as PE (Duncan et al., 2003; Zubik and Meydani, 2003).

16.5 PHYTOESTROGENS AND CANCER

16.5.1 BREAST CANCER

16.5.1.1 Epidemiological and Clinical Studies

The association between soy food intake and breast cancer risk is controversial. Although isoflavones, such as those found in soy, have been shown to inhibit breast cancer in laboratory studies, associations between consumption of isoflavone-containing foods and breast cancer risk have been inconsistent in United States epidemiological studies. However, a population-based case-control study of breast cancer among Chinese, Japanese, and Filipino women in Los Angeles County showed that high soy intake, particularly in childhood, is associated with reduced risk of breast cancer (Wu et al., 2002; Jakes et al., 2002).
In Japanese studies, consumption of miso soup and isoflavones, but not of soyfoods, was inversely correlated with the risk of breast cancer (Yamamoto et al., 2003).

### 16.5.1.2 Experimental Carcinogenesis

Conflicting reports exist on the effect of soy and its components on mammary gland carcinogenesis, probably because different rodent models have been used in chemoprevention studies. Soy protein isolate and DAI and, to a lesser extent, GST were effective inhibitors of dimethylbenz[a]anthracene (DMBA)-induced mammary tumors in adult female Sprague-Dawley rats (Constantinou et al., 2001). Transgenic MMTV-neu mice, which spontaneously develop mammary tumors due to the overexpressed ErbB-2/neu/HER2 oncogene, showed an increased tumor latency as a result of isoflavone feeding. Once tumors were formed, however, the isoflavones did not reduce their number or size (Jin and McDonald, 2002).

By contrast, dietary GST did not affect DMBA-induced malignant mammary adenocarcinoma development in wild-type mice (Allred et al., 2001) No tumors were observed in ERα knockout mice. As no protective effect of GST on DMBA-induced mammary tumors in mice was found, a suggestion emerges of a potential adverse effect on tumor development when high levels of GST are consumed (Day et al., 2001). It has been also shown that GST stimulates growth of estrogen-dependent human breast cancer cells (MCF-7) in nude mice. The conversion of genistein in the lower gut is sufficient to produce a level of GST capable of stimulating estrogen-dependent breast cancer cell growth in athymic mice bearing xenografted estrogen-dependent breast tumors (MCF-7). Removal of GSI or GST from the diet caused tumors to regress (Allred et al., 2001).

Studies on the antimetastatic activity of GST in mice transplanted with mammary cancer 16/C showed that GST did not affect lung metastasis but reduced primary tumor recurrence (Wietrzyk et al., 2000). Ipriflavone, a synthetic isoflavone used for treatment of osteoporosis in some countries, was shown not only to inhibit the growth of cancer cells but also prevent the soft tissue tumor burden and osteolytic bone metastases in mice (Iwasaki et al., 2002).

The molecular mechanisms underlying the effects of PE on mammary tumors are related to their ability to bind to ER and activate estrogen-responsive genes. Studies of E2, GST, DAI, and resveratrol on the activation status of signaling proteins that regulate cancer survival in nonmetastatic ER-positive T47D cells and metastatic ER-negative MDA-MB-231 indicated that these compounds may also exert, besides genomic effects, ER-independent nongenomic effects on cell survival and migration in breast cancer cells (Brownson et al., 2002).

GST has been shown to modulate genes involved in control of the cell cycle and apoptosis. It has also been found that GST inhibits the activation of the nuclear transcription factor, NFκB, and the Akt signaling pathway, both of which are known to maintain a balance between cell survival and apoptosis (Sarkar and Li, 2002; Gong et al., 2003).

It has recently been reported that GST treatment of the immortalized but nonmalignant human mammary epithelial cell line MCF-10F resulted in growth arrest
in the G2 phase of the cell cycle and involvement of p38 mitogen-activated protein kinase in this process. These data suggest an important interplay between the p38 pathway and G2 cell cycle checkpoint control and provide insights into possible mechanisms whereby this isoflavone may inhibit early events in mammary carcinogenesis (Frey and Singletary, 2003). Searching for the proteins that are targets of GST resulted in characterization of DING protein (N-terminal amino acid sequence Ile-Asn-Gly), which binds to genistein with high affinity. Its biological role, however, remains to be defined (Belenky et al., 2003).

Studies on breast cancer cells indicated that GST affects the expression of apoptosis-related genes and induces apoptosis through a p53-independent pathway. Inhibition of matrix metalloproteinase was also observed. Thus, GST inhibits the growth of MDA-MB-435 breast cancer cells, induces apoptosis, regulates the expression of genes, and may inhibit invasion and metastasis (Li et al., 1999a).

16.5.2 PROSTATE CANCER

16.5.2.1 Epidemiological and Clinical Studies

The mortality from prostate cancer is lower in Asian than in American or European populations. Asian men typically consume more soy than their Western counterparts do; this fact led to the investigation of individual soy components, particularly PE, as protective factors against prostate cancer. Preclinical studies suggest that GST may exert chemopreventive activity toward prostate cancer via an increase of tyrosine phosphorylation in peripheral blood mononuclear cells (Takimoto et al., 2003). Moreover, these studies showed that oral administration of SIF yielded plasma concentrations of GST that have been associated with antimetastatic activity in vitro (Takimoto et al., 2003). The effects of a low-fat diet supplemented with soy were investigated in a pilot Phase II clinical study using prostate-specific antigen (PSA) as a biomarker of prostate cancer development; however, no significant decline in PSA levels was observed. A potentially undesirable effect associated with the administration of soy was an increase of insulin-like growth factor (IGF-I) levels in serum (Spentzos et al., 2003).

16.5.2.2 Experimental Studies

The potential effect of GST and the synthetic estrogen diethylstilbestrol (DES) on rat testicular development was investigated. Dietary GST uptake resulted in serum concentrations that approximate or exceed concentrations in Asian men on a soy-containing diet. While DES exposure reduced testicular weights, altered morphology, and increased apoptosis in the seminiferous tubules, GST in the diet at doses of 250 and 1000 mg/kg diet did not significantly affect these parameters. The higher concentration of GST, however, significantly reduced testicular aromatase activity, an effect that may contribute to reduced estrogen concentrations and suppression of prostate cancer development. These data suggest that exposure to GST in the diet in doses corresponding to the upper limit in humans consuming soy products does not affect testicular development but may provide health benefits (Fritz et al., 2002; 2003).
The molecular mechanism(s) by which GST elicits its effects in prostate cancer cells has not been fully elucidated. It was found that GST specifically inhibits Akt kinase activity and abrogates the epidermal growth factor--induced activation of Akt in prostate cancer cells. Thus, the inhibition of Akt and NFκB activity and their cross-talk underlies the mechanism by which GST inhibits cell growth and induces apoptotic processes in tumorigenic but not in nontumorigenic prostate epithelial cells (Li and Sarkar, 2002a).

It has been found that GST affects the expression of genes that are critically involved in the regulation of cell growth, cell cycle, apoptosis, cell signaling transduction, angiogenesis, tumor cell invasion, and metastasis. The gene expression profiles provide comprehensive molecular mechanism(s) by which GST exerts its pleiotropic effects on cancer cells (Li and Sarkar, 2002b; 2002c).

It has been shown that GST and other polyphenols from tomatoes and soy have the ability to modulate proliferation and apoptosis of a rat prostate cancer cell line (AT6.3). These compounds may counteract the ability of IGF-I to stimulate proliferation and prevent apoptosis via inhibition of multiple intracellular signaling pathways involving tyrosine kinase activity (Wang et al., 2003). It has been shown that consumption of a GST diet was positively correlated with changes in prostate DNA methylation at CpG islands of specific mouse genes. Thus, certain soy PE, such as GST, may be involved in preventing the development of certain prostate and mammary cancers by maintaining a protective DNA methylation profile (Day et al., 2002).

Zhou et al. (2003) attempted to identify possible synergistic effects between soy and tea components on prostate tumor progression in a mouse model of androgen-sensitive human prostate cancer. Soy phytochemical concentrate (SPC), black tea, and green tea significantly reduced tumorigenicity. SPC and black tea also significantly reduced final tumor weights. The combination of SPC and black tea synergistically inhibited prostate tumorigenicity, final tumor weight, and metastases to lymph nodes in vivo. The combination of SPC and green tea synergistically inhibited final tumor weight and metastasis. Inhibition of tumor progression was associated with reduced tumor cell proliferation and tumor angiogenesis. Thus, a soy and tea combination could be an effective nutritional regimen in prostate cancer prevention (Zhou et al., 2003).

16.5.3 Other Cancers

The dietary intake of seven specific compounds representing three classes of PE (isoflavones, coumestans, and lignans) was reported to be associated with reduced risk of endometrial cancer in an American population (Horn-Ross et al., 2003). The incidence of uterine adenocarcinoma is increased by 35% from exposure to GST during fetal development in animal models, which suggests that GST is carcinogenic if exposure occurs during critical periods of differentiation (Newbold et al., 2001).

Incidence rates of ovarian cancer remain lowest in Asian nations, whereas they are among the highest in the United States and other Western nations, where relatively small amounts of soy foods are consumed. Experiments have shown that GST and DAI in vitro independently modify cytokine production and reduce ovarian
cancer cell proliferation via, at least in part, an estrogen receptor–dependent pathway (Chen and Anderson, 2001).

Epidemiological and pathological data suggest that thyroid cancer may well be an estrogen-dependent disease. The relationship between thyroid cancer risk and dietary PE intake has been examined in a multiethnic population-based case-control study of thyroid cancer conducted in the San Francisco Bay area (Horn-Ross et al., 2002). The consumption of traditional and nontraditional soy-based foods and alfalfa sprouts was associated with reduced risk of thyroid cancer. Consumption of “Western” foods with added soy flour or soy protein did not affect the risk. Of the seven specific phytoestrogenic compounds examined, DAI, GST, and the lignan secoisolariciresinol were most strongly associated with risk reduction. These results suggest that thyroid cancer prevention via dietary modification of soy and/or phytoestrogen intake in other forms may be possible (Horn-Ross et al., 2002).

The relationship between intake of soy products and death from stomach cancer was examined in a community-based prospective study of Japanese men and women in Takayama, Japan. In men, the total soy product intake was inversely correlated with death from stomach cancer. The results in women were less obvious. These data suggest that soy intake may reduce the risk of stomach cancer, at least in men (Nagata et al., 2002).

Chemoprotective effects of GST, BCA, EQU, and COU on human pancreatic adenocarcinoma cells in vitro have been examined. EQU and COU inhibited the growth of female pancreatic tumor cells by 95%; however, these agents stimulated the growth of pancreatic tumor cells from the male. GST also stimulated growth of male pancreatic tumor cells but had little effect on pancreatic tumor cells from the female. Thus, the chemoprotective potential of EQU and COU against pancreatic cancer seems to be greater in females than in males (Lyn-Cook et al., 1999).

The low incidence of colon cancer in Asian countries is associated with consumption of soybean products (Gentile et al., 2003). A limited number of human and animal studies suggest that soybean consumption might prevent colon cancer; other studies do not support this conclusion.

It has been shown that GST has anti–colon cancer effects in vitro. These effects, however, are attainable only at high concentrations that are difficult to achieve in the serum in vivo (Gentile et al., 2003).

Epidemiological data also suggest that consumption of soy products may be associated with a decreased risk of lung cancer. It has been shown that GST can inhibit the growth of H460 non–small-cell lung cancer (NSCLC) cells in vitro (Lian et al., 1999). The results of Finnish epidemiological studies have shown that high isoflavonoid consumption correlates with lower lung cancer incidence (Knekt et al., 2002). GST applied alone or in combined therapy with cyclophosphamide has shown antitumor and antiangiogenic activity in the mouse tumor model of Lewis lung carcinoma (LL2) (Wietrzyk et al., 2001). On the other hand, it has been reported that GST and nonylphenol increase the incidence of lung adenomas and carcinomas in male F344 rats treated with five different carcinogens (Seike et al., 2003). It has been assumed that the above effect is due to the stimulation of cell proliferation and DNA damage caused by oxygen radicals (Seike et al., 2003).
Dietary supplementation with isoflavones reduced primary tumor growth and pulmonary metastasis of B16BL6 murine melanoma cells in C57BL/6 mice. Tumor size and the number of lung metastases of melanoma cells were significantly decreased compared to the control values (Li et al., 1999). The antitumor and antiangiogenic effects in vivo of GST, applied alone or in combined therapy with cyclophosphamide, have been shown in the B16 melanoma model (Wietrzyk et al., 2001).

16.6 SUMMARY AND CONCLUSIONS

Introduction of scientific measurement for intake of nonnutritional dietary components and quantification of their pharmacological action provided the first step for investigation of medicinal (prophylactic as well as therapeutic) potential of PE and other secondary plant metabolites present in the human diet. Although our knowledge of the chemical and analytical characteristics of individual PE has already been improved, our knowledge of their multiple targets of biological action, pharmacokinetics, and metabolism is still rather poor. Not surprisingly, there are no Daily Recommended Intake values for phytochemicals in general.

The question whether PE may be used as anticancer therapeutic and/or chemopreventive agents remains unanswered. It is generally agreed that much more information has to be provided, especially concerning the safety of their use. It seems very difficult to predict the effects of supplementation of human diets with PE mixtures. Long-term studies (in vitro, animal, clinical, and epidemiological) with well-standardized PE preparations are necessary to establish the potential beneficial and adverse effects for healthy subjects and HRT users. Also, concerning high risk patients, with current knowledge, responsible judgment as to whether consumption of soy or SIF-supplemented food or use of particular isoflavones as experimental therapeutics will have positive, null, or even adverse effects on cancer (particularly, steroid hormone-dependent) risk and treatment is extremely difficult.

PE are supposed to be specifically useful in prevention of breast cancer. Although most animal studies have shown cancer-preventive effects, recent studies also suggest that soy phytoestrogens may stimulate breast cancer cell growth under certain circumstances. Thus, until safety with respect to breast cancer is established, phytoestrogen supplements should not be recommended, particularly for women at high risk of breast cancer (Kurzer, 2003).

REFERENCES


Carcinogenic and Anticarcinogenic Food Components


17 Diet and Cancer Prevention: Current Knowledge and Future Direction

Elizabeth H. Jeffery and John A. Milner

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17.1 INTRODUCTION: DIETARY FACTORS ASSOCIATED WITH CANCER PREVENTION

It is now more than two decades since Doll and Peto suggested that dietary choices might modulate cancer risk (Doll and Peto, 1981). Whereas their assumption was
based chiefly on epidemiological findings, evidence from a variety of sources during the past 20 years has largely substantiated the idea that diet can significantly influence cancer incidence and tumor behavior. Detailed mechanistic studies have identified multiple effects for a number of individual food components on various cancer-related processes (Figure 17.1). However, the absolute response to these bioactive food components is likely dependent on multiple factors, including a host of dietary and environmental interactions as well the genetic profile of the consumer. Thus, it is logical to expect considerable variation, as is evident in the published literature.

Overall it is becoming increasingly recognized that a diet rich in fruits and vegetables is linked with decreased risk for a broad range of cancers (Steinmetz and Potter, 1996). In addition, increased consumption of whole grains and dietary fiber may also provide protection from a number of cancers (Jacobs et al., 1998). Basic research into possible mechanisms, using purified bioactive food components in both cell culture models and transgenic/knockout animals, is beginning to identify those who might benefit most from dietary intervention and potentially any that might be placed at risk from dietary change. Other chapters in this volume review in detail some of the anticarcinogenic food components that have been identified to date and arise from fruits, grains, vegetables, herbs, and spices. This chapter is aimed at providing the reader with a global perspective on the importance of the total diet, factors that need to be considered when evaluating individual dietary components, and why individuals may vary in their responses to foods and their constituents.

The finding that foods contain bioactive components that may be responsible for health effects has spawned three key areas of research and development:

1. The use of purified bioactive components or analogs as cancer preventative drugs
2. The use of concentrates, extracts, or partially purified bioactive components as dietary supplements and potential adjuvant therapy
3. The use of whole foods containing bioactive components as part of a diet to protect against cancer

Examples exist in which the isolated component does not provide the same biological response as the more intact preparation, indicating that the food matrix may be important in determining bioavailability or in providing additional components that
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may increase or decrease the response (Amagase and Milner, 1993; Thiagarajan et al., 1998; Boileau et al., 2003). Undeniably, more research is needed to evaluate the impact of the food matrix on the response to bioactive food components.

The study of lycopene is an example of the types of challenges faced by producers, scientists, regulators, and consumers. Positive findings about a possible relationship between plasma lycopene, reflective of a diet rich in tomato products, and a lowered risk for prostate cancer have been surfacing (Miller et al., 2002). Despite the many encouraging cell culture and animal studies, the five clinical trials and several case reports conducted lately do not provide conclusive evidence as to whether dietary lycopene can or cannot slow and/or prevent prostate cancer (Kristal, 2004). Nevertheless, lycopene, purified originally from the tomato, continues to be under study for possible prevention and/or treatment of prostate cancer, and a “Lycored” extract from tomato is available on the market as a dietary supplement and food ingredient for consumers (Etminan et al., 2004).

The challenge in the tomato/lycopene field, as with the field of functional foods in general, is to understand how to translate epidemiological findings to mechanistic studies, mechanistic studies to clinical studies, and finally clinical trials to detailed information on the use of whole functional foods and isolated dietary bioactive components for improved health (Figure 17.2). Today, there are a number of examples where exciting research is accumulating and linking hypotheses based on epidemiological studies of diet and cancer risk to biochemical mechanisms of action of isolated bioactive components in cell culture studies. In many cases, confirmation of mechanisms in animal model feeding studies is also underway. However, the translation of these findings to clinical settings with proven effects lags far behind. It is becoming increasingly obvious that short- and long-term intervention studies need to receive greater attention in the coming decade to help provide the public with detailed information on cancer prevention and use of functional foods. The real dilemmas are:

At what point does information become sufficient for people to consider changing their dietary habits?

What criteria are needed to indicate the likelihood of response to populations or individuals?

When should industry begin to make such products available?

A program of discovery, development, and delivery that seeks to promote the translation of bench science into clinical settings, as proposed by the National Cancer

---

**FIGURE 17.2** From discovery to delivery of bioactive food components.
Institute, should enhance the understanding of the role that diet and dietary components have in cancer prevention (Greenwald, 2002). As the discovery of the precise sites of action of bioactive food components in the cancer process is revealed it will become increasingly important to accelerate the development of interventions, new technologies, and strategies through translational research. Finally, effective collaborations will need to be developed to assist in the delivery of these interventions in the appropriate clinical and/or public health programs. While the translation of basic research into practical interventions will not be simple, the return on investment in terms of health and disease prevention will surely be indisputable.

17.2 ADOPTING A HEALTHIER DIET

A diet low in fatty meat, yet high in fruits, vegetables, and whole grains, is proposed to delay or prevent cancer and other chronic diseases. The merits of such dietary habits were recently demonstrated in a study in which people who adhered to this type of diet, as recommended by the American Institute for Cancer Research, independently and in conjunction with not smoking, had substantially reduced cancer incidence and, to a lesser degree, cancer mortality (Cerhan et al., 2004). Unfortunately, most individuals continue to eat relatively few fruits and vegetables; the average American, for example, eats between three and four servings a day (Johnston et al., 2000). This low intake may be partly due to the fact that obtaining calories from calorie-dense foods high in fats and refined sugars is cheaper than obtaining similar calories from foods that are lower density, such as vegetables (Drewnowski and Specter, 2004).

Throughout the world, as societies have increased in wealth, they have tended to change from rural to urban living, from manual labor to deskwork, from vegetable protein to high-fat meat protein, and from complex carbohydrates to refined sugars (Dirks, 2003). Persuading individuals to modify their diets appears to be a substantial challenge, although hopefully not an insurmountable problem. Fresh, high-quality fruits and vegetables that are flavorful are needed, as are clear instructions as to how much is needed and in what manner foods can be prepared to provide optimal health benefits. Although producers and grocers have the technologies to significantly modify the quality of foods that are available, the science lags behind in establishing what specific dietary components are needed to slow or prevent chronic disease, including cancer. Unfortunately, far too often proclamations are made about the health benefits of specific foods and components without appropriate evidence. Such widespread assertions lead to mistrust in those associated with the food/dietary supplement industry and also to confusion among consumers about what an individual should do to promote health.

Some individuals change their diets when diagnosed with cancer. Unfortunately, these changes may be too little, too late, or even inappropriate. Although some of these changes may be beneficial, it is becoming increasingly clear that some dietary bioactive components that are associated with prevention may have no therapeutic effect or even interact negatively with chemotherapeutic drugs (Norman et al., 2003). To successfully impact world health, the field of cancer prevention should provide scientific evidence-based data about appropriate dietary change that needed to opti-
mize health for the general public and, where possible, specific information for tailored approaches to meet individual needs that incorporate lifestyle and genetic profiles. The challenge is for food scientists, nutritionists, cancer biologists, and others to collaborate to identify those who will benefit from consumption of specific foods and food components and those who might be placed at risk because of a dietary change.

17.2.1 Caloric Intake and Cancer Risk

Obesity has increasingly been identified as an epidemic within the United States, as well as in many locations throughout the developed world. It is rapidly overtaking smoking as the leading healthcare factor in the United States today (Stein and Colditz, 2004). Currently in America, over 65% of all adults are considered overweight; about half of whom are considered obese. Excess calories are associated with not only increased cancer at a number of sites (Carroll 1998; Key et al., 2004), but also with a variety of other conditions including type II diabetes, hypertension, coronary heart disease, gallbladder disease, and osteoarthritis (Hill et al., 2003). Historically, calorie restriction has been recognized to be associated with decreased risk for cancer (Hill et al., 2003; Hursting et al., 2003). The data are clear that overweight, defined as a body mass index (BMI) > 25, is related to enhanced cancer risk at several sites (Key et al., 2004). The mechanism(s) relating overweight to cancer risk are not clearly established but can be expected to be multifactorial, associated with both genetics and lifestyle choices, as is the case with cardiovascular disease (CVD) (Rackley, 2004).

Research continues to suggest that caloric restriction can influence several cancer processes including the mitogenic response, apoptosis, DNA repair, drug metabolism, and cell-mediated immune function (Frame et al., 1998; Hursting et al., 2003). At least part of this anticancer action associated with caloric restriction likely involves the insulin-like growth factor 1 (IGF-1) pathway (Hursting et al., 2003). While there are many areas that should be pursued to explain the effects of bioenergetics and cancer, one that likely deserves special attention is the interrelationship among calorie intake, inflammation, and cancer. Inflammation, characterized by the increased production of cytokines and subsequent elevations in reactive oxygen and nitrogen species in response to the stimuli, is a common event in epithelial cancers including cancers of the breast, prostate, colon, lung, cervix, and pancreas (Erlinger et al., 2004). About one-fourth of all cancer cases worldwide are thought to arise as a consequence of chronic inflammatory processes. For example, inflammatory bowel disease has been linked to colorectal cancer, and chronic pancreatitis has been linked to pancreatic cancer (Farrow et al., 2004; Itzkowitz and Yio, 2004).

A key enzyme, one that has been identified as indicative of precancerous/cancerous tissues and that several bioactive components are thought to inhibit, is cyclooxygenase 2 (COX-2). Upregulation of COX-2 frequently occurs in response to the transcription factor NFκB and is central to the inflammatory response, for example, in colorectal cancer (Allgayer, 2003). Changes in synthesis, translocation to the nucleus, and stability of NFκB, along with other transcription factors, may prove useful in probing the relationship between inflammation and cancer.
17.2.2 Inclusion of Specific Foods in a Healthful Diet: Fruits, Vegetables, Fish, and Grains

Evidence continues to accumulate that showcases an inverse relationship between total fruit and vegetable intake and cancer incidence at many sites (Table 17.1). It is likewise evident that considerable variability occurs in the response to these food items. Several factors may account for this variability including total calories consumed, amounts of grains and dairy products consumed, the presence and interactions of a host of bioactive food components, the consumer’s genetic background, or a combination of all.

Unfortunately in some cases the amounts of food items, such as spices, are not adequately evaluated. In other cases, although the intake of the food is adequately monitored, information about its composition is absent. The development of databases may be useful for comparing different foods but must take into consideration growing conditions, including soil and water, as well as plant genomics. Although a flavonoid database is currently under development at the U.S. Department of Agriculture (USDA), many foods have bioactivity unrelated to the content of this bioactive group. Thus, several databases that include multiple bioactive food components will logically be needed to adequately evaluate dietary exposures and ultimately the biological consequences of these exposures. Furthermore, new bioinformatic tools may be needed to integrate information about the response to multiple bioactive food components as factors that influence cancer incidence and tumor behaviors.

Teleologically, some foods should be more effective than others since the response depends on the consumer’s lifestyle and genetic background (Ferguson, 1999). Defining which foods, and under what circumstances, is the major challenge that currently exists within the scientific, public health, and regulatory communities. The literature on some foods, for example soy, garlic, green tea, broccoli, and tomatoes, is far more extensive than on others. However, the amount of research is not necessarily related to the extent or degree of their bioactivity.

One of the food groups that frequently surfaces as providing some protection against several diseases is the intake of fish and fish products. While concerns about environmental contamination should not be minimized, ecological mortality data for breast cancer from 24 European countries suggest that an inverse relationship exists between incidence of colon cancer and fish/fish oil consumption, when expressed as a proportion of dietary animal fat, and that a high dietary ratio of n-6 to n-3 fatty acids is associated with a decreased risk for colon cancer (Simonsen et al., 1998; Roynette et al., 2004). The n-3 fatty acids may function at various phases of the cancer process, through decreased tumor cell proliferation, enhanced apoptosis, enhanced cell differentiation, and retarded angiogenesis (Nkondjock et al., 2003; Roynette et al., 2004). Nevertheless, not all tissues respond identically to dietary n-3 fatty acids. A recent meta-analysis of nine cohort and case-control studies provides evidence that increased α-linolenic acid may actually increase prostate cancer risk (relative risk [RR] = 1.7) (Brouwer et al., 2004). Thus, moving research findings from a generalizable public health approach to one that addresses site-specific
### Table 17.1
Examples of Plant-Based Anticarcinogenic Foods

<table>
<thead>
<tr>
<th>Food</th>
<th>Cancer Site</th>
<th>Human Studies: RR/OR, (95% Confidence Interval), p-Value</th>
<th>References Supporting Animal Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broccoli</td>
<td>All</td>
<td>RR = 0.8 (0.4 to 1.6)</td>
<td>Colditz et al., 1985</td>
</tr>
<tr>
<td></td>
<td>Bladder</td>
<td>OR = 0.61 (0.42 to 0.87); p = 0.009</td>
<td>Michaud et al., 1999</td>
</tr>
<tr>
<td></td>
<td>Breast</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colon</td>
<td>OR = 0.18 (0.06 to 0.58)</td>
<td>Hara et al., 2003</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>OR = 0.6 (0.5 to 0.8)</td>
<td>Fontham et al., 1988</td>
</tr>
<tr>
<td></td>
<td>Stomach</td>
<td>OR = 0.60 (0.34 to 1.08)</td>
<td>Hara et al., 2003</td>
</tr>
<tr>
<td>Cabbage</td>
<td>Bladder</td>
<td>OR = 0.57 (0.33 to 0.97); p = 0.05</td>
<td>Michaud et al., 1999</td>
</tr>
<tr>
<td></td>
<td>Breast</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>RR = 0.6</td>
<td>Kvale et al., 1983</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>OR = 0.70; p = 0.05</td>
<td>Graham et al., 1978</td>
</tr>
<tr>
<td></td>
<td>Rectum</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Skin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carrots</td>
<td>Breast</td>
<td>OR = 0.56 (0.34 to 0.91)</td>
<td>Longnecker et al., 1997</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>OR = 0.45</td>
<td>Lemarchand et al., 1989</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cruciferous</td>
<td>Bladder</td>
<td>OR = 0.49 (0.32 to 0.75); p = 0.008</td>
<td>Michaud et al., 1999</td>
</tr>
<tr>
<td>vegetables (all)</td>
<td>Breast</td>
<td>OR = 0.58 (0.42 to 0.79)</td>
<td>Terry et al., 2001</td>
</tr>
<tr>
<td></td>
<td>Colorectal</td>
<td>OR = 0.85 (0.74 to 0.98)</td>
<td>Levi et al., 1999</td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>OR = 0.50; p = 0.001</td>
<td>Lemarchand et al., 1989</td>
</tr>
<tr>
<td></td>
<td>Prostate</td>
<td>OR = 0.59 (0.39 to 0.90)</td>
<td>Cohen et al., 2000</td>
</tr>
<tr>
<td></td>
<td>Stomach</td>
<td>RR = 0.7 (0.4 to 1.2)</td>
<td>Chyou et al., 1990</td>
</tr>
</tbody>
</table>
TABLE 17.1 (Continued)
Examples of Plant-Based Anticarcinogenic Foods

<table>
<thead>
<tr>
<th>Food</th>
<th>Human Studies: RR/OR(^a), (95% Confidence Interval), p-Value</th>
<th>References</th>
<th>Supporting Animal Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garlic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>OR = 0.7 (0.4 to 1.1); p &lt; 0.01</td>
<td>Levi et al., 1993</td>
<td>Schaffer et al., 1996</td>
</tr>
<tr>
<td>Colon</td>
<td>OR = 0.42 (0.2 to 0.9); p &lt; 0.001</td>
<td>Iscovich et al., 1992</td>
<td>Cheng et al., 1995</td>
</tr>
<tr>
<td>Lung</td>
<td>OR = 0.93 (0.5 to 1.9)</td>
<td>Dorant et al., 1994</td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>OR = 0.47 (0.31 to 0.71); p &lt; 0.01</td>
<td>Hsing et al., 2002</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>OR = 0.89 (0.6 to 1.2)</td>
<td>Hansson et al., 1993</td>
<td>Sparnins et al., 1988</td>
</tr>
<tr>
<td>Tea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>RR = 0.57 (0.33 to 0.98) F</td>
<td>Imai et al., 1997</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RR = 0.68 (0.39 to 1.21) M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>OR = 0.53 (0.35 to 0.78); p = 0.001</td>
<td>Wu et al., 2003</td>
<td>Kavanagh et al., 2001</td>
</tr>
<tr>
<td>Colon</td>
<td>OR = 0.59 (0.35 to 1.00)</td>
<td>Su and Arab, 2002</td>
<td>Jia and Han, 2000</td>
</tr>
<tr>
<td>Esophagus</td>
<td>OR = 0.50 (0.30 to 0.83); p = 0.01</td>
<td>Gao et al., 1994</td>
<td>Morse et al., 1997</td>
</tr>
<tr>
<td>Lung</td>
<td>OR = 0.65 (0.45 to 0.93)</td>
<td>Zhong et al., 2001</td>
<td>Chung et al., 1998</td>
</tr>
<tr>
<td>Stomach</td>
<td>OR = 0.39 (0.1 to 1.01); p = 0.048</td>
<td>Setiawan et al., 2001</td>
<td>Yamane et al., 1995</td>
</tr>
<tr>
<td>Tomato</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>OR = 0.5</td>
<td>Bidoli et al., 1992</td>
<td>Narisawa et al., 1998</td>
</tr>
<tr>
<td>GI (upper)</td>
<td>OR = 0.30</td>
<td>De Stefani et al., 2000</td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>RR = 0.77 (0.66 to 0.90); p &lt; 0.001</td>
<td>Giovannucci et al., 2002</td>
<td>Boileau et al., 2003</td>
</tr>
<tr>
<td>Rectum</td>
<td>OR = 0.4</td>
<td>Bidoli et al., 1992</td>
<td></td>
</tr>
<tr>
<td>Soy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>OR = 0.66 (0.46 to 0.95); p = 0.28</td>
<td>Dai et al., 2001</td>
<td>Gallo et al., 2002</td>
</tr>
<tr>
<td>Prostate</td>
<td>OR = 0.51 (0.28 to 0.95)</td>
<td>Lee et al., 2003</td>
<td>Landstrom et al., 1998</td>
</tr>
<tr>
<td>Spinach</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>OR = 0.56 (0.34 to 0.91)</td>
<td>Longnecker et al., 1997</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>OR = 0.45</td>
<td>Rijken et al., 1999</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) RR = relative risk; OR = odds ratio.
action(s) of individual food components will be critical for developing personalized dietary intervention strategies.

Several types of dietary fatty acids may actually modify cancer risk and tumor behaviors. Fatty acid structures typically exist in foods as \( cis \) isomers; however, \( trans \) isomers are found in the diet largely as a result of partial hydrogenation of vegetable oils. Although data in humans are limited, the intake of \( trans \) fatty acids has been suggested to promote tumorigenesis (Bakker et al., 1997). The Euramic Study (European Community Multi Center Study on Antioxidants, Myocardial Infarction, and Breast Cancer) reported a positive association between \( trans \) fatty acids in adipose tissue and the incidence of cancers of the breast and colon, but not of the prostate, again raising issues about tissue specificity in response to dietary components. Likewise, it is unclear whether \( trans \) fatty acids are all equivalent in bringing about a biological response. For example, conjugated linoleic acids (CLA) have been reported to be protective against some cancer models (Belury, 2002). It is unclear how to interpret these results, since a weak positive association was found between CLA intake and the incidence of breast cancer (RR = 1.24) (Voorrips et al., 2002). Overall, the effect of \( trans \) fatty acids on cancer remains an area of active investigation and of considerable controversy.

Refined, white flour has been known for many years to lack the tocopherols and carotenoids of whole grains since most nutrients located in the germ, or embryo, and husk or bran is lost (Slavin et al., 2001). More recently, studies have refocused on whole grains, finding a relationship between their intake and decreased risk for colorectal cancer and identifying that there are many more bioactive food components within whole grains than simply the antioxidant vitamins (Jacobs et al., 1995; Slavin et al., 2001). Evaluation of the flavonoid levels of different plant parts have documented that flavonoids are present in the peel of citrus (Manthey and Grohmann, 2001), the hull of cocoa beans (Richelle et al., 2001), and the skin of apples (Eberhardt et al., 2000), in addition to the bran of grains (Adom et al., 2003). These hulls, or bran, are also rich in fiber, metabolized by the colonic microflora to produce short-chain fatty acids, including butyrate, that appear to support a healthy colon and may be responsible at least in part for the decreased risk for colon cancer in those eating whole-grain foods (Augenlicht et al., 2002).

### 17.2.3 Tools and Biomarkers for Changing to a Healthier Diet

The tools that pharmacognostists have used for drug discovery have proven useful for identifying bioactivity-directed fractionation of extracts from a range of functional foods including resveratrol from wine/grapes (Waffo-Teguo et al., 2001) and sulforaphane from broccoli (Zhang et al., 1992). However, some foods have proven refractory to such approaches since the biological activity is lost during fractionation, whereas other foods may yield a broad spectrum of bioactive components, making the identification of truly active components exceedingly complicated. To determine how bioactive food components are effective as part of a cancer-preventive diet, it becomes necessary to compare the effects of reassociated fractions, to confirm
bioavailability, and to determine whether the effect can be gained by consuming reasonable dietary levels of the food.

The cancer process involves a wide range of cellular pathways that can be influenced by diet, including carcinogen bioactivation, cellular differentiation, DNA repair, cellular proliferation/signaling, and apoptosis. Recently, six critical elements were suggested as hallmarks of cancer: self-sufficiency in growth signals, insensitivity to antitumor signals, tissue invasion and metastasis, limitless replication potential, sustained angiogenesis, and evasion of apoptosis (Hanahan and Weinberg, 2000). Evidence already exists that each of these “elements” can be modified by specific dietary components and/or by caloric restriction. Nevertheless, more attention is needed to verify the physiological significance of diet-induced alterations in specific biomarkers that reflect efficacy on each of these elements.

These efficacy biomarkers will be key to monitoring and building incentives for promoting changes in dietary habits. Unfortunately, the number of biomarkers of efficacy against which an individual can gauge success in changing his/her dietary habits remain woefully inadequate. For cardiovascular disease, blood cholesterol has historically served as such a biomarker. Blood cholesterol is monitored by many with clear indications from physicians and other health professionals about dietary strategies that may be useful to lower its level toward a healthy range, often using the Step One diet or the American Heart Association’s dietary guidelines (Krauss et al., 2000). Few such biomarkers exist for monitoring and evaluating the influence of diet on cancer risk or tumor behavior, and thus guidelines cannot be as detailed or personalized. The one example that remains most compelling for reducing cancer risk is to maintain a BMI at or below 25. Cancer-specific or process-specific markers are much more challenging to establish.

Many publications are appearing that highlight changes in biomarkers without a clear indication whether the response is primary or secondary to other changes, or most importantly whether a change in the biomarker has any long-term implications in the progress or prognosis of the disease. For example, while prostate-specific antibody (PSA), has been evaluated for its relationship to dietary intakes in patients already diagnosed with prostate cancer, little information exists about the impact of diet and risk for prostate cancer using this as a bio- or surrogate marker. Evidence that a diet high in tomato products lowers the presurgical PSA value in men with prostate cancer is already available, although an appropriate control group was not available and thus confounds interpretation of this study (Bowen et al., 2002; Kristal, 2004). Nevertheless, one must question whether this change is a transitory change or one that has a lasting effect on the progression of the cancer. Recently, a diet low in fat and increased in fruits, vegetables, and fiber was reported to have no impact on serum PSA levels in men (Shike et al., 2002). The study also offers no evidence that this dietary intervention, over a 4-year period, affected the incidence of prostate cancer during the 4 years.

Similarly, shifts in the urinary production of estrogen metabolites may be another biomarker, especially for evaluating the influence of dietary intervention strategies on hormone-sensitive cancers. Studies increasingly indicate that dietary indole-3-carbinol (I3C) prevents the development of estrogen-enhanced cancers including breast, endometrial, and cervical cancers (Auborn et al., 2003). Animal
studies reveal that the urinary ratio of 2- to 16α-hydroxy estradiol is increased when rats are given the carcinogen dimethylbenzanthracene plus a crucifer diet, compared to the carcinogen alone (Bradlow et al., 1995). Women with breast cancer frequently have a lower 2- to 16α-hydroxy estradiol ratio. Interestingly, women fed a high crucifer diet had an altered urinary profile, with an increase in 2- to 16 α-hydroxy estradiol ratio. Whereas these data do not prove that a diet-induced elevation in the 2- to 16α-hydroxy estradiol ratio will have an impact on breast cancer risk, this is certainly an area worth further investigation, especially if it can be linked to true endpoints. In rodents, a shift in estrogen metabolism is not always correlated with a change in chemically induced tumors, suggesting that I3C may have its impact on normal cells prior to their exposure to a carcinogen (Zhang and Malejka-Giganti, 2003). Other markers that have been used and could be further developed are the shift in metabolites or adducts from cigarette carcinogens following a watercress meal (Hecht et al., 1995) and a shift in excretion of the heterocyclic amine 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) from a roast meat meal following consumption of Brussels sprouts and broccoli (Walters et al., 2004).

The attractiveness of developing and using biomarkers will likely continue to expand in the coming years and will likely incorporate new technologies in cellular and molecular biology. Whereas these indicators may provide the correct answers about intervention strategies and their effects, the uncertainty of their relevance to the cancer process may make many surrogate measurements problematic. Unfortunately, until clear outcome biomarkers exist it will be virtually impossible to generate models for individuals to use to predict and evaluate their success in changing dietary habits. Being on the causal pathway to cancer is in itself inadequate to constitute validity of a surrogate in cancer prevention. It is the totality of causal connections that is critical. Thus, multiple rather than single biomarkers will, in all likelihood, be critical for evaluating the significance of dietary change. Furthermore, the inherent limitations with the use of biomarkers or surrogate endpoints reaffirm the need to continue to conduct large clinical intervention studies with explicit cancer endpoints.

17.3 DIETARY REQUIREMENTS FOR VITAMINS AND MINERALS IN CANCER PREVENTION

A number of nutrient deficiencies have been related to increased cancer risk, including deficiencies of selenium and folic acid (Ames and Wakimoto, 2002). Very low dietary levels of vitamins have long been associated with deficiency diseases such as scurvy and beriberi, giving rise to Recommended Dietary Allowances (RDA) that have relatively good agreement around the world. Yet even in the United States, as many as 50% of adult women may not receive even 50% of the RDA for folate in their diets, and many other population groups are consuming less than optimal amounts of many vitamins and minerals (Ames and Wakimoto, 2002). Ames (2004) has suggested that approximately 50 human genetic diseases result in a poorer binding affinity (Km) of the mutant enzyme for its B vitamin coenzyme, and may thus be remedied by feeding high-dose B vitamins.
Over the last 10 or 15 years, it has become evident that, although incompletely defined at this time, vitamins may function in preventing several chronic diseases, including cancer. Deficiencies of a number of nutrients have been found to be associated with DNA damage, providing a link between nutritional needs and cancer prevention. Cell culture and animal studies have identified a number of metabolic effects apparently unrelated to those of the classic vitamin actions and typically requiring greatly elevated doses. Niacin, for example, lowers cholesterol when present at a 100- to 1000-fold greater dose than the RDA. Unfortunately, in the area of cancer prevention, increasing vitamin levels in the diet has not proven the panacea that was expected just a few years ago (Giacosa et al., 1997).

Research into the mechanism of action of vitamins in the cancer process is still largely in its infancy but now has the hurdle of understanding ineffective and even negative trials, such as the α-tocopherol, β-carotene trial, or ATBC trial, that found β-carotene enhanced slightly the appearance of lung cancer in smokers. Together with these discouraging results, there is concern that vitamin megadoses may be associated with toxicity. In the United States, the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences, recently updated advice on RDAs and included information on recommended tolerable upper intake levels (UL), defined as “the highest level of nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals in the general population. As intake increases above the UL, the risk of adverse effects increases.” Thus with nutrients, as with all compounds foreign to the body, the physiological response varies with dose and duration of exposure. Many of the “antioxidant” vitamins appear to share a common series of dose-dependent effects, although the exact dose ranges for efficacy and toxicity will vary among individuals (see Figure 17.3). A few examples of vitamins and minerals in common use at higher than RDA levels are discussed in more detail, in order to address some of the key concerns and research needs in this area.

**FIGURE 17.3** Physiological effects of antioxidant vitamins: dose dependency and individual variability.
17.3.1 **Vitamin E**

RRR-α-tocopherol (2,5,7,8-tetramethyl-2R-(4’R, 8”R, 12” trimethyltridecyl-6-chromanol), also known as vitamin E, functions primarily as an antioxidant. Because many chronic diseases are associated with oxidative damage, it has been proposed that dietary vitamin E levels greater than the RDA may protect against abnormal bursts of peroxidative damage, possibly related to the pathogenesis of chronic diseases such as cancer. Of four published large CVD prevention trials, only one showed protection by vitamin E (400 or 800 IU RRR-α-tocopherol) (Stephens et al., 1996). Similarly, data are not consistent in showing vitamin E protection against other chronic diseases such as diabetes. The data evaluating protection against cancers are also inconclusive. In the ATBC trial, the primary endpoint (lung cancer) was unaffected by vitamin E status, although in the same study, prostate and renal cancers appeared decreased in those receiving vitamin E (50 mg/day) (Heinonen et al., 1998). The positive results relating vitamin E status to prostate cancer were instrumental in including vitamin E in the SELECT trial, an ongoing randomized, prospective double-blind study to evaluate the effects of vitamin E (400 mg/day) and selenium in the prevention of prostate cancer (Klein et al., 2003). Recently, significant interest has developed in vitamin E succinate (VES) as a cancer preventive agent. Preliminary data suggest that VES, which is not redox active, selectively causes apoptosis in cancer cells prior to metabolism to vitamin E (Neuzil, 2003). It will be interesting to see how this field develops since the response may relate to a number of cellular events that may be independent of its antioxidant properties.

17.3.2 **β-Carotene and Related Compounds**

Carotenoids include both vitamin A precursors (α- and β-carotene and β-cryptoxanthin) and several additional plant, algal, fungal, and bacterial products, such as lycopene, lutein, and zeaxanthin. Plasma β-carotene is used as a measure of dietary fruit and vegetable intake (e.g., Hak et al., 2004). Over the years, the idea has surfaced that β-carotene might be causative in explaining the decrease in cancer incidence reported for diets high in fruits and vegetables (Doll and Peto, 1981). For example, a recent study of prostate cancer found that in younger men, diets “rich in β-carotene” may protect against prostate cancer (Wu et al., 2004). Yet clinical findings are inconclusive (Clarke and Armitage, 2002; Greenwald, 2003). Two of three major lung cancer prevention trials evaluating a role for supplemental β-carotene (20 or 30 mg/day or 50 mg alternate days) were abandoned because the β-carotene arm had a greater incidence of cancer. The third (the Physicians’ Health Study) showed no effect of β-carotene on the primary endpoint, lung cancer. While more information about β-carotene is needed, it is also likely that other compounds occurring in fruits and vegetables may have contributed to their links with health.

Preclinical evidence continues to accumulate that tomatoes and tomato products rich in lycopene may slow or prevent prostate cancer (Miller et al., 2002). Lycopene does appear to accumulate preferentially in a few tissues, including prostate. The possible mechanism(s) of action of lycopene in promoting health, other than as an antioxidant, are unclear. *In vitro* studies with lycopene suggest that, while it is not
oxidized to retinol, some 15, 15'-dioxygenase oxidation products may have bioac-
tivity similar to that of retinoids (Nagao, 2004). As with β-carotene, clinical studies
do not reflect the encouraging preclinical results, and there is insufficient evidence
to support a protective role for lycopene at this time (Kristal, 2004). Regardless,
considering the possibility of additional bioactive components within tomatoes, the
fact that plasma lycopene decreases and that risk for prostate cancer increases with
age, a recommendation of five servings of a tomato product per week for improved
prostate health among older men may prove of benefit (Miller et al., 2002).

### 17.3.3 Folate

Folate deficiency has been linked to a number of adverse health effects, including
an increased risk for several cancers (Ames and Wakimoto, 2002). Conversely, folate
supplementation is associated with a significant decrease in risk for colon cancer.
Folate deficiency is associated with an increased incorporation of uracil, in place of
thymidine, into DNA, which is associated with increased instability of DNA and
chromosomal breaks. This is reversed by increasing dietary folate, although no
greater effect is seen with amounts greater than the RDA. There are a large number
of single nucleotide polymorphisms associated with folate metabolism, particularly
methylenetetrahydrofolate reductase (MTHFR) (see Section 17.4 below). Because
of this, some studies of human folate deficiency/sufficiency are confounded. Animal
studies, where genetic differences are controlled, can show clearly the effect of folate
deficiency for a single phenotype. However, as our knowledge grows of the preva-
lence of polymorphisms and the role they can play in development of chronic
diseases such as cancer, it behooves us to ensure that animal models used to deter-
mine dietary needs to minimize risk for cancer, whether they are outbred, inbred,
or transgenic models, correctly reflect human phenotypes.

### 17.3.4 Selenium

More than three decades ago, evidence was provided that increasing dietary selenium
(Se) consumption might be a potent deterrent to cancer (Shamberger, 1970; Schrau-
zer, 1976). Impressively, the protection associated with greater selenium intake is
not limited to a single tumor site, but appears experimentally in the breast, prostate,
lung, colon, pancreas, and liver (Milner, 1986; Combs, 2001). Various forms of
selenium can alter carcinogen metabolism and DNA adduct formation, and influence
cell division (Fico et al., 1986; El-Bayoumy and Sinha, 2004). Some of the most
compelling evidence that diet has an influence on cancer comes from studies by
Clark and colleagues (1996). While this study was designed to examine the effect
of selenium-enriched yeast on skin cancer, which was not influenced, a secondary
analysis of data indicated that supplementation was associated with significant
depressions in lung, prostate, and colon cancer.

Recent studies using microarray analysis indicate that the expression of many
genes, perhaps as many as 2500, can be influenced by supplemental selenium (Dong
et al., 2002; 2003). These genes are known to be involved with DNA damage
processing, oxidative stress, and cell-cycle control, which suggests that multiple
targets for selenium probably exist. In precipitating so many changes, the impact of selenium is likely amplified over and over again. Bioinformatics will be increasingly needed to analyze the functional significance of selenium and other bioactive food components in terms of the phenotypic responses that arise because of nutrigenomic, proteomic, and metabolomic effects (Davis and Milner, 2004).

17.4 RELATIONSHIP BETWEEN FOOD AND GENETIC MAKEUP

Providing individuals with the incentive to improve their diets should be based on their genetic makeup and/or on their genetic–environmental interaction. People across the globe are considered to have very similar nutrient needs for normal development and body function, allowing the calculation of RDAs. However, the ability of bioactive food components to delay or prevent chronic diseases such as cancer may vary considerably from person to person because the response depends on both genetic makeup and environmental exposure (Chadwick, 2004; Davis and Milner, 2004; Mathers, 2004).

Genetic diversity and personal environmental exposures likely account for the wide variation in risks for cancers throughout the world. These factors may also be responsible for greatly differing responses to dietary intervention. As described in the preceding chapters, dietary preventive components appear to act through a variety of mechanisms, suggesting that their efficacy in preventing cancers may vary depending upon the molecular lesion responsible for a particular cancer and the multitude of factors that influence phenotypes. Furthermore, nutrikinetics (the rate and extent of absorption, distribution, metabolism, and excretion of a given dietary component) will vary across individuals, and with the formulation/processing of the diet. Thus, an individual dietary component may be more potent and/or effective for some people than for others because of variation in the expression of genes associated with transport proteins, receptors, catabolic enzymes, etc. For these reasons, it becomes clear that a single approach to dietary intervention for prevention of cancer will not work for all; approaches will need to vary among individuals and be multifactorial. To determine which dietary intervention would be most appropriate for a specific individual, or even how much of a food should be consumed and how frequently, requires individual DNA genotyping and knowledge about DNA and other epigenetic events and how these lead to transcriptional changes that influence formation of specific proteins and small molecular weight molecules. Such information may ultimately provide important information about effective biomarkers that will identify those who might benefit or be placed at risk because of dietary strategies.

New technologies are helping to answer many questions about responders and nonresponders. With the completion of the Human Genome Project, together with the identification of a number of genetic polymorphisms that modulate risk for cancer, individual genetic profiling and resulting personalized risk evaluation will soon be commonplace. The field of nutrigenomics is rapidly developing, founded in basic research that identifies specific nutrient–gene interactions that can be utilized to decrease risk for individuals where known polymorphisms are associated with an
enhanced risk for cancer (Davis and Milner, 2004; Sharp and Little, 2004; Slattery et al., 2004). To determine whether nutritional intervention is effective, biomarkers of phenotypic change are being developed. Biomarkers can be identified through a comprehensive evaluation of the proteome and/or metabolome, typically evaluating samples of plasma and urine available through relatively noninvasive methods (Go et al., 2003). The integration of nutritional genomics, proteomics, and metabolomics will permit the optimization of anticarcinogenic diets for lowering cancer risk in individuals (see Figure 17.4). Some specific examples of polymorphisms that impact upon cancer risk and that exhibit nutrient–gene interactions follow. These findings suggest that dietary intervention may be a more successful strategy for prevention of cancer among some genotypes than others.

17.4.1 Glutathione S-Transferase (GST)

The superfamily of glutathione S-transferase enzymes includes a number of common polymorphisms that influence the phenotype (approximately 50% of the Caucasian U.S. population is GSTM1 null, about 20 to 25% are GSTT1 null). Such variation has an impact on xenobiotic metabolism, considered as the primary role for this family of proteins. This polymorphism has been linked to altered risk for cancer at a number of sites, including the lung, cervix, and breast (Zhao et al., 2001; Fowke et al., 2003; Sharma et al., 2004). In a study evaluating the interaction between GSTM1-null genotype, broccoli intake, and colon cancer, broccoli preferentially protected the GSTM1-null group (Lin et al., 1998). Similarly, the risk for lung cancer among Chinese women living in Singapore was more profoundly lowered by a diet rich in cruciferous vegetables if they were GSTM1 and/or GSTT1 null than if they expressed GSTM1 and GSTT1 enzymes (Zhao et al., 2001). It has been suggested
that this response might occur because isothiocyanates (ITC), the bioactive compo-
nents in cruciferous vegetables, are metabolized by glutathione conjugation, and
individuals missing GST enzymes metabolize and excrete ITC more slowly (Seow
et al., 1998). However, in several studies, the interaction between GST genotype
and dietary crucifer intake did not appear to be significant, even when there was an
overall beneficial effect of a high crucifer diet on breast and lung cancer incidence
(Spitz et al., 2000; Fowke et al., 2003).

The GST enzymes have roles other than as detoxification enzymes, which may
be equally important in cellular biochemistry and phenotypic cancer risk develop-
ment. GST enzymes of the alpha class (GSTA1 in humans) act as peroxidases,
supplementing the action of glutathione peroxidase and preventing oxidative dam-
age; GSTPi has recently been shown to bind and suppress c Jun NH2-kinase (Elsby
et al., 2003; Gate et al., 2004). Overexpression of GSTPi, previously used as an
early biomarker for foci of transformed cells, may have the potential to inhibit
apoptosis and permit the proliferation of tumor cells through suppression of Jun
kinase (Elsby et al., 2003). Most epidemiological studies evaluating the interaction
between diet and cancer incidence report a decrease of no greater than approximately
0.6 in RR for the highest compared to the lowest intake group, irrespective of the
dietary component under study (see Table 17.1). However, when the interaction
between environmental risk (smoking) and genetic risk (GSTM1-null) was evaluated
for incidence of lung cancer, a threefold change in RR was seen with the change in
diet. Thus, individuals with multiple risk factors may experience a greater impact
from beneficial nutrient–gene interactions.

Observations that individual variation in GST expression suggest GST genotype
alone is not an accurate predictor of GST expression (Tempfer et al., 2004). What
is clear is that GST expression is highly tissue-specific, and individual expression
can vary considerably even in normal tissue. Thus, populations with the same
genotype are actually heterogeneous in expression. Since GST induction is not
independent of genotype, the quantity of individual bioactive food components and
the methods used to prepare the food source may play a key role in the phenotypic
response associated with this enzyme (Lampe and Peterson, 2002).

17.4.2 GLUTATHIONE PEROXIDASE

Glutathione peroxidases (GPx) are known for their ability to reduce organic and
inorganic hydroperoxides in mammalian cells (Sunde, 1990). The family of glut-
athione peroxidases comprises four different selenoperoxidases including cytosolic
 glutathione peroxidase (cGPx), phospholipid hydroperoxide glutathione peroxidase
 (PHGPx), gastrointestinal glutathione peroxidase, and plasma glutathione peroxidase.

There is a common polymorphism for glutathione peroxidase 1 (GPx-1), where
a proline is replaced by a leucine. The presence of the leucine allele of GPx-1 has
been correlated with increased lung (Ratnasinghe et al., 2000) and breast cancer risk
(Hu and Diamond, 2003). Part of the explanation for the link between this polymor-
phism and cancer may relate to observations that the allele directing synthesis of
leucine at position 198 of glutathione peroxidase is less responsive to selenium than
the proline-containing protein (Hu and Diamond, 2003). More recently, the hetero-
geneous GPx-1 Pro/Leu genotype was found to be associated with increased bladder cancer risk compared to the homogeneous Pro/Pro genotype. Furthermore, this risk might be further modified by an Ala-9Val manganese superoxide dismutase polymorphism (Ichimura et al., 2004).

Selenium deficiency in rat basophilic leukemia cells results in a 99% loss of cGPx and a 65% loss of PHGPx activities. Interestingly, these changes are accompanied by an eightfold increase in the release of metabolites from 5-lipoxygenase, including 13-S-hydroxyoctadecadienoic acid, which has been shown to increase extracellular signal-related kinase (ERK) and decrease p21, supporting tumor proliferation. Addition of 0.25 μg/ml selenium to selenium-deficient cells decreased the amounts of lipoxygenase metabolites back to control values within 12 h, and the activities of cGPx and PHGPx in these supplemented cells were 3 and 100% of normal values, respectively. These and other results suggest PHGPx and not cGPx is responsible for silencing of 5-lipoxygenase activity (Weitzel and Wendel, 1993; Ghosh, 2004). Thus, individual glutathione peroxidases, as well as other selenoproteins, are unique in their roles and can influence a number of processes involved with cancer, including those associated with inflammatory responses. A total of 23 variant sites have been detected in PHGPx (Maiorino et al., 2003). How diet might influence each of these remains to be determined.

17.4.3 Methylene Tetrahydrofolate Reductase (MTHFR)

Homozygosity for a common polymorphism in MTHFR (677C to T), causes expression of an enzyme with decreased activity. When folate is limiting, this condition results in less substrate being reduced and a decreased availability of methyl tetrahydrofolate for production of methionine and S-adenosyl methionine, potentially causing DNA hypomethylation. This can be overcome by elevating dietary folate so that individuals with this TT genotype likely have a higher requirement for folate if they are to minimize risk for colon cancer (Marugame et al., 2003). However, increasing plasma folate above that required to meet the RDA offers no additional protection against cancer risk in individuals with the CC or CT MTHFR genotype (Marugame et al., 2003). This relationship between dietary folate, the TT genotype, and risk for cancer is also seen for acute lymphocytic leukemia. However, the TT genotype appears to enhance risk for elevated homocysteine levels, a risk factor for CVD, making genetic counseling premature until these interactions are better understood (Amouzou et al., 2004).

One-carbon metabolism, which is associated with folate, may have a pivotal role in the etiology of some cancers. In vitro studies have found that not only can folate stabilize the TT enzyme product, but riboflavin and S-adenosylmethionine can also bring about this response (Slattery et al., 1999). Polymorphisms have been identified in cytosolic serine hydroxymethyltransferase, methylenetetrahydrofolate dehydrogenase, and glutamate carboxypeptidase II, key genes involved in one-carbon metabolism, as well as polymorphisms in DNA repair mechanisms (XRCC1, XRCC2, and XRCC3), which may markedly influence the overall phenotypic response to folate (Chen et al., 2004; Han et al., 2004). Future discoveries resulting from genetic, proteomic, and metabolomic technologies will allow a more complete picture to
unfold about the dynamic interactions between polymorphisms and nutrient deficiencies or excesses and ultimately pave the way for improved dietary recommendations.

17.5 FUNCTIONAL FOODS AND DIETARY SUPPLEMENTS

Whole functional foods are recognized to contain many bioactive components, although the relative concentrations may point to one as being the most important. It is unwise to use concentration alone as the criterion for evaluating the efficacy of a food. For example, green tea contains multiple phenolics that may act as antioxidants and appear to work most effectively when consumed together rather than separately. Epigallocatechin gallate (EGCG) is the predominant component in green tea, and its pure form is currently being examined in clinical trial (Moyers and Kumar, 2004). However, the synergy among catechins in bringing about a physiological response is also evident (Williams et al., 2003). For some foods, the evidence implicating a specific component over others is less compelling or lacking. For example, processed garlic contains several bioactive compounds but it is not clear which one is the most potent, or most important, in bringing about a change in cancer incidence or tumor behavior. Different laboratories are studying particular components (Thomson and Ali, 2003; Milner, 2004). What is clear is that both lipid- and water-soluble allyl sulfur compounds are effective in retarding some chemically induced tumors, but the greatest effects on tumor proliferation are observed with lipid-soluble metabolites (Milner, 2004). Similarly, soy is thought to modulate several cancers through the presence of the isoflavones genistein and daidseine and/or their metabolites, although lignans such as enterolactone are also present in soy and may add to the hormonal activity of soy products (Thompson et al., 1991; Bowey et al., 2003).

When uncertainty exists about the identification of the bioactive component, the whole plant food may be more reflective of effects seen in epidemiological studies than a purified component, even if the amount ingested is limited by bulk. One approach is to first identify and study effects of the individual components, then combine these in the proportions found in the food that was found effective in epidemiological studies, to determine possible interactions and whether these components account for the effect of the whole food. Even where there are strong mechanistic data supporting the bioactivity of a single component from a whole food, such as lycopene from tomato or sulforaphane from broccoli, it appears that, at least in some instances, there may be health advantages obtained from the whole plant over the isolated component (Miller et al., 2002). This may be due to the presence of vitamins or other bioactive components, to the presence of components that enhance or synergize with the key component, or to plant matrix effects that improve bioavailability of the key component (Keck et al., 2003). Dietary supplements, taken as an addition to the normal diet, are frequently concentrates of plant foods rather than purified products. For this reason, they may sometimes retain the mixture of components present in the whole food and even some of the characteristics of the food matrix. However, if the supplement is a byproduct of processing, coming from a portion of the edible food or even from the waste stream, it may contain very different mixtures of bioactive components, with very different resulting bioactivities.
Another considerable cause for differences in bioactivity between different whole foods and different extracts and products is that the genetic profile and/or growing environment for the plant or animal food may vary, causing variation in the content of bioactive components (Brown, 2002). The content of bioactive components within a plant varies substantially with the plant variety, growing conditions, harvest and storage conditions, and processing methods. Research is being conducted to optimize the content of bioactive components within the plant, both by traditional means and by genetic engineering to enhance synthetic pathways or to incorporate pathways from a less popular plant food into a more popular one (Mithen et al., 2003). Growing conditions, such as stress due to salinity of the water, can up-regulate synthesis of some bioactive components (Leonardi et al., 2000), and storage conditions can modify content of bioactive components (Gunes et al., 2002). Due to so many variables, it is unlikely that growers will provide fresh fruit or vegetables with standardized contents of bioactive components. The variation among different plant foods can be seen by studying the USDA database on vitamin content of plant foods, which provides detail on variation in vitamin levels due to variety, or to cooking, or preparation such as peeling (www.nal.usda.gov/fnic/foodcomp/search/). A new project is underway to expand the USDA database to include flavonoids in plant foods (www.nal.usda.gov/fnic/foodcomp/Data/Flav/flav.html).

Articles describing the benefits of specific foods, such as blueberries or soy, often report the daily amount of that food necessary to see a health effect. Yet it is uncommon for people to eat the same foods every day. Research is desperately needed to determine whether foods or food components that appear to act through a similar mechanism can actually replace one another, so that a meal containing broccoli one day might be replaced with one containing turmeric the next, since both are thought to exert their benefits by upregulating detoxification enzymes and other proteins through a single mechanism, Nrf2-dependent up-regulation of the antioxidant response element (Morimitsu et al., 2002; Balogun et al., 2003). If this were found to be the case, substantial dietary freedom could be granted the individual, who could pick and choose among a list of foods containing anticarcinogenic agents, varying intake from day to day. This concept is consistent with the National Cancer Institute (NCI)/Produce for Better Health Foundation message to eat five to nine fruits and vegetables a day, and with the epidemiological data showing decreased risk for cancer with increased fruit and vegetable intake (Table 17.1) (Heimendinger and Chapelsky, 1996).

For some people, eating certain foods may be unpleasant. For example, “super tasters” may dislike eating strongly flavored vegetables such as cruciferous vegetables (Drewnowski, 2001). Even for those who enjoy a certain plant food, drinking multiple cups of tea or eating large servings of a specific plant food every day may not only be disruptive to normal dietary habits but may prove impossible when eating outside the home. Thus, new and novel foods may be needed to meet the sensitivities and preferences among individuals.

Dietary supplements are a broad category of products, including both concentrates of whole foods and purified components. Supplements have the potential to be far more completely characterized than whole foods, ensuring a specific dietary intake. The disadvantages are that regulations do not require characterization in this
manner and that, at least in the United States, complete safety studies or efficacy evaluations are not required, based on the legal definition of dietary supplements as foods. Since the epidemiological studies that are the foundation of this science are based on intake of whole foods, it is sometimes inappropriate to assume that a purified component, extract, or even a dried food product will provide health effects similar to those of the whole food. It is only necessary to look at the popularity of multivitamin tablets to know that it is possible for supplements to reach a significant fraction of the general public. Improving the characterization of dietary supplements, in terms of content, bioavailability, efficacy, and safety, would surely greatly enhance public health and help individuals meet their specific needs based on their genetic background and environmental exposures.

An additional issue in considering dietary prevention of cancer is whether it is effective if one improves one’s dietary habits later in life. Animal studies and epidemiological research indicate that early exposure to carcinogens might be a particularly sensitive period for breast cancer initiation (van’t Veer et al., 1989; Bhatia et al., 1996; Hilakivi-Clarke et al., 2001; Kwon et al., 2004). It is known that early postnatal exposure to genistein, the flavonoid in soy, enhances mammary gland differentiation, decreases cell proliferation, and reduces susceptibility for mammary cancer in adult animals (Lamartiniere, 2002). Whereas consumption of a high-soy diet has been linked to the substantially lower breast cancer risk in Asian countries as compared to Caucasian women, recent findings suggest that soy does not reduce breast cancer risk if consumed only in adulthood (Wu et al., 2002). Evidence also exists that timing of caloric restriction may impact the influence that calorie restriction has on risk for breast cancer in humans (Michels and Ekbom, 2004).

Mammary tissue is not unique in its temporal response to food components. Studies have revealed that rats are particularly sensitive to CLA during the postweaning and pubertal periods of life (Ip et al., 1995). Also, data indicate that age has a significant role to play in the ability of curcumin, a naturally occurring dietary antioxidant and antiinflammatory agent, to inhibit colon cancer in rodents (Kwon et al., 2004). Historically, developing mammary tissue has been found to be more responsive to some carcinogens than tissue from mature animals is (Warner and Warner, 1975). However, the aging response appears to be highly dependent on the tissue. For example, urinary bladder hyperplasia was less severe when young mice were treated with 2-acetylaminofluorene than when mid-aged or old mice were treated (Greenman et al., 1987). Overall, much more attention needs to be given to the influence of time of exposure to bioactive food components as a factor in accounting for variability in response. Last-minute attempts to correct inappropriate dietary habits may be without utility and may, in some cases, even bring about undesirable effects.

17.6 ESTABLISHING DIETARY INTERVENTIONS TO REDUCE CANCER IN THE GENERAL POPULATION AND AMONG INDIVIDUALS

The prevention of cancer, through diet or through any other means, has developed slowly compared to prevention of CVD. During the latter half of the last century,
Carcinogenic and Anticarcinogenic Food Components

age-adjusted deaths per 100,000 due to CVD have decreased to almost one-third of their earlier number. In contrast, for many cancers age-adjusted deaths have changed little over the same time period. A substantial breakthrough in the prevention of CVD occurred with the discovery that blood cholesterol levels could be used as a readily measurable, reversible measure of risk. No counterpart to blood cholesterol is available for monitoring changes in cancer risk in response to dietary intervention. Although treatment of cancers continues to develop, prevention lags behind, needing a stronger scientific basis and clear translation to prevention strategies that, like prevention of CVD, will feature dietary intervention. At least part of the confusion in setting dietary strategies for cancer prevention resides in the fact that not all cancers respond similarly to dietary intervention. For example, calcium is reported to decrease risk for colon cancer, while potentially increasing prostate cancer risk (Lamprecht and Lipkin, 2003; Rodriguez et al., 2003). Although much progress has been made in the identification of sites of action of bioactive food components, much more information will be needed to truly evaluate the effects of specific foods and food components as modifiers of cancer risk. Until that occurs, it remains prudent to eat a variety of foods and maintain a healthy weight.

Biomarkers that reliably inform people of their individual risks for cancer are under constant development, as are dietary strategies to decrease this risk. The Pap smear surveillance program for premalignant change in cervical cells, leading to prompt surgical removal of abnormal tissue prior to development of cancer, has led to an ~80% decrease in deaths due to cervical cancer in the United States (Sawaya et al., 2003). Similarly, surveillance programs monitoring precancerous changes in the colon, skin, and oral mucosa have led to successful surgical removal prior to development of cancer. Dysplastic changes detected by histopathology are considered sufficiently far along the disease pathway to require surgical removal, rather than dietary intervention. However, following surgery these same individuals are at high risk for additional primary cancers, making them candidates for preventive measures. The tamoxifen story is an example of preventive measures following breast cancer therapy (Coombes et al., 2004). Many of the earlier chapters in this book suggest that dietary components, such as tea or cruciferous vegetables, may decrease risk for cancer, making them potential candidates for dietary prevention strategies. However, the dose and frequency required, the variation in dose within the whole-food or dietary supplement products on the market, and even the efficacy are greatly in need of a stronger scientific basis before the message is clear.

Emerging technologies suggest that genetic polymorphisms may be useful for identifying those who will benefit most from intervention. However, many checks and balances exist, and thus a single mutation is likely not going to provide an adequate snapshot of the condition or the response to a bioactive food component. Clearly, as the genomic area unfolds, there will need to be continued vigilance in making sure that decisions are being made responsibly and ethically. Although genetic markers are not reversible, related phenotypic changes in proteomic or metabolomic endpoints are under development that may provide insights into dietary intervention that can be easily and cheaply provided and monitored in a surveillance program.

Developing the message that smoking is related to cancer has taken considerable time, government involvement, money and, yes, in some cases, lawsuits. In contrast,
developing the message about the cancer-fighting health benefits of foods may emanate not from the government, but from demands by consumers for more scientifically valid information. The recent response of the food industry to the public’s interest in low-carbohydrate foods shows clearly that public demand can substantially influence industry’s research and development for new products, without the need for government intervention. The general public is constantly searching for more healthful foods, providing a challenge to scientists, food manufacturers, and restaurants to provide tasty foods that are also good for you. Many individuals start their day with orange juice, not particularly for the flavor, but because they believe in it as a functional food, providing vitamin C, flavonoids that are possibly beneficial and, depending upon the product, added calcium. This “bundling” of bioactive components, whether in a whole food, a fortified food, or a supplement, may best meet the needs of a hurried population concerned about the lack of health-promoting components in their diet. Using the modern technologies available today, of genomics, proteomics, and metabolomics, and working closely with the food industry, we can expect the science of cancer prevention to translate into effective dietary strategies that substantially decrease cancer deaths.

17.7 SUMMARY

Evidence from a variety of sources suggests that what one eats can influence the risk of several chronic diseases, including cancer. It is refreshing that a variety of different types of foods are linked to improved health and disease resistance. At this point it seems most prudent to enjoy eating a variety of foods, while maintaining a healthy weight. The new food guide pyramid (http://www.mypyramid.gov/), based on the 2005 dietary guidelines (http://www.health.gov/dietaryguidelines/) promotes eating a variety of fruits and vegetables. The variety of foods with potential health benefits offers exciting opportunities for developing new and novel prepared foods that will meet consumer likes and demands. While it may be prudent to consume some foods in greater quantities for improved health, not all individuals will respond equally well — in fact, their responses may not even be in the same direction. Unraveling who will benefit most and those who might be placed at risk will necessitate a greater understanding of genomic and environmental factors that influence the response to a food and its constituents. Clearly, quantity, duration, and timing of the introduction of foods and food components are key to determining the overall response within an individual. Whereas public health approaches and strategies for disease resistance will continue to be critical for populations, the future of nutrition likely resides in a personalized approach that blends information about the consumer’s genomic background, behaviors, and environment. What is already clear is that genes and gene expression patterns can vary among individuals, thus influencing the requirements for essential nutrients and the biological response to nonessential dietary components. This need for a personalized approach will become more obvious as additional information surfaces about the sites of action of the various bioactive food components and as factors that influence their efficacy in producing phenotypic change are elucidated. As new biomarkers emerge that build on genomic technologies, it should become easier to distinguish responders to
intervention from nonresponders. As these technologies are used, it will be critical that they are used wisely and within a bioethical framework.

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