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Review

Oxidants, antioxidants, and the degenerative diseases of aging

(cancer/mutation/endogenous DNA adducts/oxygen radicals)

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ABSTRACT **Metabolism, like other aspects of life, involves tradeoffs. Oxidant by-products of normal metabolism cause extensive damage to DNA, protein, and lipid. We argue that this damage (the same as that produced by radiation) is a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, immune-system decline, brain dysfunction, and cataracts. Antioxidant defenses against this damage include ascorbate, tocopherol, and carotenoids. Dietary fruits and vegetables are the principal source of ascorbate and carotenoids and are one source of tocopherol. Low dietary intake of fruits and vegetables doubles the risk of most types of cancer as compared to high intake and also markedly increases the risk of heart disease and cataracts. Since only 9% of Americans eat the recommended five servings of fruits and vegetables per day, the opportunity for improving health by improving diet is great.**

The degenerative diseases associated with aging include cancer, cardiovascular disease, immune-system decline, brain dysfunction, and cataracts. The functional degeneration of somatic cells during aging appears, in good part, to contribute to these diseases. The relationship between cancer and age in various mammalian species illustrates this point. Cancer increases with about the fifth power of age in both short-lived species, such as rats, and long-lived species, such as humans. Thus a marked decrease in age-specific cancer rates has accompanied the marked increase in lifespan that has occurred in 60 million years of mammalian evolution; i.e., cancer rates are high in a 2-year-old rat, but low in a 2-year-old human. One important factor in longevity appears to be basal metabolic rate, which is about 7 times higher in a rat than in a human and which could markedly affect the level of endogenous oxidants and other mutagens produced as by-products of metabolism. The level of oxidative DNA damage appears to be roughly related to metabolic rate in a number of mammalian species (1–3).

Oxidation and Damage to DNA, Protein, and Lipids

Oxidative damage to DNA, proteins, and other macromolecules accumulates with age and has been postulated to be a major, but not the only, type of endogenous damage leading to aging (4–9). Superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (·OH), which are mutagens produced by radiation, are also by-products of normal metabolism (10–12). Lipid peroxidation gives rise to mutagenic lipid epoxides, lipid hydroperoxides, lipid alkoxyl and peroxyl radicals, and enals (α,β-unsaturated aldehydes) (13, 14). Singlet oxygen, a high-energy and mutagenic form of oxygen, can be produced by transfer of energy from light, the respiratory burst from neutrophils, or lipid peroxidation (15).

Animals have numerous antioxidant defenses, but since these defenses are not perfect, some DNA is oxidized. Oxidatively damaged DNA is repaired by enzymes that excise the lesions, which are then excreted in the urine. Methods have been developed to assay several of these excised damaged bases in the urine of rodents and humans (1, 16), almost all of which appear as the free base from repair by glycosylases. We estimate that the number of oxidative hits to DNA per cell per day is about 100,000 in the rat and about 10,000 in the human. DNA-repair enzymes efficiently remove most, but not all, of the lesions formed (4). Oxidative lesions in DNA accumulate with age, so that by the time a rat is old (2 years) it has about 2 million DNA lesions per cell, which is about twice that in a young rat. Mutations also accumulate with age (17, 18). For example, the somatic mutation frequency in human lymphocytes, of which the contribution of oxidative DNA lesions is unknown, is about 9 times greater in elderly people than in neonates (17). The importance of oxidative DNA lesions in cancer and aging is underscored by the existence of specific repair glycosylases that excise these lesions from DNA. In the case of 8-oxo-2′-deoxyguanosine, a lesion formed from oxidative damage to guanine residues in DNA, loss of a specific glycosylase activity leads to an appreciable increase in the spontaneous mutation rate (19), indicating the intrinsic mutagenic potential of this DNA lesion. Many other oxidative DNA lesions are likely to be important as well (10).

Mitochondrial DNA (mtDNA) from rat liver has more than 10 times the level of oxidative DNA damage than does nuclear DNA from the same tissue (20). This increase may be due to a lack of mtDNA repair enzymes, a lack of histones protecting mtDNA, and the proximity of mtDNA to oxidants generated during oxidative phosphorylation. The cell defends itself against this high rate of damage by a constant turnover of mitochondria, thus presumably removing those damaged mitochondria that produce increased oxidants. Despite this turnover, oxidative lesions appear to accumulate with age in mtDNA at a higher rate than in nuclear DNA (Fig. 1). Oxidative damage could also account for the mutations in mtDNA that accumulate with age (21, 22).

Endogenous oxidants also damage proteins and lipids. Stadtman and his colleagues (7, 23, 24) have shown that the activity of proteolytic enzymes that hydrolyze oxidized proteins is not sufficient to prevent an age-associated increase of oxidized proteins. In two human diseases associated with premature aging, Werner syndrome and progeria, oxidized proteins increase at a much higher rate than is normal (7). Fluorescent pigments, which are thought to be due in part to crosslinks between protein and lipid peroxidation products, also increase with age (14, 25).

Sources and Effects of Oxidants

Four endogenous sources appear to account for most of the oxidants produced by cells. (*i*) As a consequence of normal aerobic respiration, mitochondria consume O_2, reducing it by sequential steps to produce H_2O (Fig. 2). Inevitable by-products of this process, as stated above,

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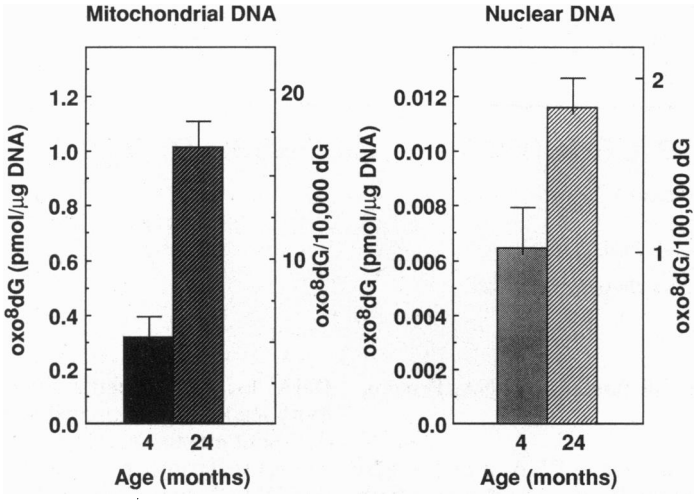

FIG. 1. Steady-state oxidative DNA damage increases with age. 8-Oxo-2'-deoxyguanosine (oxo^8dG) was analyzed (as in refs. 5 and 20) in nuclear and mitochondrial DNA from the livers of young and old rats.

are O_2^-, H_2O_2, and ·OH. About 10^{12} O_2 molecules are processed by each rat cell daily, and the leakage of partially reduced oxygen molecules is about 2%, yielding about 2×10^{10} O_2^- and H_2O_2 molecules per cell per day (26). (*ii*) Phagocytic cells destroy bacteria or virus-infected cells with an oxidative burst of nitric oxide (NO), O_2^-, H_2O_2, and OCl^-. Chronic infection by viruses, bacteria, or parasites results in a chronic phagocytic activity and consequent chronic inflammation, which is a major risk factor for cancer. Chronic infections are particularly prevalent in third-world countries (see below). (*iii*) Peroxisomes, which are organelles responsible for degrading fatty acids and other molecules, produce H_2O_2 as a by-product, which is then degraded by catalase. Evidence suggests that, under certain conditions, some of the peroxide escapes degradation, resulting in its release into other compartments of the cell and in increased oxidative DNA damage (27). (*iv*) Cytochrome P450 enzymes in animals constitute one of the primary defense systems against natural toxic chemicals from plants, the major source of dietary toxins. The induction of these enzymes prevents acute toxic effects from foreign chemicals but also results in oxidant by-products that damage DNA (J.-Y. K. Park and B.N.A., unpublished work).

Three exogenous sources may significantly increase the large endogenous oxidant load. (*a*) The oxides of nitrogen (NO_x) in cigarette smoke (about 1000 ppm) cause oxidation of macromolecules (28–31) and deplete antioxidant levels (32–34). This is likely to contribute significantly to the pathology of smoking (see below). Smoking is a risk factor for heart disease as well as a wide variety of cancers in addition to lung cancer (35–38). (*b*) Iron (and copper) salts promote the generation of oxidizing radicals from peroxides (Fenton chemistry). Men who absorb significantly more than normal amounts of dietary iron due to a genetic defect (hemochromatosis disease) are at an increased risk for both cancer and heart disease (39). It has therefore been argued that too much dietary copper or iron, particularly heme iron (which is high in meat), is a risk factor for cardiovascular disease and cancer in normal men (39–42). (*c*) Normal diets contain plant food with large amounts of natural phenolic compounds, such as chlorogenic and caffeic acid, that may generate oxidants by redox cycling (43, 44).

Chronic Infection, Inflammation and Cancer. Leukocytes and other phagocytic cells combat bacteria, parasites, and virus-infected cells by destroying them with NO, O_2^-, H_2O_2, and OCl^-, a powerful oxidant mixture (45, 46). These oxidants protect humans from immediate death from infection but cause oxidative damage to DNA and mutation (47, 48), thereby contributing to the carcinogenic process. Antioxidants appear to inhibit some of the pathology of chronic inflammation (see below) (49–55).

Chronic infections contribute to about one-third of the world's cancer. Hepatitis B and C viruses infect about 500 million people, mainly in Asia and Africa, and are a major cause of hepatocellular carcinoma (56–58). Another major chronic infection is schistosomiasis, which is caused by a parasitic worm that is widespread in China and Egypt. The Chinese worm lays its eggs in the colon, producing inflammation that often leads to colon cancer (59). The Egyptian worm lays eggs in the bladder, promoting bladder cancer (60). *Opisthorchis viverrini* and *Chlonorchis sinensis* are liver flukes that infect millions of people in China, Thailand, Laos, and Malaysia. These worms cause chronic inflammation of the biliary tract and markedly increase the risk for developing cholangiocarcinoma (61, 62). *Helicobacter pylori* bacteria, which infect the stomachs of over one-third of the world population, appear to be the major cause of stomach cancer, ulcers, and gastritis (53, 54, 63–65, 215). In wealthy countries the disease is usually asymptomatic, which indicates that the effects of inflammation are at least partially suppressed, possibly, in part, by adequate levels of dietary antioxidants (66).

Chronic inflammation resulting from noninfectious sources also contributes to various pathological conditions leading to cancer. For example, asbestos exposure causing chronic inflammation may be in good part the reason it is a significant risk factor for cancer of the lung (51, 52).

Tobacco, Cancer, and Heart Disease. Smoking, which we and others argue is a major oxidative stress in addition to a source of mutagens, contributes to about one-third of U.S. cancer, about one-quarter of U.S. heart disease, and about 400,000 premature deaths per year in the U.S. (38). Tobacco is a major global cause of cancer, but it causes even more deaths by other diseases. Tobacco will cause about 3 million deaths per year worldwide in the 1990s and will, at present rates of smoking, cause about 10 million deaths per year a few decades from now (38).

Aging and Dietary Restriction

Evolutionary biologists have argued that aging is inevitable because of several tradeoffs (67–70). One tradeoff is that a considerable proportion of an animal's resources is devoted to reproduction at a cost to maintenance, which means that the maintenance of somatic tissues is less than that required for indefinite survival. Of the vast array of maintenance processes that are necessary to sustain normal function in somatic cells, those that defend the cell against metabolism-derived oxidants are likely to play an important role. Metabolism has costs: oxidant by-products of normal energy metabolism extensively damage DNA, proteins, and other molecules in the cell,

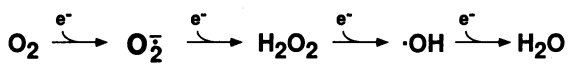

FIG. 2. Oxidants from normal metabolism. The formation of O_2^-, H_2O_2, and ·OH occurs by successive additions of electrons to O_2. Cytochrome oxidase adds four electrons fairly efficiently during energy generation in mitochondria, but some of these toxic intermediates are inevitable by-products.

Table 1. Review of epidemiological studies by Block *et al.* (93) showing protection by fruits and vegetables against cancer

Cancer site	Fraction of studies showing protection	Relative risk (median)
Epithelial		
Lung	24/25	2.2
Oral	9/9	2.0
Larynx	4/4	2.3
Esophagus	15/16	2.0
Stomach	17/19	2.5
Pancreas	9/11	2.8
Cervix	7/8	2.0
Bladder	3/5	2.1
Colorectal	20/35	1.9
Miscellaneous	6/8	—
Hormone-dependent		
Breast	8/14	1.3
Ovary/endometrium	3/4	1.8
Prostate	4/14	1.3
Total	129/172	

and this damage accumulates with age (4–7). Another tradeoff is that nature selects for many genes that have immediate survival value but that may have long-term deleterious consequences. The oxidative burst from phagocytic cells, for example, protects against death from bacterial and viral infections but contributes to DNA damage, mutation, and cancer (71, 72).

Aging Is Slowed by Calorie or Protein Restriction. In rodents a calorie-restricted diet significantly increases lifespan, decreases reproduction, and markedly decreases cancer rates (73–76). It has been suggested that Darwinian fitness in animals is increased by the delay of reproductive function during periods of low food availability (77) and that the saved resources are invested in maintenance of the body until food resources are available for successful reproduction (78). Protein restriction appears to have the same effects on rodents as calorie restriction, though it is less well studied (79). An understanding of mechanisms for this marked effect on aging and cancer is becoming clearer and may in good part be due to reduced oxidative damage. The suggestion that maintenance functions are enhanced in calorie-restricted rats, thus resulting in less oxidative damage, is supported by the findings of more efficient DNA repair, better coupled mitochondrial respiration, and a delay in the age-dependent decline of some antioxidant defenses (80–82). The higher level of antioxidant defenses could also account for the enhanced immune response in restricted animals (83). Work in this laboratory has shown that either calorie or protein restriction decreases the rate of accumulation of oxidized protein that accompanies aging in rats (79), and preliminary results suggest a decrease in preneoplastic foci and oxidative lesions in DNA as well. Thus, the overall effect of these enhanced maintenance activities appears to be a reduction in oxidative damage to DNA and protein, a decrease in DNA and protein lesions, and a decrease in somatic mutations. Markedly lower mitotic rates are observed in a variety of tissues in calorie-restricted rodents compared with rodents fed *ad libitum* (84, 85), which may also contribute to the decrease in tumor incidence† as discussed below.

Antioxidants Protect Against Disease

Many defense mechanisms within the organism have evolved to limit the levels of reactive oxidants and the damage they inflict (89). Among the defenses are enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. The glutathione *S*-transferases inactivate reactive electrophilic mutagens, including the aldehyde products of lipid peroxidation. There are also many structural defenses, such as sequestration of H_2O_2-generating enzymes in peroxisomes and chelation of any free iron or copper salts in transferrin and ferritin or ceruloplasmin to avoid Fenton chemistry. O_2^-, however, can release iron from ferritin (90).

†Dietary restriction activates the pituitary–adrenocorticotropic axis, resulting in a decrease in the release of reproductive and mitogenic hormones. Decreases in mitogenic hormones such as insulin, thyrotropin, growth hormone, estrogen, and prolactin decrease the likelihood of hormone-induced cancers, as has been shown in various animal studies (86). This is consistent with suppression of mitogenic hormones and decreased protooncogene expression (87). The lowered incidence of mammary tumors observed in calorie-restricted rats has been attributed to reduced circulating levels of the mammotropic hormones estrogen and prolactin (88).

Oxidized DNA is repaired by a series of glycosylases that are specific for particular oxidized bases and possibly by nonspecific excision repair enzymes. In the absence of cell division these oxidative lesions are removed from DNA quite effectively and the mutation rate is kept to a minimum. Oxidized proteins are degraded by proteases. Lipid hydroperoxides are destroyed by glutathione peroxidase. Almost all of these defenses appear to be inducible, as are most other types of defenses—i.e., the amounts increase in response to damage. There is a large literature showing that cells respond to low levels of radiation, an oxidative mutagen, by inducing antioxidant defenses that help to protect them against mutation by high levels of radiation (91, 92).

In addition to the protective effects of endogenous enzymatic antioxidant defenses, consumption of dietary antioxidants appears to be of great importance. Fruits and vegetables, the main source of antioxidants in the diet, are associated with a lowered risk of degenerative diseases. Block and her colleagues (93) have recently reviewed 172 studies in the epidemiological literature that relate, with great consistency, the lack of adequate consumption of fruits and vegetables to cancer incidence (Table 1). The quarter of the population with low dietary intake of fruits and vegetables has double the cancer rate for most types of cancer (lung, larynx, oral cavity, esophagus, stomach, colon and rectum, bladder, pancreas, cervix, and ovary) when compared with the quarter with high intake. Data on the types of cancer known to be associated with hormone levels are not as consistent and show less protection by fruits and vegetables: for breast cancer the protective effect was about 30% (93, 94). There is also literature on the protective effect of fruit and vegetable consumption on cardiovascular disease and stroke (95, 96). Only 9% of Americans eat five servings of fruits and vegetables per day, the intake recommended by the National Cancer Institute and the National Research Council (93, 97). European countries with low fruit and vegetable intake (e.g., Scotland) are generally in poorer health and have higher rates of cardiovascular disease and cancer than countries with high intake (e.g., Greece) (98).

The cost of fruits and vegetables is an important factor in discouraging consumption. Poorer people spend a higher percentage of their income on food, eat less fruits and vegetables (99), and have shorter life expectancy than wealthier people. A major contributor to health in this century was synthetic pesticides, which markedly decreased the cost of food production and ensured that most of the crops planted would be eaten by

humans rather than insects (100). Synthetic pesticide residues do not appear in the general population to be a significant cause of cancer (44).

Dietary Antioxidants. The effect of dietary intake of the antioxidants ascorbate, tocopherol, and carotenoids is difficult to disentangle by epidemiological studies from other important vitamins and ingredients in fruits and vegetables (93, 101). Nevertheless, several arguments suggest that the antioxidant content of fruits and vegetables is a major contributor to their protective effect. (*i*) Biochemical data, discussed above, show that oxidative damage is massive and is likely to be the major endogenous damage to DNA, proteins, and lipids. (*ii*) Oxidative damage to sperm DNA is increased when dietary ascorbate is insufficient (see below). (*iii*) Epidemiological studies and intervention trials on prevention of cancer and cardiovascular disease in people taking antioxidant supplements are suggestive, though larger studies need to be done (95, 102, 130, 146, 147, 172). Clinical trials using antioxidants will be the critical test for many of the ideas discussed here. (*iv*) Studies on oxidative mechanisms and epidemiology on antioxidant protection for individual degenerative diseases are discussed below.

Small-molecule dietary antioxidants such as vitamin C (ascorbate), vitamin E (tocopherol), and carotenoids have generated particular interest as anticarcinogens and as defenses against degenerative diseases (103–106). Most carotenoids have antioxidant activity, particularly against singlet oxygen, and many, including β-carotene, can be metabolized to vitamin A (retinal) (107–110). Earlier papers have called attention to a number of previously neglected physiological antioxidants, including urate, bilirubin, carnosine, and ubiquinol (111–114). Ubiquinone (CoQ_{10}), for example, is the critical small molecule for transporting electrons in mitochondria for the generation of energy. Its reduced form, ubiquinol, is an effective antioxidant in membranes (112, 115–117). Optimal levels of dietary ubiquinone/ubiquinol could be of importance in many of the degenerative diseases.

Antioxidants and Cancer. A critical factor in mutagenesis is cell division (72, 118, 119). When the cell divides, an unrepaired DNA lesion can give rise to a mutation. Thus an important factor in mutagenesis, and therefore carcinogenesis, is the cell division rate in the precursors of tumor cells. Stem cells are important as precursor cells in cancer because they are not on their way to being discarded. Increasing their cell division rate would increase mutation. As expected, there is little cancer in nondividing cells. Such diverse agents as chronic infection (see above), high levels of particular hormones (120), or chemicals at doses that cause cell death (72, 119, 121, 122) result in increased cell division and therefore an increased risk for cancer.

Oxidants form one important class of agents that stimulate cell division (123, 124, 216). This may be related to the stimulation of cell division that occurs during the inflammatory process accompanying wound healing (72). Antioxidants therefore can decrease mutagenesis, and thus carcinogenesis, in two ways: by decreasing oxidative DNA damage and by decreasing cell division. Of great interest is the understanding of mechanisms by which tocopherol and carotenoids can prevent cell division (125–128).

There is an increasing literature on the protective role of dietary tocopherol, ascorbate, and β-carotene in lowering the incidence of a wide variety of human cancer (129–132, 217) (see also Table 1). Antioxidants can counteract the induction of cancer in rodents by a variety of carcinogens (133–135). Two of the major causes of cancer—cigarette smoke and chronic inflammation—appear to involve oxidants in their mechanism of action. Almost all of the epidemiological studies that examined the relation between antioxidant levels and cigarette-induced lung cancer showed a statistically significant protective effect of antioxidants (93, 129, 131). Antioxidants inhibit much of the pathology of cigarette smoke in rodents (136, 137). Inflammatory reactions release large amounts of NO, a radical, nitrosating agent, and indirect mutagenic oxidant (55, 138, 139). Ascorbate inhibits nitrosation under physiological conditions (140). Antioxidants help to protect against the carcinogenic effects of chronic inflammation, as discussed above.

Antioxidants and Cardiovascular Disease. A major development in cardiovascular disease research is the finding that oxidation reactions play a central role in atherogenesis (141) and that in epidemiological studies (reviewed in refs. 95 and 96) cardiovascular disease is associated with low plasma concentrations of ascorbate, tocopherol, and β-carotene (95, 130, 142–148). A wealth of evidence suggests that oxidative modification of apolipoprotein B100 plays a key role in recognition of low density lipoprotein (LDL) and that LDL uptake by scavenger receptors in macrophages leads to foam-cell formation and atherosclerotic plaques (refs. 40, 141, and 149–154; reviewed in ref. 155). Apolipoprotein B100 can be altered by reactive products of lipid peroxidation that causes a net decrease in positive charge, a modification that leads to its recognition by the scavenger receptors. The beneficial effects of dietary antioxidants are also strengthened by animal (ref. 156; reviewed in ref. 96) and biochemical (103, 115, 155, 157–160) studies.

Antioxidants and the Immune System. The proliferation of T and B cells, natural killer cells, and lymphokine-activated killer cells that is required to mount an effective defense against pathogens and tumor cells appears to be inhibited markedly with age (161) and upon exposure to oxidants (162, 163). These effects can, in part, be counteracted in elderly individuals by dietary antioxidant supplementation (164–166). The endogenous sources of oxidants that lead to the suppression of lymphocyte-dependent immunity are not known. *In vitro* studies, however, have demonstrated that both polymorphonuclear leukocytes and macrophages can inhibit proliferation of various lymphocyte subpopulations through the production of reactive oxygen intermediates and prostaglandin E_2 (167) and NO (168). This suggests that conditions that involve infiltration of polymorphonuclear leukocytes and macrophages (i.e., chronic inflammatory diseases) could result in compromised lymphocyte function. The suppressive effects of macrophages on mitogen-induced lymphocyte proliferation can be reversed partially by thiol reagents (169), by catalase or indomethacin (167), or by N^G-monomethyl-L-arginine, a competitive inhibitor of NO synthesis (170). The age-associated decrease in cell-mediated immunity may be due to a decreased level of certain small-molecule antioxidants and antioxidant enzyme activities. Calorie restriction, a dietary regimen that increases maximal lifespan in rodents, also enhances T-lymphocyte responsiveness (171), possibly by slowing the rate of thymus involution and by increasing the level of cellular antioxidant defenses (82).

Antioxidants and Cataracts. Cataract removal is the most common operation in the U.S. (1.2 million per year) with costs of over 3 billion dollars. Taylor (172) has recently reviewed the impressive evidence that cataracts have an oxidative etiology and that dietary antioxidants can prevent their formation in humans. Five epidemiological studies that have examined the effect of dietary antioxidants on cataracts show strong preventative effects of ascorbate, tocopherol, and carotenoids (173–177). Those individuals taking daily supplements of ascorbate or tocopherol had about one-third the risk of developing cataracts. Smoking, a severe oxidative stress, is a major risk factor for cataracts, and radiation, an oxidative mutagen, is well known to cause cataracts (178, 179). Eye proteins show an increased level of methionine sulfoxide with age, and proteins in human cataracts have >60% of their methionine residues oxidized (180). Pregnant mice depleted of glutathione, the main sulfhydryl antioxidant in cells, produce offspring with cataracts (181). The most promising preventative strategy against cataracts appears

to be to increase dietary antioxidants and to decrease smoking (172).

Antioxidants and Brain Dysfunction. Biochemical studies suggest that oxidation may be important in a number of brain pathologies (182–188). The few epidemiological studies are consistent with a protective effect of fruits and vegetables or antioxidants (104, 105, 189) in a number of neurological pathologies, including brain ischemia, Parkinson disease, and familial amyotrophic lateral sclerosis (Lou Gehrig's disease), a degenerative disorder of motor neurons (190, 191). Ischemic episodes liberate iron, an important catalyst in reactions forming oxygen radicals; iron chelators reduce neuron loss following this trauma (192). In individuals suffering from Parkinson disease, oxidative DNA damaged is elevated within brain regions rich in dopaminergic neurons (E. Övervik, J. Sanchez-Ramos, and B.N.A., unpublished work). The most convincing evidence so far for a link between neurological disorders and oxygen radical formation is the strong association found between familial amyotrophic lateral sclerosis and mutations in the Cu/Zn superoxide dismutase gene, suggesting that oxygen radicals might be responsible for the selective degeneration of motor neurons occurring in this fatal disease (191). The protective role of superoxide dismutase against brain injury due to ischemia is supported by the finding that its overproduction is protective in a transgenic mouse model (193). Based on the similar protective effects against ischemia-induced brain injury by inhibition of NO formation, and the recent evidence implicating these two radical species in cytotoxicity of neuronal cells (194, 195), it would appear that peroxynitrite, a powerful oxidant formed from the combination of O_2^- and NO (196), plays an important role in neuronal injury following ischemia and reperfusion (197).

Oxidant Stress, Birth Defects, and Childhood Cancer. Oxidative lesions in sperm DNA are increased 250% when levels of dietary ascorbate are insufficient to keep seminal fluid ascorbate at an adequate level (198) (Fig. 3). A sizable percentage of the U.S. population ingests inadequate levels of dietary ascorbate, particularly single males, the poor, and smokers (199). The oxidants in cigarette smoke deplete the antioxidants in plasma. Smokers must eat 2–3 times more ascorbate than nonsmokers to achieve the same level of ascorbate in blood (32–34), but they rarely do. In comparisons of sperm from smokers and nonsmokers (200, 201) the number of sperm and the percentage of motile sperm decrease significantly in smokers, and this decrease was dependent on the dose and duration of smoking. Paternal smoking, in particular, appears to increase the risk of birth defects and childhood cancer in offspring (200, 202–211). One expects, and finds, a much larger contribution to the germ-line mutation rate from the father than from the mother, with the age of the father being an important risk factor (212). Thus, inadequate diets (and smoking) of fathers appear to result in damage not only to their own DNA but also to the DNA of their sperm, an effect that may reverberate down future generations.

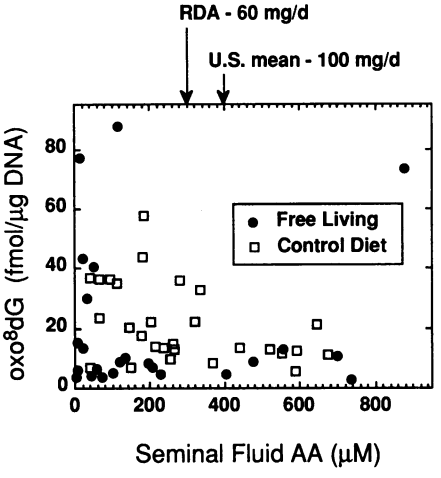

FIG. 3. Relation of seminal-fluid ascorbic acid (AA) to oxidative DNA damage in sperm. Data from ref. 198 were replotted by P. Motchnik. RDA, recommended daily allowance.

The Optimum Level of Antioxidants. The epidemiological evidence and the guidelines of the National Cancer Institute and the National Research Council/National Academy of Sciences suggest that at least two fruits and three vegetables per day is a desirable intake. Since ascorbate, tocopherol, and β-carotene supplements are inexpensive and high doses are remarkably nontoxic, there is a school that believes that supplements, in addition to a diet containing recommended levels of fruits and vegetables, are desirable. There is suggestive but inadequate epidemiological and biochemical evidence bearing on the question (102, 130, 146, 147). What is clear is that fruits and vegetables contain many necessary micronutrients in addition to antioxidants, some of which can also prevent mutations. Folic acid, for example, is required for the synthesis of the nucleotides in DNA. Inadequate intake has been shown to cause chromosome breaks and increased cancer and birth defects (104, 213). Folate deficiency may be a risk factor for myocardial infarction as well (214). Niacin is required for making poly(ADP-ribose), a component of DNA repair. Other micronutrients are also likely to be part of our defense systems.

The U.S. recommended daily allowances for ascorbate and tocopherol intake—there is no guideline for β-carotene independent of its provitamin A activity—are not adequate for at least two reasons. (*i*) The amount recommended (e.g., 60 mg/day for ascorbate) is primarily for avoiding an observable deficiency syndrome (e.g., scurvy) and is not necessarily the amount for optimum lifetime health, which is usually not known. (*ii*) A recommended blood level of each antioxidant (e.g., 60 μM ascorbate) would be a more desirable standard. People vary considerably in the intake required to keep their blood level adequate. A smoker, for example, needs to take in several times as much ascorbate as a nonsmoker to keep the blood level the same. Infections may also cause an oxidative stress that leads to antioxidant depletion by activating phagocytic cells. The observation that antioxidant inadequacy is associated with oxidative damage to DNA of the germ line as well as somatic cells emphasizes the urgency of defining adequate blood levels (198).

Since only 9% of Americans, and fewer in most other countries, are eating five fruits and vegetables per day, there is a great opportunity to improve health by increasing consumption.

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