Dietary Carcinogens and Anticarcinogens

Oxygen radicals and degenerative diseases

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Comparison of data from different countries reveals wide differences in the rates of many types of cancer. This leads to hope that each major type of cancer may be largely avoidable, as is the case for cancers due to tobacco, which constitute 30 percent of the cancer deaths in the United States and the United Kingdom (1). Despite numerous suggestions to the contrary, there is no convincing evidence of any generalized increase in U.S. (or U.K.) cancer rates other than what could plausibly be ascribed to the delayed effects of previous increases in tobacco usage (1–3). Thus, whether or not any recent changes in life-style or pollution in industrialized countries will substantially affect future cancer risks, some important determinants of current risks remain to be discovered among long-established aspects of our way of life. Epidemiologic studies have indicated that dietary practices are the most promising area to explore (1, 4). These studies suggest that a general increase in consumption of fiber-rich cereals, vegetables, and fruits and decrease in consumption of fat-rich products and excessive alcohol would be prudent (1, 4). There is still a lack of definitive evidence about the dietary components that are critical for humans and about their mechanisms of action. Laboratory studies of natural foodstuffs and cooked food are beginning to uncover an extraordinary variety of mutagens and possible carcinogens and anticarcinogens. In this article I discuss dietary mutagens and carcinogens and anticarcinogens that seem of importance and speculate on relevant biochemical mechanisms, particularly the role of oxygen radicals and their inhibitors in the fat-cancer relationship, promotion, anticarcinogenesis, and aging.

Natural Mutagens and Carcinogens in Food

Plant material. Plants in nature synthesize toxic chemicals in large amounts, apparently as a primary defense against the hordes of bacterial, fungal, and insect and other animal predators (5–40). Plants in the human diet are no exception. The variety of these toxic chemicals is so great that organic chemists have been characterizing them for over 100 years, and new plant chemicals are still being discovered (12, 24, 25). However, toxicological studies have been completed for only a very small percentage of them. Recent widespread use of short-term tests for detecting mutagens (41, 42) and the increased number of animal cancer tests on plant substances (6) have contributed to the identification of many natural mutagens, teratogens, and carcinogens in the human diet (5–40). Sixteen examples are discussed below.

1) Safrole, estragole, methyleugenol, and related compounds are present in many edible plants (5). Safrole, estragole, and methyleugenol are carcinogens in rodents, and several of their metabolites are mutagens (5). Oil of sassafras, which had been used in "natural" sarsaparilla root beer, is about 75 percent safrole. Black pepper contains small amounts of safrole and large amounts (close to 10 percent by weight) of the closely related compound piperine (26). Extracts of black pepper cause tumors in mice at a variety of sites at a dose of extract equivalent to 4 mg of dried pepper per day (about 160 mg/kg per day) for 3 months; an estimate of the average human intake of black pepper is over 140 mg per day (about 2 mg/kg per day) for life (26).

2) Most hydrazines that have been tested are carcinogens and mutagens, and large amounts of carcinogenic hydrazines are present in edible mushrooms. The widely eaten false morel (Gyromitra esculenta) contains 11 hydrazines, three of which are known carcinogens (28). One of these, N-methyl-N-formylhydrazine, is present at a concentration of 50 mg per 100 g and causes lung tumors in mice at the extremely low dietary level of 20 μg per mouse per day (28). The most common commercial mushroom, Agaricus bisporus, contains about 300 mg of agaritine, the 8-glutamyl derivative of the mutagen 4-hydroxy-

Summary. The human diet contains a great variety of natural mutagens and carcinogens, as well as many natural antimutagens and anticarcinogens. Many of these mutagens and carcinogens may act through the generation of oxygen radicals. Oxygen radicals may also play a major role as endogenous initiators of degenerative processes, such as DNA damage and mutation (and promotion), which may be related to cancer, heart disease, and aging. Dietary intake of natural antioxidants could be an important aspect of the body's defense mechanism against these agents. Many antioxidants are being identified as anticarcinogens. Characterizing and optimizing such defense systems may be an important part of a strategy of minimizing cancer and other age-related diseases.

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rashes on their arms when exposed to diseased celery (19). Oil of bergamot, a citrus oil, is very rich in a psoralen and was used in the leading suntan lotion in France (17). Psoralens, when activated by sunlight, damage DNA and induce tanning more rapidly than the ultraviolet component of sunlight, which is also a carcinogen (17). Psoralens (plus light) are also effective in producing oxygen radicals (18).

4) The potato glycoalkaloids solanine and chaconine are strong cholinesterase inhibitors and possible teratogens and are present at about 15 mg per 200 g of potato (12, 13). When potatoes are diseased, bruised, or exposed to light, these and other (24) glycoalkaloids reach levels that can be lethal to humans (12). Plants typically respond to damage by making more (and often different) toxic chemicals as a defense against insects and fungi (19, 24, 25). The different cultivars of potatoes vary in the concentration of these toxic glycoalkaloids (the concentration is a major determinant of insect and disease resistance); one cultivar bred for insect resistance had to be withdrawn from use because of its toxicity to humans (> 40 mg of glycoalkaloids in a 200-g potato is considered to be a toxic level) (12).

5) Quercetin and several similar flavonoids are mutagens in a number of short-term test systems. Flavonoids are extremely widespread (daily levels close to 1 g) in the human diet (8, 16, 20, 21). There is evidence for the carcinogenicity of quercetin in two strains of rats (8), although it was negative in other experiments (21).

6) Quinones and their phenol precursors (9, 14, 16, 23, 45) are widespread in the human diet. Quinones are quite toxic as they can act as electrophiles or accept a single electron to yield the semiquinone radical, which can either react directly with DNA (14, 46) or participate in a redox cycle of superoxide radical generation by transferring the electron to O₂ (47). The superoxide radical and its metabolic product H₂O₂ can, in turn, lead to the oxidation of fat in cellular membranes by a lipid peroxidation chain reaction, thus generating mutagens and carcinogens, as discussed below. A number of quinones and dietary phenols have been shown to be mutagens (7, 9, 16, 23, 44). Mutagenic anthraquinone derivatives are found in plants such as rhubarb and in mold toxins (7, 16, 48). Many dietary phenols can spontaneously autoxidize to quinones, generating hydrogen peroxide at the same time [examples are catechol derivatives such as the caffeic acid component of chlorogenic acid (9), which is present at about 250 mg per cup of coffee]. The amounts of these phenols in human urine (and in the diet) are appreciable (45). Catechol, for example, is excreted in urine at about 10 mg per day and appears to be mainly derived from metabolism of plant substances (45). Catechol is a potent promoter of carcinogenesis (45), an inducer of DNA damage, a likely active metabolite of the carcinogen benzene (46), and a toxic agent in cigarette smoke (45). Catecholamine induction of cardiomyopathy is thought to occur through generation of oxygen radicals (49).

7) Theobromine, a relative of caffeine, has been shown to be genotoxic in a variety of tests, to potentiate (as does caffeine) DNA damage by various carcinogens in human cells, and to cause testicular atrophy and spermatogenic cell abnormalities in rats (27). Cocoa powder is about 2 percent theobromine, and therefore humans may consume hundreds of milligrams of theobromine a day from chocolate. Theobromine is also present in tea.

8) Pyrrolizidine alkaloïds are carcinogenic, mutagenic, and teratogenic and are present in thousands of plant species (often at > 1 percent by weight), some of which are ingested by humans, particularly in herbs and herbal teas and occasionally in honey (7, 29). Pyrrolizidine alkaloid poisonings in humans (as well as in other mammals) cause lung and liver lesions and are commonly misdiagnosed (29).

9) The broad (fava) bean (Vicia faba), a common food of the Mediterranean region, contains the toxins vicine and convicine at a level of about 2 percent of the dry weight (30). Pythagoras forbade his followers to eat the beans, presumably because he was one of the millions of Mediterranean people with a deficiency of glucose-6-phosphate dehydrogenase. This deficiency results in a low glutathione concentration in blood cells, which causes increased resistance to the malarial parasite, probably accounting for the widespread occurrence of the mutant gene in malarial regions. However, the low glutathione concentration also results in a marked sensitivity to agents that cause oxidative damage, such as the fava bean toxins and a variety of drugs and viruses. Sensitive individuals who ingest fava beans develop a severe hemolytic anemia caused by the enzymatic hydrolysis of vicine to its glycone, divicine, which forms a quinone that generates oxygen radicals (30).

10) Allyl isothiocyanate, a major flavor ingredient in oil of mustard and horseradish, is one of the main toxins of the mustard seed and has been shown to cause chromosome aberrations in hamster cells at low concentration (50) and to be a carcinogen in rats (31).

11) Gossypol is a major toxin in cottonseed and accounts for about 1 percent of its dry weight (32). Gossypol causes pathological changes in rat and human testes, abnormal sperm, and male sterility (32, 33). Genetic damage has been observed in embryos sired by gossypol-treated male rats; dominant lethal mutations in embryos were measured after males were taken off gossypol treatment and allowed to mate (33). Gossypol appears to be a carcinogen as well: it has been reported to be a potent initiator and also a promoter of carcinogenesis in skin painting studies with mice (34). Crude, unrefined cottonseed oil contains considerable amounts of gossypol (100 to 750 mg per 100 ml). Thus human consumption may be appreciable in countries, such as Egypt, where fairly crude cottonseed oil is commonly used in cooking. Gossypol is being tested as a male contraceptive in over 10,000 people in China (at an oral dose of about 10 mg per person per day), as it is inexpensive and causes sterility during use (33). Gossypol's mode of action as a spermicide may be through the production of oxygen radicals (35).

Plant breeders have developed "glandless cotton," a new strain with low levels of gossypol, but seeds from this strain are much more susceptible to attack by the fungus Aspergillus flavus, which produces the potent carcinogen aflatoxin (36).

12) Sterculic acid and malvalic acid are widespread in the human diet. They are toxic cyclopropenoid fatty acids present in cottonseed oil and other oils from seeds of plants in the family Malvaceae (for instance, cotton, kapok, okra, and durian) (51). Another possible source of human exposure is consumption of fish, poultry, eggs, and milk from animals fed on cottonseed (51). Cyclopropenoid fatty acids are carcinogens in trout, markedly potentiate the carcinogenicity of aflatoxin in trout, cause atherosclerosis in rabbits, are mitogenic in rats, and have a variety of toxic effects in farm animals (51). The toxicity of these fatty acids could be due to their ease of oxidation to form peroxides and radicals (51).

13) Leguminous plants such as lupine contain very potent teratogens (22). When cows and goats forage on these plants, their offspring may have severe teratogenic abnormalities; an example is
the characteristic "crooked calf" abnormality due to the ingestion of anagyrine from lupine (22). In addition, significant amounts of these teratogens are transferred to the animals' milk, so that drinking the milk during pregnancy is a serious teratogenic hazard (22). In one rural California family, a baby boy, a litter of puppies, and goat kids all had "crooked" bone birth-defect abnormalities. The pregnant mother and the dog had both been drinking milk obtained from the family goats, which had been foraging on lupine (the main forage in winter) (22). It was at first mistakenly thought that the birth defects were caused by spraying of 2,4-D.

14) Sesquiterpene lactones are widespread in many plants (37), although because they are bitter they are not eaten in large amounts. Some have been shown to be mutagenic (37). They are a major toxin in the white sap of Lactuca virosa (poison lettuce), which has been used as a folk remedy. Plant breeders are now transferring genes from this species to commercial lettuce to increase insect resistance (38).

15) The phorbol esters present in the Euphorbiaceae, some of which are used as folk remedies or herb teas, are potent promoters of carcinogenesis and may have been a cause of nasopharyngeal cancer in China and esophageal cancer in Curaçao (39).

16) Alfalfa sprouts contain canavanine, a highly toxic arginine analog that is incorporated into protein in place of arginine. Canavanine, which occurs in alfalfa sprouts at about 1.5 percent of their dry weight (40), appears to be the active agent in causing the severe lupus erythematosus-like syndrome seen when monkeys are fed alfalfa sprouts (40). Lupus in man is characterized by a defect in the immune system which is associated with autoimmunity, antinuclear antibodies, chromosome breaks, and various types of pathology (40). The chromosome breaks appear to be due to oxygen radicals as they are prevented by superoxide dismutase (52). The canavanine—alfalfa sprout pathology could be due in part to the production of oxygen radicals during phagocytization of antibody complexes with canavanine-containing protein.

The 16 examples above, plus coffee (discussed below), illustrate that the human dietary intake of "nature's pesticides" is likely to be several grams per day—probably at least 10,000 times higher than the dietary intake of man-made pesticides (53).

Levels of plant toxins that confer insect and fungal resistance are being increased or decreased by plant breeders (38). There are health costs for the use of these natural pesticides, just as there are for man-made pesticides (41, 54), and these must be balanced against the costs of producing food. However, little information is available about the toxicology of most of the natural plant toxins in our diet, despite the large doses we are exposed to. Many, if not most, of these plant toxins may be "new" to humans in the sense that the human diet has changed drastically with historic times. By comparison, our knowledge of the toxicological effects of new man-made pesticides is extensive, and general exposure is exceedingly low (53).

Plants also contain a variety of anticarcinogens (55), which are discussed below.

Alcohol. Alcohol has long been associated with cancer of the mouth, esophagus, pharynx, larynx, and, to a lesser extent, liver (1, 56), and it appears to be an important human teratogen, causing a variety of physical and mental defects in babies of mothers who drink (57). Alcohol drinking causes abnormalities in mice (57a) and is a synergist for somesome damage in humans (58). Alcohol metabolism generates acetaldehyde, which is a mutagen and teratogen (59), a cocarcinogen, and possibly a carcinogen (60), and also radicals that produce lipid hydroperoxides (61) and other mutagens and carcinogens (62; see below). In some epidemiological studies on alcohol (56), it has been suggested that dietary green vegetables are a modifying factor in the reduction of cancer risk.

Mold carcinogens. A variety of mold carcinogens and mutagens are present in mold-contaminated food such as corn, grain, nuts, peanut butter, bread, cheese, fruit, and apple juice (15, 63). Some of these, such as sterigmatocystin and aflatoxin, are among the most potent carcinogens and mutagens known (15, 63). Dietary glutathione has been reported to counteract aflatoxin carcinogenicity.

Nitrite, nitrate, and nitrosamines. A number of human cancers, such as stomach and esophageal cancer, may be related to nitrosamines and other nitroso compounds formed from nitrate and nitrite in the diet (64, 65). Beets, celery, lettuce, spinach, radishes, and rhubarb all contain about 200 mg of nitrate per 100-g portion (65). Anticarcinogens in the diet may be important in this context as well (66).

Fat and cancer: possible oxidative mechanisms. Epidemiologic studies of cancer in humans suggest, but do not prove, that high fat intake is associated with colon and breast cancer (1, 4, 67). A number of animal studies have shown that high dietary fat is a promoter and a presumptive carcinogen (4, 67, 68). Colon and breast cancer and lung cancer (which is almost entirely due to cigarette smoking) account for about half of all U.S. cancer deaths. In addition to the cyclopตนเอง phosphoryl fatty acids already discussed, two other plausible mechanisms involving oxidative processes could account for the relation (69) between high fat and both cancer and heart disease.

1) Rancid fat. Fat accounts for over 40 percent of the calories in the U.S. diet (67), and the amount of ingested oxidized fat may be appreciable (70, 71). Unsaturated fatty acids and cholesterol in fat are easily oxidized, particularly during cooking (70, 71). The lipid peroxidation chain reaction (rancidity) yields a variety (71–73) of mutagens, promoters, and carcinogens such as fatty acid hydroperoxides (62), cholesterol hydroperoxide (74), endoperoxides, cholesterol and fatty acid epoxides (74–77), enals and other aldehydes (44, 59, 78), and alkoxy and hydroperoxy radicals (44, 72). Thus the colon and digestive tract are exposed to a variety of fat-derived carcinogens. Human breast fluid can contain enormous levels (up to 780 μM) (75) of cholesterol epoxide (an oxidation product of cholesterol), which could originate from either ingested oxidized fat or oxidative processes in body lipids. Rodent feeding studies with oxidized fat (79) have not yielded definitive results.

2) Peroxisomes oxidize an appreciable percentage of dietary fatty acids, and removal of each two-carbon unit generates one molecule of hydrogen peroxide (a mutagen, promoter, and carcinogen) (80, 81). Some hydrogen peroxide escapes the catalase in the peroxisome (80, 82, 83), thus contributing to the supply of oxygen radicals, which also come from other metabolic sources (72, 83–85). Hydroperoxides generate oxygen radicals in the presence of iron-containing compounds in the cell (72). Oxygen radicals, in turn, can damage DNA and can start the rancidity chain reaction which leads to the production of the mutagens and carcinogens listed above (72). Drugs such as clofibrate, which cause lowering of serum lipids and proliferation of peroxisomes in rodents, result in age pigment (lipofuscin) accumulation (a sign of lipid peroxidation in tissues) and liver tumors in animals (80). Some fatty acids, such as C22:1 and certain trans fatty acids, appear to cause peroxisomal proliferation because they are poorly oxidized in mitochondria and are preferentially oxidized in the peroxisomes, although they may be selective for heart or
liver (86). There has been controversy about the role of trans fatty acids in cancer and heart disease, and recent evidence suggests that trans fatty acids might not be a risk factor for atherosclerosis in experimental animals (87). Americans consume about 12 g of trans fatty acids a day (87) and a similar amount of unnatural cis isomers (which need further study (88)), mainly from hydrogenated vegetable fats. Dietary C22:1 fatty acids are also obtained from rapeseed oil and fish oils (86). Thus oxidation of certain fatty acids might generate grams of hydrogen peroxide per day within the peroxisome (86). Another source of fat toxicity could be perturbations in the mitochondrial or peroxisomal membranes caused by abnormal fatty acids, yielding an increased flux of superoxide and hydrogen peroxide. Mitochondrial structure is altered when rats are fed some abnormal fatty acids from partially hydrogenated fish oil (89). Dietary C22:1 fatty acids and coloflbrate also induce ornithine decarboxylase (86), a common attribute of promoters.

A recent National Academy of Sciences committee report suggests that a reduction of fat consumption in the American diet would be prudent (4), although other scientists argue that, until we know more about the mechanism of the fat-cancer relation and about which types of fat are dangerous, it is premature to recommend dietary changes (90).

Cooked Food as a Source of Ingested Burnt and Browned Material

Work of Sugimura and others has indicated that the burnt and browned material from heating protein during cooking is highly mutagenic (21, 91). Several chemicals isolated on the basis of their mutagenicity from heated protein or pyrolyzed amino acids were found to be carcinogenic when fed to rodents (21). In addition, the browning reaction products from the caramelization of sugars or the reaction of amino acids and sugars during cooking (for instance, the brown material on bread crusts and toasted bread) contain a large variety of DNA-damaging agents and presumptive carcinogens (23, 38, 92). The amount of burnt and browned material in the human diet may be several grams per day. By comparison, about 500 mg of burnt material is inhaled each day by a smoker using two packs of cigarettes (at 20 mg of tar per cigarette) a day. Smokers have more easily detectable levels of mutagens in their urine than nonsmokers (92), but so do people who have consumed a meal of fried pork or bacon (94). In the evaluation of risk from burnt material it may be useful (in addition to carrying out epidemiologic studies) to compare the activity of cigarette tar to that of the burnt material from cooked food (or polluted air) in short-term tests and animal carcinogenicity tests involving relevant routes of exposure. Route of exposure and composition of the burnt material are critical variables. The risk from inhaled cigarette smoke can be one reference standard: an average life shortening of about 8 years for a two-pack-a-day smoker. The amount of burnt material inhaled from severely polluted city air, on the other hand, is relatively small: it would be necessary to breathe smoggy Los Angeles air (111 µg/m³ total particulates; 31 µg/m³ soluble organic matter) for 1 to 2 weeks to equal the soluble organic matter of the particulates or the mutagenicity from one cigarette (20 mg of tar) (95).

Epidemiologic studies have not shown significant risks from city air pollution alone (1, 96). In the houses of smokers is considerably more polluted than city air outside (97).

Coffee, which contains a considerable amount of burnt material, including the mutagenic pyrolysis product methylglyoxal, is mutagenic (21, 98). However, one cup of coffee also contains about 250 mg of the natural mutagen chlorogenic acid (9) [which is also an antinitrosating agent (66)], highly toxic atracylosides (10), the glutathione transference inducers kahweal palmitate and caffeol palmitate (11), and about 100 mg of caffeine [which inhibits a DNA-repair system and can increase tumor yield (99) and cause birth defects at high levels in several experimental species (100)]. There is preliminary, but not conclusive, epidemiologic evidence that heavy coffee drinking is associated with cancer of the ovary, bladder, pancreas, and large bowel (101). Cooking also accelerates the rancidity reaction of cooking oils and fat in meat (70, 71), thus increasing consumption of mutagens and carcinogens.

Anticarcinogens

We have many defense mechanisms to protect ourselves against mutagens and carcinogens, including continuous shedding of the surface layer of our skin, stomach, cornea, intestines, and colon (102). Understanding these mechanisms should be a major goal of cancer, heart, and aging research. Among the most important defenses may be those against oxygen radicals and lipid peroxidation if, as discussed here, these agents are major contributors to DNA damage (103). Major sources of endogenous oxygen radicals are hydrogen peroxide (83) and superoxide (72, 104) generated as side products of metabolism, and the oxygen radical burst from phagocytosis after viral or bacterial infection or the inflammatory reaction (105). A variety of environmental agents could also contribute to the oxygen radical load, as discussed here and in recent reviews (72, 106). Many enzymes protect cells from oxidative damage; examples are superoxide dismutase (104), glutathione peroxidase (107), DT-diaphorase (108), and the glutathione transferases (109). In addition, a variety of small molecules in our diet are required for antioxidative mechanisms and appear to be anticarcinogens; some of these are discussed below.

1) Vitamin E (tocopherol) is the major radical trap in lipid membranes (72) and has been used clinically in a variety of oxidation-related diseases (110). Vitamin E ameliorates both the cardiac damage and carcinogenicity of the quinones adriamycin and daunomycin, which are mutagenic, carcinogenic, cause cardiac damage, and appear to be toxic because of free radical generation (111). Protective effects of tocopherols against radiation-induced DNA damage and mutation and dimethylhydrazine-induced carcinogenesis have also been observed (112). Vitamin E markedly increases the endurance of rats during heavy exercise, which causes extensive oxygen radical damage to tissues (113).

2) β-Carotene is another antioxidant in the diet that could be important in protecting body fat and lipid membranes against oxidation. Carotenoids are free-radical traps and remarkably efficient quenchers of singlet oxygen (114). Singlet oxygen is a very reactive form of oxygen which is mutagenic and particularly effective at causing lipid peroxidation (114). It can be generated by pigment-mediated transfer of the energy of light to oxygen, or by lipid peroxidation, although the latter is somewhat controversial. β-Carotene and similar polyenes are present in carrots and in all food that contains chlorophyll, and they appear to be the plants’ main defense against singlet oxygen generated as a byproduct from the interaction of light and chlorophyll (115). Carotenoids have been shown to be anticarcinogens in rats and mice (116). Carotenoids (in green and yellow vegetables) may be anticarcinogens in humans (1, 56, 117). Their protective effects in smokers might be related to the high level of oxidants in both cigarette smoke and tar (45, 118). Carotenoids have been used medically in
the treatment for some genetic diseases, such as porphyrias, where a marked photosensitivity is presumably due to singlet oxygen formation (119).

3) Selenium is another important dietary anticarcinogen. Dietary selenium (usually selenite) significantly inhibits the induction of skin, liver, colon, and mammary tumors in experimental animals by a number of different carcinogens, as well as the induction of mammary tumors by viruses (120). It also inhibits transformation of mouse mammary cells (121). Low selenium concentrations may be a risk factor in human cancer (122). A particular type of heart disease in young people in the Keshan area of China has been traced to a selenium deficiency, and low selenium has been associated with cardiovascular death in Finland (123). Selenium is in the active site of glutathione peroxidase, an enzyme essential for destroying lipid hydroperoxides and endogenous hydrogen peroxide and thus helping to prevent oxygen radical–induced lipid peroxidation (107), although not all of the effects of selenium may be accounted for by this enzyme (120). Several heavy-metal toxins, such as Cd$^2^+$ (a known carcinogen) and Hg$^2^+$, lower glutathione peroxidase activity by interacting with selenium (107). Selenite (and vitamin E) has been shown to counter the oxidative toxicity of mercuric salts (124).

4) Glutathione is present in food and is one of the major antioxidants and anti- mutagens in the soluble fraction of cells. The glutathione transferases (some of which have peroxidase activity) are major defenses against oxidative and alkylating carcinogens (109). The concentration of glutathione may be influenced by dietary sulfur amino acids (125, 126). N Acetylcysteine, a source of cysteine, raises glutathione concentrations and reduces the oxidative cardiotoxicity of adriamycin and the skin reaction to radiation (127). Glutathione concentrations are raised even more efficiently by 1,2 oxothiazolidine-4-carboxylate, which is an effective antagonist of acetaminophen-caused liver damage (126). Acetaminophen is thought to be toxic through radical and quinine oxidizing metabolites (128). Dietary glutathione may be an effective anticarcinogen against aflatoxin (129).

5) Dietary ascorbic acid is also important as an antioxidant. It was shown to be anticarcinogenic in rodents treated with ultraviolet radiation, benzo[a]pyrene, and nitrite (forming nitroso carcinogens) (64, 65, 130), and it may be inversely associated with human uterine cervical dysplasia (although this is not proof of a cause-effect relationship) (131). It was recently hypothesized that ascorbic acid may have been supplemented and perhaps partially replaced in humans by uric acid during primate evolution (132).

6) Uric acid is a strong antioxidant present in high concentrations in the blood of humans (132). The concentration of uric acid in the blood can be increased by dietary purines; however, too much causes gout. Uric acid is also present in high concentrations in human saliva (132) and may play a role in defense there as well, in conjunction with lactoperoxidase. A low uric acid level in blood may possibly be a risk factor in cigarette-caused lung cancer in humans (133).

7) Edible plants and a variety of substances in them, such as phenols, have been reported to inhibit (cabbage) or to enhance (beets) carcinogenesis (11, 55, 134) or mutagenesis (23, 66, 92, 135) in experimental animals. Some of these substances appear to inhibit by inducing cytochrome P-450 and other metabolic enzymes ([134]; see also [11]), although on balance it is not completely clear whether it is generally helpful or harmful for humans to ingest these inducing substances.

The hypothesis that as much as 80 percent of cancer could be due to environmental factors was based on geographic differences in cancer rates and studies of migrants (136). These differences in cancer rates were thought to be mainly due to life-style factors, such as smoking and dietary carcinogens and promoters (136), but they also may be due in good part [see also (1)] to less than optimum amounts of anticarcinogens and protective factors in the diet.

The optimum levels of dietary antioxidants, which may vary among individuals, remain to be determined; however, at least for selenium (120), it is important to emphasize the possibility of deleterious side effects at high doses.

Oxygen Radicals and Degenerative Diseases Associated with Aging

**Aging.** A plausible theory of aging holds that the major cause is damage to DNA (102, 137) and other macromolecules and that a major source of this damage is oxygen radicals and lipid peroxidation (43, 84, 103, 138–141). Cancer and other degenerative diseases, such as heart disease (102), are likely to be due in good part to this same fundamental destractive process. Age pigment (lipofuscin) accumulates aging in all mammalian species and has been associated with lipid peroxidation (73, 84, 138, 139). The fluorescent products in age pigment are thought to be formed by malondialdehyde (a mutagen and carcinogen and a major end product of rancidity) cross-linking protein and lipids (138). Metabolic rate is directly correlated with the rate of lipofuscin formation (and inversely correlated with longevity) (139).

Cancer increases with about the fourth power of age, both in short-lived species such as rats and mice (about 30 percent of rodents have cancer by the end of their 2- to 3-year life-span) and in long-lived species such as humans (about 30 percent of people have cancer by the end of their 85-year life-span) (142). Thus, the marked increase in life-span that has occurred in 60 million years of primate evolution has been accompanied by a marked decrease in age-specific cancer rates; that is, in contrast to rodents, 30 percent of humans do not have cancer by the age of 3 (142). One important factor in longevity appears to be basal metabolic rate (139, 141), which is much lower in man than in rodents and could markedly affect the level of endogenous oxygen radicals.

Animals have many antioxidant defenses against oxygen radicals. Increased levels of these antioxidants, as well as new antioxidants, may also be a factor in the evolution of man from short-lived prosimians (143). It has been suggested that an increase in superoxide dismutase is correlated (after the basal metabolic rate is taken into account) with increased longevity during primate evolution, although this has been disputed (141). Ames et al. proposed (132) that as uric acid was an antioxidant and was present in much higher concentrations in the blood of humans than in other mammals, it may have been one of the innovations enabling the marked increase in life span and consequent marked decrease in age-specific cancer rates which occurred during primate evolution. The ability to synthesize ascorbic acid may have been lost at about the same time in primate evolution as uric acid levels began to increase (144).

**Cancer and promotion.** Both DNA-damaging agents (initiating mutants) (21, 41, 42) and promoters (145) appear to play an important role in carcinogenesis (21, 146). It has been postulated that certain promoters of carcinogenesis act by generation of oxygen radicals and resultant lipid peroxidation (73, 146–149). Lipid peroxidation cross-links proteins (43, 150) and affects all aspects of cell organization (72), including membrane and surface structure, and the mi-
that benign or from this have also could have arisen in evolution, oxidation, peroxides, and carcinogenicity of the free-radical-generating quinones adriamycin and daunomycin; N-acetylcysteine reduces the cardiotoxicity of adriamycin; and selenium is an antirisk factor for one type of heart disease.

Other diseases. The brain uses 20 percent of the oxygen consumed by man and contains an appreciable amount of unsaturated fat. Lipid peroxidation (with consequent age pigment) is known to occur readily in the brain (72), and possible consequences could be senile dementia or other brain abnormalities (84). Several inherited progressive diseases of the central nervous system, such as Batten's disease, are associated with lipofuscin accumulation and may be due to a lipid peroxidation caused by a high concentration of unbound iron (170). Mental retardation is one consequence of an inherited defective DNA repair system (XP complementation group D) for depurinated sites in DNA (171).

Senile cataracts have been associated with light-induced oxidative damage (172). The retina and an associated layer of cells, the pigment epithelium, are extremely sensitive to degeneration in vitamin E and selenium deficiency (173). The pigment epithelium accumulates massive amounts of lipofuscin in aging and dietary antioxidant deficiency (173). The eye is well known to be particularly rich in antioxidants.

The testes are quite prone to lipid peroxidation and to the accumulation of age pigment. A number of agents, such as gossypol, which cause genetic birth defects (dominant lethals) may be active by this mechanism. The various agents known to cause cancer by oxidative mechanisms are prospective mutagenic agents for the germ line. Thus, vitamin E, which was discovered 60 years ago as a fertility factor (72), and other antioxidants such as selenium (174), may help both to engender and to protect the next generation.

Risks

There are large numbers of mutagens and carcinogens in every meal, all perfectly natural and traditional [see also (21, 23)]. Nature is not benign. It should be emphasized that no human diet can be entirely free of mutagens and carcinogens and that the foods mentioned are only representative examples. To identify a substance, whether natural or man-made, as a mutagen or a carcinogen, is just a first step. Beyond this, it is necessary to consider the risks for alternative courses of action and to quantitate the approximate magnitude of the risk, although the quantification of risk poses a major challenge. Carcinogens differ in their potency in rodents by more than a millionfold (175), and the levels of particular carcinogens to which humans are exposed can vary more than a billionfold. Extrapolation of risk from rodents to humans is difficult for many reasons, including the longevity difference, antioxidant factors, and the probable multi-causal nature of most human cancer.

Tobacco smoking is, without doubt, a major and well-understood risk, causing about 30 percent of cancer deaths and 25 percent of fatal heart attacks (as well as other degenerative diseases) in the United States (1). These percentages may increase even more in the near future as the health effects of the large increase in women smokers become apparent (1). Diet, which provides both carcinogens and anticarcinogens, is extremely likely to be another major risk factor. Excessive alcohol consumption is another risk, although it does not seem to be of the same general importance as smoking and diet. Certain other high-dose exposures might also turn out to be important for particular groups of people—for instance, certain drugs, where consumption can reach hundreds of milligrams per day; particular cosmetics; and certain occupational exposures (2), where workers inhale dusts or solvents at high concentration. We must also be prudent about environmental pollution (41, 54).

Despite all of these risks, it should be emphasized that the overall trend in life expectancy in the United States is continuing steadily upward (176).

The understanding of cancer and degenerative disease mechanisms is being aided by the rapid progress of science and technology, and this should help to dispel confusion about how important health risks can be identified among the vast number of minor risks. We have many methods of attacking the problem of environmental carcinogens (and anticarcinogens), including human epidemi-
ology (J), short-term tests (41, 42, 177), and animal cancer tests (175). Powerful new methods are being developed [for instance, see (58, 177)] for measuring DNA damage or other pertinent factors with great sensitivity in individuals. These methods, which are often noninvasive as they can be done on blood or urine (even after storage), can be combined with epidemiology to determine whether particular factors are predictive of disease. Thus, more powerful tools will be available for optimizing antioxidants and other dietary anti-risk factors, for identifying human genetic variants at high risk, and for identifying significant health risks.

References and Notes

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RESEARCH ARTICLE

Imaging Dopamine Receptors in the Human Brain by Positron Tomography

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One of the most intriguing problems in biomedical research today is that of relating manifestations of neuropsychiatric diseases to chemical processes in different parts of the brain. The neurotransmitter dopamine appears to be associated with abnormalities related to disorders such as Parkinson's disease and schizophrenia. The highest density of dopamine neurons occurs in the nigrostriatal dopamine pathway which degenerates in Parkinson's disease (1). Neuroleptic drugs elicit extrapyramidal parkinsonian side effects by blocking dopamine receptors in the corpus striatum and also exert antischizophrenic action by blocking dopamine receptors, perhaps in limbic areas (2). Numbers of dopamine receptors are increased by chronic neuroleptic treatment (3) and are also increased in some schizophrenics, perhaps as a result of neuroleptic therapy (4). The development of positron emission tomography (PET) and appropriate radioactive tracers labeled with positron-emitting radionuclides has now made it possible to relate regional biochemistry within the human brain to measurements of behavior in normal subjects and to elucidate abnormalities in patients with Alzheimer's disease (5), Huntington's disease (6), depression (7), and multiple infarct dementia (8). The technique consists of intravenous injection of a substance such as $^{18}$F-labeled deoxyglucose, $[^11]$C-carboxyhemoglobin, and ionic rubidium-82, $^{90}$Ga-labeled EDTA, and other radiopharmaceuticals, and subsequent imaging of the distribution of the radioactive label in the brain by means of the tomographic method, based on detection of the annihilation radiation produced during positron emission (9).

The butyrophenone neuroleptic drug spiperone has been useful in binding studies for measuring dopamine receptors both in vitro (10) and in vivo (11). We now report initial results obtained with $^{3}$-N-$[^11]$C-methylspiperone ($[^11]$C-NMSP), a spiperone derivative, in PET scanning studies to visualize the distribution of dopamine receptors in the brains of baboons and a human being. All studies were performed with a NeuroECAT scanner (Ortec Inc., Oak Ridge, Tennessee), which has a spatial resolution of approximately 8 mm (full width at half maximum) in the plane of the slice. The distance between slices is 3 cm.

The newly developed tracer $[^11]$C-NMSP was synthesized by N-alkylation of spiperone with $[^11]$C-methyl iodide; the iodide was produced from $^{13}$CO$_2$, which in turn had been produced with an in-hospital cyclotron (model RNP-16, Scanditronix Cyclotron, Sweden). Carbon-11 is a positron-emitting isotope with a physical half-life of 20 minutes. The entire synthesis was accomplished with material ready for injection within 55 minutes after the end of the cyclotron.

Abstract. Neuror-transmitter receptors may be involved in a number of neuropsychiatric disease states. The ligand 3-N-$[^11]$C-methylspiperone, which preferentially binds to dopamine receptors in vivo, was used to image the receptors by positron emission tomography scanning in baboons and in humans. This technique holds promise for noninvasive clinical studies of dopamine receptors in humans.

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178. I. am indebted to C. Fortunucci, A. Blum, L. Gold, P. Hartman, W. Havender, N. K. Hooper, G. W. Ivey, J. McCann, J. Mead, R. Olson, R. Peto, A. Tappel, and numerous other colleagues for their criticisms. This work was supported by DOE contract DE-AT03-76EV01560 to B.A.N and by National Institute of Environmental Health Sciences Center Grant ES01896. This article has been expanded from a talk presented at the 12th European Environmental Mutagen Society Conference, Espoo, Finland, June 1982 [in Mutagens in Our Environment, M. Sorsa and H. Vainio, Eds. (Liss, New York, 1982)]. I wish to dedicate this article to the memory of Philip Handler, pioneer in the field of oxygen radicals.