Mutation Research, 250 (1991) 3–16

ADONIS 002751079100160O

MUT 02534

Endogenous mutagens and the causes of aging and cancer

Bruce N. Ames [1] and Lois Swirsky Gold [2]

[1] *Division of Biochemistry and Molecular Biology, Barker Hall, University of California, Berkeley, CA 94720 (U.S.A.)* and [2] *Cell and Molecular Biology Division, Lawrence Berkeley Laboratory, Berkeley, CA 94720 (U.S.A.)*

(Accepted 3 May 1991)

Keywords: Mutation; Cancer; Aging; Promotion

Summary

A very large oxidative damage rate to DNA occurs as part of normal metabolism. In each rat cell the steady-state level is estimated to be about 10^6 oxidative adducts and about 10^5 new adducts are formed daily. It is argued that this endogenous DNA damage is a major contributor to aging and the degenerative diseases of aging, such as cancer. The oxidative damage rate in mammalian species with a high metabolic rate, short life span, and high age-specific cancer rate is much higher than the rate in humans, a long-lived creature with a lower metabolic rate and a lower age-specific cancer rate. It is argued that deficiency of micronutrients, such as dietary antioxidants or folate, is a major contributor to human cancer and degenerative diseases.

Understanding the role of mitogenesis in mutagenesis is critical for clarifying the mechanisms of carcinogenesis and interpreting high-dose animal cancer tests. High-dose animal cancer tests have been done mainly on synthetic industrial chemicals, yet almost all of the chemicals humans are exposed to are natural. About half of natural chemicals tested in high-dose animal cancer tests are rodent carcinogens, a finding that is consistent with the view that high-dose tests frequently increase mitogenesis rates. Animals have numerous defenses against toxins that make them very well buffered against low doses of almost all toxins, whether synthetic or natural.

Aging, cancer, and endogenous sources of DNA damage

A marked decrease in age-specific cancer rates has accompanied the marked increase in life span that has occurred in 60 million years of primate evolution, e.g., at two years of age, cancer rates are high in rodents, but are extremely low in humans. Cancer incidence increases with approximately the fourth power of age, both in short-lived species such as rats and mice and in long-lived species such as humans (Portier et al., 1986; Doll, 1971). Thus, cancer is one of the degenerative diseases of old age, though exogenous factors can substantially increase it (e.g., cigarette smoking in humans) or decrease it (e.g., calorie restriction in rodents). One important factor in longevity appears to be basal metabolic rate (Cutler, 1984), which is much lower in man than in rodents and

Correspondence: Bruce N. Ames, Division of Biochemistry and Molecular Biology, Barker Hall, University of California, Berkeley, CA 94720 (U.S.A.).

could markedly affect the level of endogenous mutagens produced by normal metabolism.

Aging is thought to occur because nature selects for many genes that have immediate survival value, but that have long-term deleterious consequences (Williams and Nesse, 1991). The burst of NO, O_2^-, H_2O_2, and OCl^- from white blood cells, for example, protects against bacterial and virus infections, but contributes to DNA damage and mutation. It seems plausible that DNA damage is likely to be critical for both cancer and aging. One view of the somatic damage theory of aging is that the amount of maintenance of somatic tissues is always less than that required for indefinite survival because a considerable proportion of an animal's resources is devoted to reproduction at a cost to maintenance. Thus, some DNA damage that is induced in somatic cells by endogenous mutagens will accumulate with time and contribute to aging and the degenerative diseases associated with aging such as cancer.

Four endogenous processes leading to significant DNA damage are likely to be oxidation (Harman, 1981; Totter, 1980; Ames, 1983), methylation, deamination, and depurination (Saul and Ames, 1986). The importance of these processes is supported by the existence of specific DNA repair glycosylases for oxidative, methylated and deaminated adducts, and a repair system for apurinic sites that are produced by spontaneous depurination (Lindahl, 1982). The measurement of DNA adducts by new methods shows that DNA damage produced by oxidation (see below) could be the most significant endogenous damage.

Oxidative DNA damage

Oxidants are produced as by-products of normal metabolism and of lipid peroxidation (Figs. 1 and 2).

Non-specific DNA repair enzymes excise DNA adducts to release deoxynucleotides, and specific DNA repair glycosylases release free bases. Deoxynucleotides are enzymatically hydrolyzed to deoxynucleosides that are not usually further metabolized, and both these and the free bases may be recovered in the urine. Two products of oxidative damage to DNA are thymine glycol and

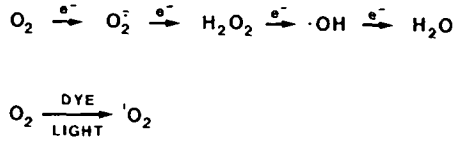

Fig. 1. Oxidants from normal metabolism. The formation of superoxide, hydrogen peroxide, and hydroxyl radicals by successive additions of electrons to oxygen. Cytochrome oxidase adds 4 electrons fairly efficiently during energy generation in mitochondria, but some of these toxic intermediates are inevitable by-products. The same oxidants are produced in copious quantities from phagocytic cells. Singlet oxygen is generated from oxygen by the absorption of energy from a dye activated by light.

5-hydroxymethyluracil. A specific DNA repair enzyme, a DNA glycosylase in mouse cells, repairs 5-hydroxymethyluracil and differs from the specific DNA glycosylase repair enzyme for thymine glycol in mouse cells (Hollstein et al., 1984). The existence of these *specific* repair enzymes points to the importance of this type of DNA damage in vivo.

The postulated importance of endogenously produced oxidative damage to DNA in aging and age-related degenerative pathologies such as can-

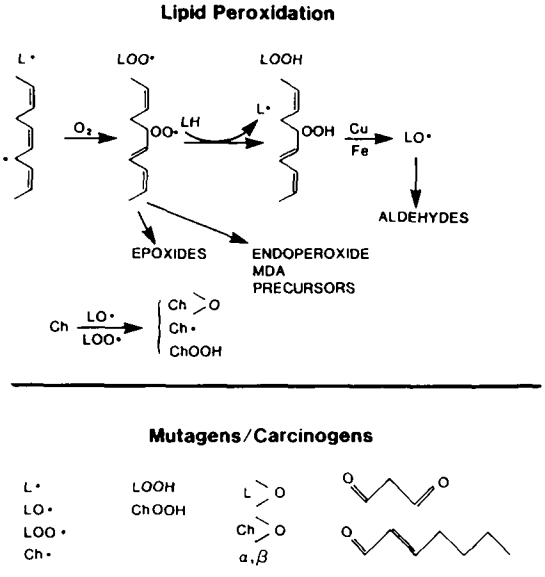

Fig. 2. Lipid peroxidation (Ames, 1988). L = lipid radical; L' = alkoxy lipid radical; LOO· = hydroperoxy lipid radical; LOOH = lipid hydroperoxide; Ch = cholesterol; Ch > O = cholesterol epoxide; L > O = lipid epoxide; MDA = malondialdehyde.

cer has prompted efforts to develop rapid methods that measure this damage (Cathcart et al., 1984; Ames, 1989; Adelman et al., 1988; Richter et al., 1988; Fraga et al., 1990). Endogenously produced oxidative damage to DNA has been assayed by measuring the urinary levels of the known radiation damage products thymine glycol, thymidine glycol, hydroxymethyluracil, and hydroxymethyldeoxyuridine by HPLC with UV detection (Cathcart et al., 1984; Saul et al., 1987; Adelman et al., 1988). Our results indicate that normal humans excrete a total of about 100 nmol/day of the first three compounds. We have considerable evidence that most of this total is derived from repair of oxidized DNA, rather than from alternative sources, such as diet or bacterial flora (Cathcart et al., 1984; Saul et al., 1987; Ames and Saul, 1988). This 100 nmol may therefore represent an average of about 10^3 oxidized thymine residues per day for each of the body's 6×10^{13} cells. Because these products are only three of > 20 products of oxidative damage of DNA (Cadet and Berger, 1985; von Sonntag, 1987), the total number of all types of oxidative hits to DNA per cell per day may be about 10^4 in man and about 10^5 in the rat.

A more easily assayed product of oxidative DNA damage is 8-hydroxydeoxyG (oh^{8}dG) which can be measured with great sensitivity by HPLC-EC (Floyd et al., 1986). oh^{8}dG is a mutagen (Kuchino et al., 1987; Shibutani et al., 1991) formed in DNA by γ-irradiation (Dizdaroglu, 1985) and various carcinogens (Kasai et al., 1987; Fiala et al., 1989; Rosier and VanPeteghem, 1989). Polyclonal antibodies that recognize oh^{8}dG have been produced and their binding properties characterized (Degan et al., 1991). An immunoaffinity column facilitates the isolation of oh^{8}dG, oh^{8}Gua, and oh^{8}G from urine (Shigenaga et al., 1989; Degan et al., 1991) (Fig. 3). We have now produced monoclonal antibodies to oh^{8}dG that are even more effective. The steady-state level of oh^{8}dG lesions in liver DNA from 1-year-old Fischer 344 rats is 64,000 residues/cell or about 1/224,000 bases; about 4000 molecules of oh^{8}dG and oh^{8}Gua are excreted per cell per day (Fraga et al., 1990). Taking into account that this lesion is only one of > 20 known γ-irradiation-induced DNA damage products (von Sonntag, 1987), we estimate that each rat cell contains approximately 10^6 oxidatively damaged bases in its DNA (Richter et al., 1988; Fraga et al., 1990) and that about 10^5 oxidative hits to the DNA occur per rat cell per day (Cathcart et al., 1984; Fraga et al., 1990). This estimate based on oh^{8}dG is in agreement with the earlier estimate based on thymidine glycol. The repair rate almost equals the damage rate; however, we estimate in DNA isolated from kidney of young and old rats that 80 oh^{8}dG residues accumulate per cell per day (Fraga et al., 1990).

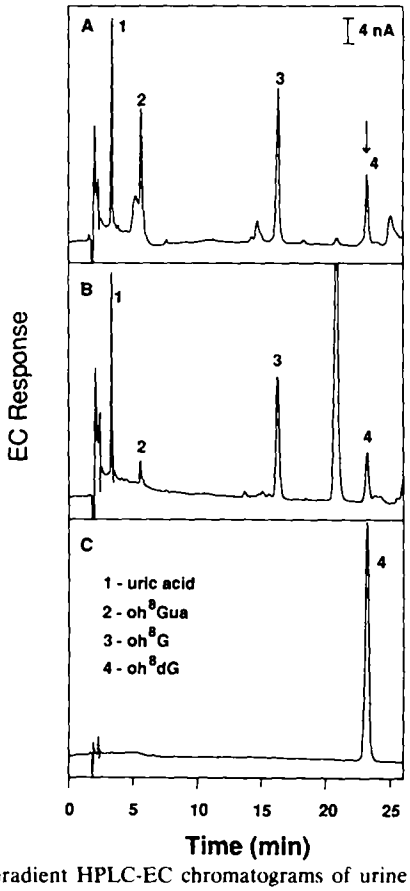

Fig. 3. Gradient HPLC-EC chromatograms of urine samples processed by C18/OH solid phase extraction and the anti-oh^{8}dG immunoaffinity columns. (A) Rat urine. (B) Human urine. (C) oh^{8}dG standard (5 pmol). The amounts of urine analyzed were equivalent to 0.26 and 0.34 ml of urine for the rat and human samples, respectively. The arrow denotes the retention time of oh^{8}dG (peak 4). Peaks 1, 2, and 3 correspond to the retention times for uric acid, oh^{8}Gua, and oh^{8}G, respectively (Degan et al., 1991).

The oxidative DNA damage rate as measured by thymidine glycol excretion in urine for mouse, rat, monkey, and man is related to the metabolic rate and is inversely related to the age-specific cancer rate and life span (Adelman et al., 1988). We have found a similar relation with oh^{8}dG excretion (Shigenaga et al., 1989).

Defenses against oxidants

Many defense mechanisms within the organism have evolved to limit the levels of reactive oxidants and the damage they induce. Among the defenses are enzymes such as SOD, catalase, and GSH peroxidase as well as the dietary antioxidants β-carotene, α-tocopherols, and vitamin C. We have been particularly interested in antioxidant micronutrients because their level in humans can be altered, and we have discussed five previously unappreciated antioxidants.

(1) *Uric acid* is a powerful antioxidant that appeared in primate evolution concomitantly with the development of a longer life span and a large, metabolically active brain (Ames et al., 1981). Uric acid is the main antioxidant in saliva. Its concentration in human blood is 300 μM, and it is present in lower concentrations in prosimians. Since uric acid levels increased in primate evolution at about the same time that the ability to synthesize ascorbic acid was lost, these events may be related.

(2, 3) Heme is degraded to *biliverdin*, and in mammals biliverdin is converted to *bilirubin*, both of which have been shown to be powerful antioxidants (Stocker and Ames, 1987; Stocker et al., 1987a,b). The bilirubin in human blood is bound at a specific site on albumin at a concentration of 20 μM (Stocker and Ames, 1987; Stocker et al., 1987a,b). This is a much higher level than in rat blood. Conjugated bilirubin also appears to be the most important antioxidant in bile, and when combined with copper ions present in bile, forms a powerful redox system for oxidizing xenobiotics and destroying hydroperoxides (Stocker and Ames, 1987).

(4) Ubiquinone-10 (coenzyme Q_{10}) acts as an electron carrier of the respiratory chain in mitochondria. *Ubiquinol*-10, the reduced form of ubiquinone-10, also efficiently scavenges free radicals that are generated chemically within liposomal membranes (Frei et al., 1990). Ubiquinol-10 is about as effective in preventing peroxidative damage to lipids as is α-tocopherol, which is considered the best soluble antioxidant in humans. It is known that ubiquinol-10 can be recycled by electron transport carriers present in various biomembranes and possibly by some enzymes. In contrast to tocopherol, ubiquinol-10 is not recycled by ascorbate. We have also shown that ubiquinol-10 spares α-tocopherol when both antioxidants are present in the same liposomal membranes and that ubiquinol-10, like tocopherol, does not interact with reduced glutathione. Our data, together with previous work on the antioxidant function of ubiquinol reported in the literature, strongly suggest that ubiquinol-10 is an important physiological lipid-soluble antioxidant. It is the main lipid-soluble antioxidant in *E. coli* and Salmonella, which do not contain vitamin E, and it may be particularly important in mitochondria.

(5) *Carnosine*, which is present in high concentrations in human muscle and brain, has been shown to have antioxidant properties: it chelates copper and iron ions so as to inhibit oxidative reactions and also is a moderately good radical scavenger. We have postulated that it is a physiologically significant antioxidant (Kohen et al., 1988).

Ascorbate is the most important antioxidant in the aqueous compartment of human plasma. The temporal order of antioxidant consumption in human blood plasma exposed to a constant flux of aqueous peroxyl radicals is ascorbate > bilirubin > urate > α-tocopherol; detectable lipid peroxidation starts only after ascorbate has been consumed completely (Frei et al., 1989). Plasma devoid of ascorbate, but no other endogenous antioxidant, is extremely vulnerable to oxidant stress and susceptible to peroxidative damage to lipids (Frei et al., 1989). This oxidant stress can be caused by a chemical that generate radicals, by activated white cells (Frei et al., 1988), and by cigarette smoke (Frei et al., 1991).

A study of the effect of seminal fluid ascorbic acid on the levels of oh^{8}dG in sperm DNA (Fraga et al., 1991) showed the following: (a) in individuals whose dietary intake of ascorbate was de-

creased from 250 to 5 mg/day, the levels of seminal fluid ascorbate levels were halved and the levels of oh^8dG in sperm DNA were doubled; (b) repletion of dietary ascorbate led to a decrease in the steady-state levels of oh^8dG. In another group of 26 subjects, the steady-state levels of oh^8dG correlates inversely with the content of seminal plasma ascorbate. These results indicate both that human sperm DNA is subjected to endogenous oxidative damage and that ascorbate appears to protect against such oxidative damage. The high level of oxidative damage to sperm DNA reported here could be related to a higher risk of birth defects, particularly in populations with low ascorbate levels, such as smokers.

Because of the time interval between the generation of oxidants and their destruction by various defense mechanisms, low levels of oxidants can persist for sufficient time to produce damage to cellular macromolecules (Chance et al., 1979). For nuclear DNA, however, the mammalian cell has three more levels of defense. First, nuclear DNA is compartmentalized away from mitochondria and peroxisomes where most oxidants are probably generated. Second, most nonreplicating nuclear DNA is surrounded by histones and polyamines that may protect against oxidants. Finally, most types of DNA damage can be repaired by efficient enzyme systems. The net result of this multilevel defense is that nuclear DNA is very well protected, but not completely protected from oxidants.

MITOGENESIS INCREASES MUTAGENESIS

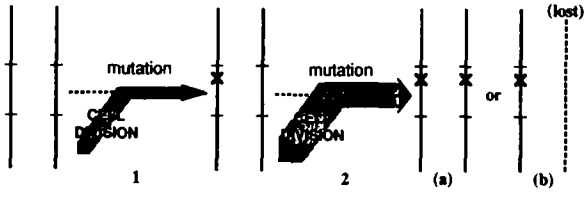

Fig. 4. Mitogenesis (induced cell division) is a major multiplier of endogenous (or exogenous) DNA damage leading to mutation. The pathway to inactivating (×) both copies of a recessive tumor suppressor gene is shown (two vertical lines represent the pair of chromosomes carrying the genes). Cell division increases mutagenesis due to the following: DNA adducts converted to mutations before they are repaired (1 and 2a); mutations due to DNA replication (1 and 2a); vulnerability of replicating DNA to damage (1 and 2a). Mitotic recombination (2a), gene conversion (2a), and nondisjunction (2b) are more frequent, and the first two give rise to the same mutation on both chromosomes. This diagram does not attempt to deal with the complex mutational pathway to tumors (Kinzler et al., 1991; Fearon et al., 1990).

Oxidants are a stimulus for mitogenesis, a major risk factor for carcinogenesis (see below). Therefore antioxidants are important for decreasing mitogenesis as well as for scavenging mutagens. Chronic inflammation is a risk factor for cancer (Weitzman and Gordon, 1990; Templeton, 1980; Lewis and Adams, 1987; Madsen, 1989). The oxidants produced by phagocytic cells during inflammation are signals for mitogenesis (to promote wound healing) (Crawford and Cerutti, 1988; Chan et al., 1986; Craven et al., 1987; Sieweke, 1989; Burdon et al., 1990). Both *fos* and *jun* oncogenes are under oxidative control (Abate et al., 1990) and could conceivably be the genes involved in the proliferative response of wound healing.

Mutagenesis, mitogenesis, and carcinogenesis

Geneticists have long known that cell division is critical for mutagenesis. If one accepts that mutagenesis is important for carcinogenesis, it follows that mitogenesis rates must be important. The inactivation of tumor suppressor genes is also known to be important in carcinogenesis, and recent evidence suggests that one of the functions of tumor suppressor genes is to inhibit mitogenesis (Stanbridge, 1990). Once the first copy of a tumor suppressor gene is mutated, the inactivation of the second copy (loss of heterozygosity) is more likely to be caused by processes whose frequency is dependent on cell division (mitotic recombination, gene conversion, and non-disjunction) than by an independent second mutation (Ames and Gold, 1990a,b). Therefore loss of heterozygosity will be stimulated by increased mitogenesis. Thus, while the stimulation of mitogenesis increases the chance of every mutational step, it is a much more important factor for tumor induction after the first mutation has occurred. This explains why mutagenesis and mitogenesis are synergistic (Ames and Gold, 1990a,b) and why mitogenesis after the first mutation is more effective than before (Fig. 4).

Thinking of chemicals as "initiators" or "promoters" confuses mechanistic issues (Iversen, 1988). The idea that "promoters" are not in themselves carcinogens is not credible on mechanistic grounds and is not correct on experimental grounds (Ames and Gold, 1990a,b; Iversen, 1988). Every classical "promoter" that has been tested adequately, e.g., phenobarbital, catechol, TPA, is a carcinogen. The very word "promoter" confuses the issue, since mitogenesis may be caused by one dose of a chemical and not by a lower dose. Dominant oncogenes and their clonal expansion by mitogenesis can clearly be involved in carcinogenesis, adding complexity; however, these mechanisms are still consistent with the view that mitogenesis is an important factor in carcinogenesis. Nongenotoxic agents, e.g., saccharin, can be carcinogens at high doses just by causing cell killing with chronic mitogenesis and inflammation, and the dose response would be expected to show a threshold (Ames and Gold, 1990a; Butterworth and Slaga, 1991; Cohen and Ellwein, 1990). Epigenetic factors are also involved in carcinogenesis. However, both mitogenesis (e.g., through mitotic recombination) and DNA damage can cause loss of 5-methylC or other epigenetic modification, as we have discussed (Ames and Gold, 1990a). Chronic mitogenesis by itself can be a risk factor for cancer: theory predicts it and a large literature supports it (Ames and Gold, 1990a; Preston-Martin et al., 1990). The 40% of rodent carcinogens that are not detectable mutagens, should be investigated to see if their carcinogenic effects at high dose result from induction of mitogenesis; if so, then such rodent carcinogens would be unlikely to be a risk at low doses.

Genotoxic chemicals, because they hit DNA, are even more effective than nongenotoxic chemicals at causing cell killing and cell replacement at high doses. Since genotoxic chemicals also act as mutagens, they can produce a multiplicative interaction not found at low doses, leading to an upward curving dose response for carcinogenicity (Ames and Gold, 1990a; Butterworth and Slaga, 1991; Cohen and Ellwein, 1990). Mitogenesis can often be the dominant factor in chemical carcinogenesis at the high, nearly toxic doses used in rodent bioassays, even for mutagens. Mitogenesis can be caused by toxicity of chemicals at high dose (cell killing and subsequent replacement), by interference with cell-cell communication at high doses (Trosko et al., 1983, 1990a,b; Trosko, 1989) by substances such as hormones binding to receptors that control cell division (Preston-Martin et al., 1990), by oxidants (the wound healing response), by viruses, etc. (Ames and Gold, 1990a). The important factor is not toxicity, but increased mitogenesis in those cells that are not discarded. Work on radiation has also supported the idea of both mutagenesis and mitogenesis being important in tumor induction (Jones et al., 1983; Jones, 1984; Little et al., 1985; Ootsuyama and Tanooka, 1991).

In rodents, calories may be the most interesting carcinogen (Roe, 1989; Boutwell and Pariza, 1987; Roe et al., 1991). A calorie-restricted diet, compared to an ad libitum diet, significantly increases the life span of rats and mice and markedly decreases the cancer rate. It is striking that in calorie-restricted animals mitogenesis rates are markedly lowered in a variety of tissues (Lok et al., 1990; Heller et al., 1990): this could in principle account for much of the decrease in the cancer rate.

Epigenetic changes in DNA such as 5-methylcytosine appear important in turning off genes in differentiation and could play a role in both cancer (Vorce and Goodman, 1989a,b; Fearon et al., 1990; Holliday, 1987a) and aging (Holliday 1987a,b). It has been observed that the 5-methylC level decreases with age (Wilson et al., 1987) and it is known that cells are de-differentiating with age (Hartman, 1983; Hartman and Morgan, 1985). Folate deficiency (Bhave et al., 1988; Cravo et al., 1991), mitogenesis, and DNA damage (Cannon et al., 1988) would all be expected to increase the rate of loss of 5-methylC.

Causes of human cancer

The high endogenous level of oxidative adducts reinforces evidence from epidemiology that both deficiency of antioxidants (National Research Council, 1989; Bendich and Butterworth, 1991) and mitogenesis (Ames and Gold, 1990a; Preston-Martin et al., 1990) are likely to be important risk factors for cancer.

Mitogenesis and cancer

Henderson and co-workers (Henderson et al., 1988; Preston-Martin et al., 1990) and others (Ames and Gold, 1990a), have discussed the importance of chronic mitogenesis for many, if not most, of the known causes of human cancer, e.g., hormones in breast cancer, hepatitis B (Dunsford et al., 1990) or C viruses or alcohol in liver cancer, high salt or Helicobacter (Campylobacter) infection in stomach cancer; papilloma virus in cervical cancer; asbestos or tobacco smoke in lung cancer; and excess animal fat and low calcium in colon cancer. For chemical carcinogens associated with occupational cancer, worker exposure has been primarily at high, near-toxic, doses that might be expected to induce mitogenesis. Permitted worker exposure levels for some rodent carcinogens are too close to the doses that induce tumors in test animals (Gold et al., 1987). For high occupational exposures little extrapolation is required from the doses used in rodent bioassays, and therefore assumptions about extrapolation are less important.

Dietary imbalances

Epidemiologists have been accumulating evidence that unbalanced diets are major contributors to heart disease and cancer and are likely to be as important as smoking. The main dietary imbalances are too few fruits and vegetables and too much fat. Particular micronutrients in fruits and vegetables that appear to be important in disease prevention are antioxidants (carotenoids, tocopherols, ascorbate) and folic acid, but many more vitamins and essential minerals may also be of interest (Bendich and Butterworth, 1991; National Research Council, 1989; Reddy and Cohen, 1986). Micronutrients are components of the defenses against oxidants and other endogenous mutagens contributing to the degenerative diseases associated with aging: cancer, heart disease, cataracts, etc. Since endogenous oxidative DNA damage is enormous, there are good theoretical reasons for thinking that antioxidants should be as important as they are being found to be. Surveys have indicated that 91% of the U.S. population is not eating sufficient fruit and vegetables; almost half of the population had eaten neither fruits nor vegetables on the day of the survey (Patterson and Block, 1991).

Work on folate deficiency in mice showing that it results in chromosome breakage (MacGregor et al., 1990) reinforces the large literature (Bendich and Butterworth, 1991; National Research Council, 1989) indicating that folate deficiency is an important cause of chromosome breaks, cancer, and birth defects. Again, a sizeable proportion of the population (30% or more) may not be ingesting sufficient folate.

In the quest to delay aging and prevent cancer and heart disease it is important to understand what level of each micronutrient is optimal for long-term effects. The RDA (U.S. Recommended Daily Allowance) is based on the level necessary to prevent an immediate pathological effect, but long-term optimal levels may be higher. The great genetic variability of the human species makes it likely that many people will require a higher than average optimal RDA for particular micronutrients. This will require the development of in vivo assays of short-term damage that can be measured in humans. The measurement of DNA damage in humans is clearly relevant (Degan et al., 1991).

Epidemiology and cancer trends

Epidemiologists are frequently discovering clues about the causes of human cancer, and the resulting hypotheses are then refined by animal and metabolic studies. Current epidemiologic data point to several risk factors for human cancer: cigarette smoking (which is responsible for 30% of cancer deaths), dietary imbalances, infections, hormones, and occupation. "The age adjusted mortality rate for all cancers combined except lung cancer has been declining since 1950 for all individual age groups except 85 and above" (National Cancer Institute, 1988). This conclusion is also supported by the West European cancer mortality data (Doll, 1990). Although incidence rates for some cancers have been rising, trends in recorded incidence rates may be biased by improved registration and diagnosis (Doll, 1990). Even though mortality rates for cancers at particular sites can be shown to be increasing (for

example, non-Hodgkins lymphoma, melanoma) or decreasing (for example, stomach, cervical, rectal), establishing causes remains difficult because of the many changing aspects of our lifestyle. Life expectancy continues to increase every year.

Cancer clusters in small areas are expected to be common by chance alone, and epidemiology lacks the power to establish causality in these cases (Higginson, 1988). It is important to show that a pollution exposure that purportedly causes a cancer cluster is significantly greater than the background of exposures to naturally occurring rodent carcinogens.

Animal cancer tests

Animal cancer tests are conducted at the maximum tolerated dose (MTD) of the test chemical for long periods of time, which can often cause chronic mitogenesis (Ames and Gold, 1990a; Butterworth and Slaga, 1991; Bernstein et al., 1985). Chronic dosing at the MTD may often be the equivalent of chronic wounding, which is known to be both a promoter of carcinogenesis in animals and a risk factor for cancer in humans (Weitzman and Gordon, 1990). Thus, a high percentage of all chemicals might be expected to be carcinogenic at chronic, near-toxic doses and this is exactly what is found. About half of all chemicals tested chronically at the MTD are carcinogens (Gold et al., 1989; Ames and Gold, 1990a; Ames et al., 1990a). It is unlikely that the high proportion of carcinogens in rodent studies is due simply to selection of suspicious chemical structures since most chemicals were selected because of their use as industrial compounds, pesticides, drugs, or food additives. Moreover, historically our knowledge to predict carcinogenicity has been inadequate.

Synthetic chemicals account for 79% (378/479) of the chemicals adequately tested in both rats and mice (Ames et al., 1990a). Despite the fact that humans eat vastly more natural than synthetic chemicals, the world of natural chemicals has never been tested systematically. Of the natural chemicals tested, approximately half are carcinogens as are synthetic chemicals (Ames et al., 1990a).

Dietary pesticides are 99.99% all natural

One major group of natural chemicals in the human diet are the chemicals that plants produce to defend themselves, the natural pesticides (Ames and Gold, 1990a). We calculate that 99.99% (by weight) of the pesticides in our diet are natural. Few natural pesticides have been tested in at least one rodent species, and again about *half* (27/52) are rodent carcinogens. These 27 occur commonly in plant foods (Ames et al., 1990a). The human diet contains thousands of natural pesticides and we estimate that the average intake is about 1500 mg per person per day (Ames and Gold, 1990a). This compares to a total of 0.09 mg per person per day of residues of about 100 synthetic pesticides (Ames and Gold, 1990a). In addition, of the mold toxins tested at the MTD (including aflatoxin), 11 out of 16 are rodent carcinogens.

Caution is necessary in interpreting the implications of ingesting natural pesticides that are rodent carcinogens when tested at high doses. It is not argued here that these dietary exposures are necessarily of much relevance to human cancer. Overall, consumption of fruits and vegetables lowers cancer rates. What is important in our analysis is that relatively high and widespread exposure to natural rodent carcinogens, and the high proportion of those tested that are rodent carcinogens, casts doubt on the relevance of far lower levels of exposures to synthetic rodent carcinogens. Particular natural pesticides that are carcinogenic in rodents can be bred out of crops if studies of mechanism indicate that they may be significant hazards to humans.

Cooking food

The cooking of food is also a major dietary source of potential rodent carcinogens. Cooking produces about 2000 mg per person per day of mostly untested burnt material that contains many rodent carcinogens – e.g., polycyclic hydrocarbons, heterocyclic amines (Sugimura, 1988; Takayama et al., 1987), furfural, nitrosamines and nitroaromatics – as well as a plethora of mutagens (Sugimura, 1988; Lucier and Hook, 1986; Hayatsu, 1991). Thus, the number and amounts of carcinogenic (or total) synthetic pesticide residues appear to be minimal compared to the

background of naturally-occurring chemicals in the diet. Roasted coffee, for example, is known to contain 826 volatile and several hundred non-volatile chemicals; 22 have been tested chronically and 17 are rodent carcinogens (Ames et al., 1990a). A typical cup of coffee contains at least 10 mg (40 ppm) of rodent carcinogens (mostly caffeic acid, catechol, furfural, hydroquinone, and hydrogen peroxide) (Ames et al., 1990a) and a thousand chemicals remain to be tested. Thus one cup of coffee contains known rodent carcinogens about equivalent in weight to the potentially carcinogenic synthetic pesticide residues one eats in a year (assuming half of the untested synthetic residue-weight will turn out to be carcinogenic in rodents) (Ames and Gold, 1990a).

The evidence on coffee and human health has been recently evaluated, but its role as a possible risk factor for cancer in humans is still unclear (International Agency for Research on Cancer, 1991). The same caution discussed above about the implications for humans of natural pesticide rodent carcinogens in the diet applies to coffee and the products of cooked food.

A broader view of the chemical world is required to identify the greatest potential human carcinogenic hazards, whether natural or synthetic; only a tiny fraction of the chemicals humans are exposed to are ever going to be tested in rodent bioassays. Recently, we compared the possible hazards of some rodent carcinogens, using the ratios Human Exposure/Rodent Potency (HERP) (Ames et al., 1987) and Permitted Exposure/Rodent Potency (PERP) (Gold et al., 1987). This HERP ranking suggests that carcinogenic hazards from current levels of pesticide residues or water pollution are likely to be of minimal concern relative to the background levels of natural substances, although one cannot say whether these natural exposures are likely to be of major or minor importance in human cancer. The PERP ranking suggests that some permitted occupational exposures rank very high.

Similarity in the toxicology of synthetic and natural toxins

It is often assumed that, because plants are part of human evolutionary history whereas synthetic chemicals are recent, the mechanisms that animals have evolved to cope with the toxicity of natural chemicals will fail to protect us against synthetic chemicals. For example, Rachel Carson stated, "For the first time in the history of the world, every human being is now subjected to contact with dangerous chemicals, from the moment of conception until death" (Carson, 1962). We find this assumption flawed for several reasons.

Defenses that animals have evolved are mostly of a general type, as might be expected, since the number of natural chemicals that might have toxic effects is so large (Ames et al., 1990b). General defenses offer protection not only against natural chemicals, but also against synthetic chemicals, making humans well buffered against toxins (Ames et al., 1990b). These defenses include the following:

(a) The continuous shedding of cells exposed to toxins: the surface layers of the mouth, esophagus, stomach, intestine, colon, skin, and lungs are discarded every few days.

(b) The induction of a wide variety of general detoxifying mechanisms, such as antioxidant defenses or the Phase II electrophile-detoxifying systems, e.g., glutathione transferases (Talalay, 1988, 1989). Cells that are exposed to small doses of an oxidant, such as radiation or hydrogen peroxide, induce antioxidant defenses and become more resistant to higher doses, whether the oxidant is synthetic or natural (Wolff et al., 1988, 1989, 1990). The induction may be effected through the oxidation of a sensor regulatory protein that binds DNA (Storz et al., 1990; Abate, 1990). Electrophiles induce Phase II detoxifying enzymes that are effective against the whole class of electrophiles whether natural or synthetic (Talalay, 1988, 1989). A wide variety of plant chemicals induce Phase II detoxifying enzymes if the chemicals can be metabolized to electrophiles (Talalay, 1988, 1989). The sensor regulatory protein may have an easily alkylated sulfhydryl group (Talalay, 1988, 1989). These plant chemicals have sometimes been considered "anticarcinogens" because low doses induce Phase II enzymes. However, it should be noted that electrophiles are usually mutagenic and that the inducing plant chemicals tested (e.g., catechol, allyl isothio-

cyanate) are, as expected for electrophiles, carcinogenic.

(c) The active excretion of planar hydrophobic molecules (natural or synthetic) out of liver and intestinal cells. This multidrug resistance (MDR) system (Kane et al., 1990) may well be a normal defense against intercalating agents, to which mitochondrial DNA is unusually sensitive (King and Attardi, 1988).

(d) DNA excision repair: this is effective against DNA adducts formed from both synthetic and natural chemicals, and is inducible in response to DNA damage.

The fact that defenses are usually general, rather than specific for each chemical, makes good evolutionary sense. The reason that predators of plants evolved general defenses against toxins is presumably to be prepared to counter a diverse and ever-changing array of plant toxins in an evolving world; if a herbivore had defenses against only a set of specific toxins it would be at a great disadvantage in obtaining new foods when favored foods became scarce or evolved new toxins.

Humans have not had time to evolve into a "toxic harmony" with all of the plants in their diet. Indeed, very few of the plants that humans eat would have been present in an African hunter-gatherer's diet. The human diet has changed drastically in the last few thousand years, and most humans are eating many recently introduced plants that their ancestors did not, e.g., coffee, cocoa, tea, potatoes, tomatoes, corn, avocados, mangoes, olives, and kiwi fruit. In addition, cruciferous vegetables such as cabbage, broccoli, kale, cauliflower, and mustard were used in ancient times primarily for medicinal purposes and were spread as foods across Europe only in the Middle Ages (Ames et al., 1990a,b). Natural selection works far too slowly for humans to have evolved specific resistance to toxins in these newly introduced plants.

DDT is often viewed as the typically dangerous synthetic pesticide because it persists for years, and bioconcentrates in the food chain due to its unusual lipophilicity. DDT was representative of a class of chlorinated pesticides. Natural pesticides, however, also bioconcentrate if lipophilic: for example, the rodent teratogens from potatoes, solanine (and its aglycone solanidine) and chaconine are found in the tissues of people who eat potatoes (Matthew et al., 1983; Claringbold et al., 1982; Harvey et al., 1985). Although DDT was unusual with respect to bioconcentration, it was remarkably non-toxic to mammals, saved millions of lives, and has not been shown to cause harm to humans (Jukes, 1974). To a large extent DDT, the first major synthetic insecticide, replaced lead arsenate, a major pesticide used before the modern era; lead arsenate is even more persistent than DDT, and although natural, both lead and arsenic are carcinogenic. When the undesirable bioconcentration and persistence of DDT and its possible lethal effects on some birds were recognized, it was prudently phased out, and less persistent chemicals were developed to replace it. Examples are the synthetic pyrethroids that disrupt the same insect sodium channel that is disrupted by DDT, that are degraded rapidly in the environment, and that can often be used in amounts as low as a few grams per acre.

Positive results are remarkably common in high-dose screening tests for carcinogens, clastogens, teratogens, and mutagens. About half of the chemicals tested, whether natural or synthetic, are carcinogens in chronic, high-dose rodent tests (Gold et al., 1989; Ames and Gold, 1990a; Ames et al., 1990a) and about half are clastogens in tissue culture tests (Ishidate et al., 1988). A high proportion of positives is also reported for rodent teratogenicity tests: 38% of the 2800 chemicals tested in laboratory animals "have been teratogenic" in the standard, high-dose protocol (Schardein, 1985). It is therefore reasonable to assume that a sizeable percentage of both synthetic and natural chemicals will be reproductive toxins at high doses. Mutagens are also common: of 384 chemicals tested for carcinogenicity in both rats and mice and for mutagenicity in Salmonella, 44% were mutagens, and mutagens were 1.6 times more likely to be carcinogenic than were non-mutagens (Gold et al., 1989, 1990). Of these 384 chemicals, 72% were either mutagens or carcinogens or both. How much this high frequency of positive results is due to bias in selecting chemicals is not known. Even if selection bias doubled the percentage of positives,

which we think is unlikely (Gold et al., 1989; Ames et al., 1990a,b), the high proportion of positives would still mean that almost everything natural we eat contains carcinogens, mutagens, teratogens, and clastogens. Thus, testing a random group of natural pesticides and pyrolysis products from cooking should be a high priority for these various tests so that an adequate comparison can be made to synthetic toxins. Synthetic pesticide residues (or water pollution) from industrial chemicals must be put in the context of the enormous background of natural substances, and there is no convincing evidence from either epidemiology or toxicology that they are of interest as causes of human cancer (Ames and Gold, 1990a; Higginson, 1988).

Acknowledgements

This work was supported by National Cancer Institute Outstanding Investigator Grant CA39910 and by National Institute of Environmental Health Sciences Center Grant ESO1896 to B.N.A. and through the Lawrence Berkeley Laboratory by U.S. Department of Energy, Contract DE-AC-03-76SF00098 and U.S. Environmental Protection Agency, Agreement R-815619-01-0 to L.S.G. This paper was adapted in part from B.N. Ames and L.S. Gold, 1990a,b; B.N. Ames, M. Profet, and L.S. Gold, 1990a,b; and B.N. Ames and L.S. Gold, "Animal cancer tests and the prevention of cancer", J. Natl. Cancer Inst., in press.

References

Abate, C., L. Patel, F.J. Rauscher III and T. Curran (1990) Redox regulation of *fos* and *jun* DNA-binding activity in vitro, Science, 249, 1157–1161.

Adelman, R., R.L. Saul and B.N. Ames (1988) Oxidative damage to DNA: Relation to species metabolic rate and life span, Proc. Natl. Acad. Sci. (U.S.A.), 85, 2706–2708.

Ames, B.N., R. Cathcart, E. Schwiers and P. Hochstein (1981) Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: A hypothesis, Proc. Natl. Acad. Sci. (U.S.A.), 78, 6858–6862.

Ames, B.N. (1983) Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases, Science, 221, 1256–1264.

Ames, B.N., R. Magaw and L.S. Gold (1987) Ranking possible carcinogenic hazards, Science, 236, 271–280.

Ames, B.N. (1988) Measuring oxidative damage in humans: Relation to cancer and aging, in: H. Bartsch, K. Hemminki and I.K. O'Neill (Eds.), Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention, IARC Scientific Publications No. 89, International Agency for Research on Cancer, Lyon, pp. 407–416.

Ames, B.N., and R.L. Saul (1988) Cancer, aging, and oxidative DNA damage, in: O.H. Iversen (Ed.), Theories of Carcinogenesis, Hemisphere, Washington, DC, pp. 203–220.

Ames, B.N. (1989) Endogenous oxidative DNA damage, aging and cancer, Free Rad. Res. Commun., 7, 121–128.

Ames, B.N., and L.S. Gold (1990a) Chemical carcinogenesis: Too many rodent carcinogens, Proc. Natl. Acad. Sci. (U.S.A.), 87, 7772–7776.

Ames, B.N., and L.S. Gold (1990b) Too many rodent carcinogens: Mitogenesis increases mutagenesis, Science, 249, 970–971.

Ames, B.N., M. Profet and L.S. Gold (1990a) Dietary pesticides (99.99% all natural), Proc. Natl. Acad. Sci. (U.S.A.), 87, 7777–7781.

Ames, B.N., M. Profet and L.S. Gold (1990b) Nature's chemicals and synthetic chemicals: Comparative toxicology, Proc. Natl. Acad. Sci. (U.S.A.), 87, 7782–7786.

Bendich, A., and C.E. Butterworth Jr. (Eds.) (1991) Micronutrients in Health and in Disease Prevention, Marcel Dekker, New York.

Bernstein, L., L.S. Gold, B.N. Ames, M.C. Pike and D.G. Hoel (1985) Some tautologous aspects of the comparison of carcinogenic potency in rats and mice, Fund. Appl. Toxicol., 5, 79–86.

Bhave, M.R., M.J. Wilson and L.A. Poirier (1988) c-H-*ras* and c-K-*ras* gene hypomethylation in the livers and hepatomas of rats fed methyl-deficient, amino acid-defined diets, Carcinogenesis, 9, 343–348.

Boutwell, R.K., and M.W. Pariza (1987) Historical perspective: Calories and energy expenditure in carcinogenesis, Am. J. Clin. Nutr., 45 (1 Suppl.), 151–156.

Burdon, R.H., V. Gill and C. Rice-Evans (1990) Oxidative stress and tumour cell proliferation, Free Rad. Res. Commun., 11, 65–76.

Butterworth, B.E., and T. Slaga (Eds.) (1991) Chemically Induced Cell Proliferation: Implications for Risk Assessment, Alan R. Liss, New York.

Cadet, J., and M. Berger (1985) Radiation-induced decomposition of the purine bases within DNA and related model compounds, Int. J. Radiat. Biol., 47, 127–143.

Cannon, S.V., A. Cummings and G.W. Teebor (1988) 5-Hydroxymethylcytosine DNA glycolases activity in mammalian tissue, Biochem. Biophys. Res. Commun., 151, 1173–1179.

Carson, R. (1962) in: Silent Spring, Houghton-Mifflin, Boston, MA, p. 15.

Cathcart, R., E. Schwiers, R.L. Saul and B.N. Ames (1984) Thymine glycol and thymidine glycol in human and rat urine: A possible assay for oxidative DNA damage, Proc. Natl. Acad. Sci. (U.S.A.), 81, 5633–5637.

Chan, T.M., E. Chen, A. Tatoyan, N.S. Shargill, M. Pleta and P. Hochstein (1986) Stimulation of tyrosine-specific pro-

tein phosphorylation in the rat liver plasma membrane by oxygen radicals, Biochem. Biophys. Res. Commun., 139, 439–445.

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

Claringbold, W.D.B., J.D. Few and J.H. Renwick (1982) Kinetics and retention of solanidine in man, Xenobiotica, 12, 293–302.

Cohen, S.M., and L. Ellwein (1990) Cell proliferation in carcinogenesis, Science, 249, 1007–1011.

Craven, P.A., J. Pfanstiel and F.R. DeRubertis (1987) Role of activation of protein kinase C in the stimulation of colonic epithelial proliferation and reactive oxygen formation by bile acids, J. Clin. Invest., 79, 532–541.

Cravo, M., J. Mason, R.N. Salomon, J. Ordovas, J. Osada, J. Selhub and J.H. Rosenberg (1991) Folate deficiency in rats causes hypomethylation of DNA, FASEB J., 5, A914.

Crawford, D., and P. Cerutti (1989) Expression of oxidant stress-related genes in tumor promotion of mouse epidermal cells JB6, in: O. Nygaard and M.G. Simic (Eds.), Anticarcinogenesis and Radiation Protection, Plenum, New York, pp. 183–190.

Cutler, R.G. (1984) Antioxidants, aging, and longevity, in: W.A. Pryor (Ed.), Free Radicals in Biology, Academic Press, New York, pp. 371–428.

Degan, P., M.K. Shigenaga, E.-M. Park, P.E. Alperin and B.N. Ames (1991) Immunoaffinity isolation of urinary 8-hydroxy-2′-deoxyguanosine and 8-hydroxyguanine and quantitation of 8-hydroxy-2′-deoxyguanosine in DNA by polyclonal antibodies, Carcinogenesis, 5, 865–871.

Dizdaroglu, M. (1985) Formation of 8-hydroxyguanine moiety in deoxyribonucleic acid on γ-irradiation in aqueous solution, Biochemistry, 24, 4476–4481.

Doll, R. (1971) The age distribution of cancer: Implications for models of carcinogenesis, J.R. Stat. Soc., A134, 133–166.

Doll, R. (1990) Are we winning the fight against cancer? An epidemiological assessment, Eur. J. Cancer, 26, 500–508.

Dunsford, H.A., S. Sell and F.V. Chisari (1990) Hepatocarcinogenesis due to chronic liver cell injury in hepatitis B virus transgenic mice, Cancer Res., 50, 3400–3407.

Fearon, E.F., K.R. Cho, J.M. Nigro, S.E. Kern, J.W. Simons, J.M. Ruppert, S.R. Hamilton, A.C. Preisinger, G. Thomas, K.W. Kinzler and B. Vogelstein (1990) Identification of a chromosome 18q gene that is altered in colorectal cancer, Science, 247, 49–56.

Fiala, E.S., C.C. Conaway and J.E. Mathis (1989) Oxidative DNA and RNA damage in the livers of Sprague-Dawley rats treated with the hepatocarcinogen 2-nitropropane, Cancer Res., 49, 5518–5522.

Floyd, R.A., J.J. Watson, P.K. Wong, D.H. Altmiller and R.C. Richard (1986) Hydroxyl free radical adduct of deoxyguanosine: Sensitive detection and mechanisms of formation, Free Rad. Res. Commun., 1, 163–172.

Fraga, C.G., M.K. Shigenaga, J.-W. Park, P. Degan and B.N. Ames (1990) Oxidative damage to DNA during aging: 8-hydroxy-2′ deoxyguanosine in rat organ DNA and urine, Proc. Natl. Acad. Sci. (U.S.A.), 87, 4533–4537.

Fraga, C.G., R. Jacob, H.J. Helbock, M.K. Shigenaga, P.A. Motchnik and B.N. Ames (1991) Endogenous oxidative damage to human sperm DNA: Antioxidant effect of ascorbic acid on the formation of 8-hydroxy-2′-deoxyguanosine, Proc. Natl. Acad. Sci. (U.S.A.), in press.

Frei, B., R. Stocker and B.N. Ames (1988) Antioxidant defenses and lipid peroxidation in human blood plasma, Proc. Natl. Acad. Sci. (U.S.A.), 85, 9748–9752.

Frei, B., L. England and B.N. Ames (1989) Ascorbate is an outstanding antioxidant in human blood plasma, Proc. Natl. Acad. Sci. (U.S.A.), 86, 6377–6381.

Frei, B., M.C. Kim and B.N. Ames (1990) Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations, Proc. Natl. Acad. Sci. (U.S.A.), 87, 4879–4883.

Frei, B., T.M. Forte, B.N. Ames and C.E. Cross (1991) Gas phase oxidants of cigarette smoke induce lipid peroxidation and changes in lipoprotein properties in human blood plasma: protective effects of ascorbic acid, Biochem. J., in press.

Gold, L.S., G.M. Backman, N.K. Hooper and R. Peto (1987) Ranking the potential carcinogenic hazards to workers from exposures to chemicals that are tumorigenic in rodents, Environ. Health Perspect., 76, 211–219.

Gold, L.S., L. Bernstein, R. Magaw and T.H. Slone (1989) Interspecies extrapolation in carcinogenesis: Prediction between rats and mice, Environ. Health Perspect., 81, 211–219.

Gold, L.S., T.H. Slone, G.M. Backman, S. Eisenberg, M. DaCosta, M. Wong, N.B. Manley, L. Rohrbach and B.N. Ames (1990) Third chronological supplement to the Carcinogenic Potency Database: Standardized results of animal bioassays through December 1986 and by the National Toxicology Program through June 1987, Environ. Health Perspect., 84, 215–285.

Harman, P. (1981) The aging process, Proc. Natl. Acad. Sci. (U.S.A.), 78, 7124–7128.

Hartman, P.E. (1983) Mutagens: Some possible health impacts beyond carcinogenesis, Environ. Mutagen., 5, 139–152.

Hartman, P.E., and R.W. Morgan (1985) Mutagen-induced focal lesions as key factors in aging: A review, in: R.S. Sohal, L.S. Birnbaum and R.G. Cutler (Eds.), Molecular Biology of Aging: Gene Stability and Gene Expression, Vol. 29, Raven, New York, pp. 93–136.

Harvey, M.H., B.A. Morris, M. McMillan and V. Marks (1985) Measurement of potato steroidal alkaloids in human serum and saliva by radioimmunoassay, Hum. Toxicol., 4, 503–512.

Hayatsu, H. (Ed.) (1991) Mutagens in Food: Detection and Prevention, CRC Press, Boca Raton, FL.

Heller, T.D., P.R. Holt and A. Richardson (1990) Food restriction retards age-related histological changes in rat small intestine, Gastroenterology, 98, 387–391.

Henderson, B.E., R. Ross and L. Bernstein (1988) Estrogens as a cause of human cancer: The Richard and Hinda

Rosenthal Foundation Award Lecture, Cancer Res., 48, 246–253.

Higginson, J. (1988) Changing concepts in cancer prevention: Limitations and implications for future research in environmental carcinogenesis, Cancer Res., 48, 1381–1389.

Holliday, R. (1987a) DNA methylation and epigenetic defects in carcinogenesis, Mutation Res., 181, 215–217.

Holliday, R. (1987b) The inheritance of epigenetic defects, Science, 238, 163–170.

Hollstein, M.C., P. Brooks, S. Linn and B.N. Ames (1984) Hydroxymethyluracil DNA glycosylase in mammalian cells, Proc. Natl. Acad. Sci. (U.S.A.), 81, 4003–4007.

International Agency for Research on Cancer (1991) Monographs on the Evaluation of Carcinogenic Risks to Humans: Coffee, Tea, Mate, Methylxanthines and Methylglyoxal, Vol. 51, International Agency for Research on Cancer, Lyon.

Ishidate Jr., M., Harnois, M.C. and T. Sofuni (1988) A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures, Mutation Res., 195, 151–213.

Iversen, O.H. (Ed.) (1988) in: Theories in Carcinogenesis, Hemisphere Publishing, Washington, DC.

Jones, T.D., G.D. Griffin and P.J. Walsh (1983) A unifying concept for carcinogenic risk assessments, J. Theor. Biol., 105, 35–61.

Jones, T.D. (1984) A unifying concept for carcinogenic risk assessments: Comparison with radiation-induced leukemia in mice and men, Health Phys., 4, 533–558.

Jukes, T.H. (1974) Insecticides in health, agriculture and the environment, Naturwissenschaften, 61, 6–16.

Kane, S.E., I. Pastan and M.M. Gottesman (1990) Genetic basis of multidrug resistance of tumor cells, J. Bioenerg. Biomembr., 22, 593–618.

Kasai, H., S. Nishimura, Y. Kurokawa and Y. Hayashi (1987) Oral administration of the renal carcinogen, potassium bromate, specifically produces 8-hydroxydeoxyguanosine in rat target organ DNA, Carcinogenesis, 8, 1959–1961.

King, M.P., and G. Attardi (1988) Injection of mitochondria into human cells leads to a rapid replacement of the endogenous mitochondrial DNA, Cell, 52, 811–819.

Kinzler, K.W., M.C. Nilbert, B. Vogelstein, T.M. Bryan, D.B. Levy, K.J. Smith, A.C. Preisinger, S.R. Hamilton, P. Hedge, A. Markham, M. Carlson, G. Joslyn, J. Groden, R. White, Y. Miki, Y. Miyoshi, I. Nishiso and Y. Nakamura (1991) Identification of a gene located at chromosome 5q21 that is mutated in colorectal cancers, Science, 251, 1366–1370.

Kohen, R., Y. Yamamoto, K. Cundy and B.N. Ames (1988) Antioxidant activity of carnosine, homocarnosine, and anserine present in muscle and brain, Proc. Natl. Acad. Sci. (U.S.A.), 85, 3175–3179.

Kuchino, Y., F. Mori, H. Kasai, H. Inoue, S. Iwai, K. Miura, E. Ohtsuka and S. Nishimura (1987) Misreading of DNA templates containing 8-hydroxydeoxyguanosine at the modified base and at adjacent residues, Nature (London), 327, 77–79.

Lewis, J.G., and D.O. Adams (1987) Inflammation, oxidative DNA damage, and carcinogenesis, Environ. Health Perspect., 76, 19–27.

Lindahl, T. (1982) DNA repair enzymes, Annu. Rev. Biochem., 51, 61–87.

Little, J.B., A.R. Kennedy and R.B. McGandy (1985) Effect of dose rate on the induction of experimental lung cancer in hamsters by radiation, Radiat. Res., 103, 293–299.

Lok, E., F.W. Scott, R. Mongeau, E.A. Nera, S. Malcolm and D.B. Clayson (1990) Calorie restriction and cellular proliferation in various tissues of the female Swiss Webster mouse, Cancer Lett., 51, 67–73.

Lucier, G., and G.E.R. Hook (1986) Dietary Mutagens: Special Issue, Environ. Health Perspect., 67.

MacGregor, J.T., R. Schlegel, C.M. Wehr, P. Alperin and B.N. Ames (1990) Cytogenetic damage induced by folate deficiency in mice is enhanced by caffeine, Proc. Natl. Acad. Sci. (U.S.A.), 87, 9962–9965.

Madsen, C. (1989) Squamous-cell carcinoma and oral, pharyngeal and nasal lesions caused by foreign bodies found in food. Cases from a long-term study in rats, Lab. Anim., 23, 241–247.

Matthew, J.A., M.R.A. Morgan, R. McNerney, H.W.-S. Chan and D.T. Coxon (1983) Determination of solandine in human plasma by radioimmunoassay, Food Chem. Toxicol., 21, 637–640.

National Cancer Institute (1988) 1987 Annual Cancer Statistics Review Including Cancer Trends: 1950–1985, NIH Publication 88–2789, National Institutes of Health, Bethesda, MD.

National Research Council (1989) Diet and Health, Implications for Reducing Chronic Disease Risk, National Academy Press, Washington, DC.

Ootsuyama, A., and H. Tanooka (1991) Threshold-like dose of local β-irradiation repeated throughout the life span of mice for induction of skin and bone tumors, Radiat. Res., 125, 98–101.

Patterson, B.H., and G. Block (1991) Fruit and vegetable consumption national survey data, in: A. Bendich and C.E. Butterworth, Jr. (Eds.), Micronutrients in Health and in Disease Prevention, Marcel Dekker, New York.

Portier, C.J., J.C. Hedges and D.G. Hoel (1986) Age-specific models of mortality and tumor onset for historical control animals in the National Toxicology Program's carcinogenicity experiments, Cancer Res., 46, 4372–4378.

Preston-Martin, S., M.C. Pike, R.K. Ross and P.A. Jones (1990) Increased cell division as a cause of human cancer, Cancer Res., 50, 7415–7421.

Reddy, B.S., and L.A. Cohen (Eds.) (1986) in: Diet, Nutrition, and Cancer: A Critical Evaluation, Vols. I and II, CRC Press, Boca Raton, FL.

Richter, C., J.-W. Park and B.N. Ames (1988) Normal oxidative damage to mitochondrial and nuclear DNA is extensive, Proc. Natl. Acad. Sci. (U.S.A.), 85, 6465–6467.

Roe, F.J.C. (1989) Non-genotoxic carcinogenesis: Implications for testing and extrapolation to man, Mutagenesis, 4, 407–411.

Roe, F.J.C., P.N. Lee, G. Conybeare, G. Tobin, D. Kelly, D.

Prentice and B. Matter (1991) Risks of premature death and cancer predicted by body weight in early adult life, Hum. Exp. Toxicol., in press.

Rosier, J.A., and C.H. VanPeteghem (1989) Peroxidative in vitro metabolism of diethylstilbestrol induces formation of 8-hydroxy-2′deoxyguanosine, Carcinogenesis, 10, 405–406.

Saul, R.L., and B.N. Ames (1986) Background levels of DNA damage in the population, in: M.G. Simic, L. Grossman and A.C. Upton (Eds.), Mechanisms of DNA Damage and Repair: Implications for Carcinogenesis and Risk Assessment, Plenum, New York, pp. 529–536.

Saul, R.L., P. Gee and B.N. Ames (1987) Free radicals, DNA damage, and aging, in: H.R. Warner, R.N. Butler, R.L. Sprott and E.L. Schneider (Eds.), Modern Biological Theories of Aging, Raven, New York, pp. 113–129.

Schardein, J.L. (1985) in: Chemically Induced Birth Defects, Marcel Dekker, New York.

Shibutani, S., M. Takeshita and A.P. Grollman (1991) Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG, Nature (London), 349, 431–434.

Shigenaga, M.K., C.J. Gimeno and B.N. Ames (1989) Urinary 8-hydroxy-2′-deoxyguanosine as a biomarker of in vivo oxidative DNA damage, Proc. Natl. Acad. Sci. (U.S.A.), 86, 9697–9701.

Sieweke, M.H., A.W. Stoker and M.J. Bissell (1989) Evaluation of the cocarcinogenic effect of wounding in Rous Sarcoma virus tumorigenesis, Cancer Res., 49, 6419–6424.

Stanbridge, E.J. (1990) Human tumor suppressor genes, Annu. Rev. Genet., 24, 615–657.

Stocker, R., and B.N. Ames (1987) Potential role of conjugated bilirubin and copper in the metabolism of lipid peroxides in bile, Proc. Natl. Acad. Sci. (U.S.A.), 84, 8130–8134.

Stocker, R., A.N. Glazer and B.N. Ames (1987a) Antioxidant activity of albumin-bound bilirubin, Proc. Natl. Acad. Sci. (U.S.A.), 84, 5918–5922.

Stocker, R., Y. Yamamoto, A.F. McDonagh, A.N. Glazer and B.N. Ames (1987b) Bilirubin is an antioxidant of possible physiological importance, Science, 235, 1043–1045.

Storz, G., L.A. Tartaglia and B.N. Ames (1990) Transcriptional regulator of oxidative stress-inducible genes: Direct activation by oxidation, Science, 248, 189–194.

Sugimura, T. (1988) Successful use of short-term tests for academic purposes: Their use in identification of new environmental carcinogens with possible risk for humans, Mutation Res., 205, 33–39.

Takayama, S., Y. Nakatsura and S. Sato (1987) Carcinogenic effect of the simultaneous administration of five heterocyclic amines to F344 rats, Jpn. J. Cancer Res. (Gann), 78, 1068–1072.

Talalay, P., M.J. De Long and H.J. Prochaska (1988) Identification of a common chemical signal regulating the induction of enzymes that protect against chemical carcinogenesis, Proc. Natl. Acad. Sci. (U.S.A.), 85, 8261–8265.

Talalay, P. (1989) Mechanisms of induction of enzymes that protect against chemical carcinogenesis, in: G. Weber (Ed.), Advances in Enzyme Regulation, Vol. 28, pp. 237–250.

Templeton, A. (1980) Pre-existing, non-malignant disorders associated with increased cancer risk, J. Environ. Pathol. Toxicol., 3, 387–397.

Totter, J.R. (1980) Spontaneous cancer and its possible relationship to oxygen metabolism, Proc. Natl. Acad. Sci. (U.S.A.), 77, 1763–1767.

Trosko, J.E., C.C. Chang and A. Medcalf (1983) Mechanisms of tumor promotion: Potential role of intercellular communication, Cancer Invest., 1, 511–526.

Trosko, J.E. (1989) Towards understanding carcinogenic hazards: A crisis in paradigms, J. Am. Coll. Toxicol., 8, 1121–1132.

Trosko, J.E., C.C. Chang and B.V. Madhukar (1990a) In vitro analysis of modulators on intercellular communications: Implications for biologically based risk assessment models for chemical exposure, Toxicol. In Vitro, 4, 635–643.

Trosko, J.E., C.C. Chang, B.V. Madhukar and J.E. Klaunig (1990b) Chemical, oncogene, and growth factor inhibition of gap junctional communication: An integrative hypothesis of carcinogenesis, Pathobiology, 58, 265–278.

von Sonntag, C. (1987) The Chemical Basis of Radiation Biology, Taylor and Francis, London.

Vorce, R.L., and J.I. Goodman (1989a) Hypomethylation of ras oncogenes in chemically induced and spontaneous B6C3F1 mouse liver tumors, Mol. Toxicol., 2, 99–116.

Vorce, R.L., and J.I. Goodman (1989b) Altered methylation of ras oncogenes in benzidine-induced B6C3F1 mouse liver tumors, Toxicol. Appl. Pharmacol., 100, 398–410.

Weitzman, S.A., and L.J. Gordon (1990) Inflammation and cancer: Role of phagocyte-generated oxidants in carcinogenesis, Blood, 76, 655–663.

Williams, G.C., and R.M. Nesse (1991) The dawn of Darwinian medicine, Q. Rev. Biol., 66, 1–22.

Wilson, V.L., R.A. Smith, S. Ma and R.G. Cutler (1987) Genomic 5-methyldeoxycytidine decreases with age, J. Biol. Chem., 262, 9948–9951.

Wolff, S., V. Afzal, J.K. Wiencke, G. Olivieri and A. Michaeli (1988) Human lymphocytes exposed to low doses of ionizing radiations become refractory as well to chemical mutagens that induce double-strand breaks in DNA, Int. J. Radiat. Biol., 53, 39–48.

Wolff, S., J.K. Wiencke, V. Afzal, J. Youngblom and F.R. Cortés (1989) The adaptive response of human lymphocytes to very low doses of ionizing radiation: A case of induced chromosomal repair with the induction of specific proteins, in: K.F. Baverstock and J.W. Stather (Eds.), Low Dose Radiation: Biological Bases of Risk Assessment, Taylor and Francis, London, pp. 446–454.

Wolff, S., G. Olivieri and V. Afzal (1990) Adaptation of human lymphocytes to radiation or chemical mutagens: Differences in cytogenetic repair, in: A.T. Natarajan and G. Obe (Eds.), Chromosomal Aberrations: Basic and Applied Aspects, Springer, New York, pp. 140–150.