Beyond Antioxidant Adaptation

A Free Radical-Hypoxia-Clonal Thesis of Cancer Causation

Stephen A. Levine, Ph.D.[1] and Parris M. Kidd, Ph.D.[2]

Abstract

We have chosen to review some of the cancer literature in the context of our thesis on antioxidant adaptation to oxidative stress (Levine and Kidd, 1984), the ubiquity of toxic carcinogens as free radical oxidants *in vivo*, and the central importance of hypoxia in degenerative disease states (Levine and Kidd, 1985). We suggest that the cancerous cell line is the end result of initial free radical oxidant damage to DNA; its subsequent fixation into the genotype by cellular replication (facilitated by cancer promoter agents), and subsequent clonal selection for an anaerobic malignant cellular lifestyle *in vivo*. This hypothesis that we have presented regarding the metabolic basis of cancer is meant to be a complete one, in that it attempts to organize many disparate observations into a unified theoretical framework. It has largely been possible because of the power inherent in viewing degenerative diseases from the expanded perspective of antioxidant defenses and free radical biochemistry.

1. Director of Research, Allergy Research Group, 400 Preda Street, San Leandro CA 94577.

2. Kidd and Associates, Biomedical Consultants, 6525 Hillmont Drive, Oakland, CA 94605

Discovery consists in seeing what everybody else has seen and thinking what nobody has thought.
ALBERT SZENT-GYORGYI

A tragic reality of modern society is that chemicals cause cancer in animals and humans. Eighty to ninety percent of all human cancers originate from exposures to chemicals. Herein we present the hypothesis that chemical carcinogenesis is a multistage process initiated and maintained by free radical oxidative stress, and opposed by the body's sophisticated antioxidant defense system. We suggest that the cancerous cell is the consequence of a process of selection caused by the loss of the cell's antioxidant defense capabilities and its assumption of an alternative, anaerobic cellular lifestyle. A corollary of our hypothesis is that a redox-modulating (i.e., antioxidant) approach utilizing redox-active agents (which takes advantage of the cancer cell's limited redox tolerance) is a strategy for cancer prevention and therapy.

Free Radicals, Antioxidant Adaptation, and Chemical Carcinogenesis

Free radicals are ubiquitous in living systems, and play both beneficial and deleterious

roles in a wide variety of biological processes. Free radicals tend to "steal" electrons from biological molecules to bring them unglued*. The oxygen molecule is a reactive "diradical" with partial free radical character (Demopoulos, 1973; Demopoulos et al., 1980). The utilization of oxygen for the burning of substrates provides higher yields of ATP and has allowed aerobic eukaryotic cells to acquire their high level of functional differentiation and specialization. However, the oxygen molecule is so highly reactive as an electron acceptor that its oxidizing power is a constant threat to the molecular integrity of organisms living in an oxygenated atmosphere (Haugaard, 1968). Hence elaborate biochemical antioxidant defenses have evolved in aerobic organisms to cope with the ever-present threat of oxygen toxicity (Di-Guiseppi and Fridovich, 1984). Acute or chronic oxidative stress resulting from oxidant free radical attack appears to be associated with macromolecular damage, abnormal metabolism, inflammatory states, immune dysfunctions and degenerative diseases (Gerschman et al., 1954; DiLuzio, 1967; Demopoulos, 1973; Freeman and Crapo, 1982; Halliwell, 1982; Levine and Reinhardt, 1983; Ames, 1983).

Free radicals are inevitable byproducts of aerobic metabolic processes in higher organisms, especially during aerobic respiration oxygen derivatives, including the superoxide (Freeman and Crapo, 1982). Thus reactive anion radical and other "activated" derivatives such as hydrogen peroxide, are generated in the mitochondrial inner membrane by premature single-electron leakage to molecular oxygen from respiratory chain components during the course of respiratory electron transport. Mitochondrial generation of oxygen radicals may account for as much as 5 percent of the oxygen consumed by the aerobic cell (Forman and Boveris, 1982).

The likelihood of damage to any living system from molecular oxygen and its activated derivatives, or from other oxidizing stressors**, appears to hinge on the balance between the cumulative oxidative challenge to the system and its antioxidant defense capabilities (Chow, 1979; Levine and Reinhardt, 1983). Peroxidative and cross-linking damage to cellular membranes (mediated by oxidant free radicals) can endanger membrane transport function, impair membrane flexibility, and eventually cause the cell to die (Noronha-Dutra and Steen 1982; Parke, 1982). We have suggested elsewhere that biochemical antioxidant defenses are significantly adaptable in higher organisms (Levine and Kidd, 1984).

According to our Antioxidant Adaptation Hypothesis, abnormally-elevated oxidative stress to the organism provokes localized augmentation of protective antioxidant molecules and enzymes to maintain or restore optimal oxidant/antioxidant (redox) balance. However, the antioxidant adaptability of a cell, tissue or organ will be limited in its breadth and magnitude by the availability of nutrient-derived antioxidant constituents (Tappel, 1980; Hafeman and Hoekstra, 1977). Dietary factors which stimulate free radical activity and lipid peroxidation will tend to increase oxidative stress to the organism. Thus increased quantities of dietary polyunsaturated fats given to rodents accelerate free radical reactions in tissues and thereby enhance chemical carcinogenesis (Demopoulos et al., 1980).

Nutrient-derived antioxidant factors and antioxidant enzymes constitute many of the "nuts and bolts" which together make up the sophisticated, adaptable antioxidant defense system (Forman and Fisher, 1981; Levine and Reinhardt, 1983; DiGuiseppi and Fridovich, 1984). In this article we will review the evidence that environmental pollutant chemicals invariably mediate free radical attack on tissues exposed to them (Demopoulos, 1980; Levine and Kidd, 1984). Viewed from the perspective of antioxidant resistance to oxidant chemical exposures, individual susceptibility to cancer may be a direct consequence of the failure of exposed "target tissues to successfully adapt to oxidant free radical attack. From this perspective we have developed a hypoxia-clonal thesis of cancer causation.

* A free radical is a substance with an unpaired electron in an outer orbital. Its inherent tendency to attain molecular stability by acquiring or donating an electron renders it highly reactive. Recall that loss of an electron amounts to oxidation, whereas the gaining of an electron amounts to reduction.

** Diverse stressors initiate biological damage through exacerbating the endogenous production of free radical species by the cell (Levine and Kidd, 1984, 1985; DiGuiseppi and Fridovich, 1984).

Key Features of the Hypothesis. Our hypothesis proposes that cumulative oxidative stress to the aerobic mammalian cell (from endogenous and/or exogenous free radical generation) mediates initial damage to the DNA which leads to somatic mutations, and eventually cripples the cell's aerobic metabolic apparatus with associated compromise of its antioxidant defense. The product of this ongoing process is a population of hypermutable cells, forced to survive anaerobically (i.e., by reliance on glycolytic metabolism) and therefore subject to selective pressures which favor the anaerobic cellular lifestyle in the aerobic multicellular organism. According to this scenario, those anaerobic clones which possess the most adaptable phenotypes would be most favored for survival. Initially, the most favored phenotypic characteristics would include the abilities to shift the glycolytic mode and function efficiently in this mode. Later, the abilities to grow aggressively and exist independently of the aerobic tissues would also favor clonal evolution. If environmental conditions *in vivo* continue to favor such clonal selection via anaerobic existence, one or more clones would progress to irreversible growth transformation (neoplasia) and eventually give rise to malignant tumors in the host organism.

The starting point for our hypothesis is that a functionally-significant oxidant/antioxidant (redox) balance is homeostatically maintained in mammalian cells and tissues (Chayen, 1982). The maintenance of a localized redox balance predicts that if oxidative stress can be minimized and antioxidant defenses optimized, there will be protection to a large degree against free radical oxidant attack by chemical carcinogens or other oxidants, and a healthy state will prevail. Conversely, *if there is significant acute or chronic exposure to oxidative stress and/or antioxidant defenses are Suboptimal, the oxidant/antioxidant balance will be shifted in an unfavorable direction (initially towards a more oxidized state, and later in hypoxic precancerous cell populations towards a highly reduced state).* Past some critical point in this redox shift, cell and tissue homeostasis will become compromised, and degenerative diseases such as cancer can ensue.

There is a good deal of evidence, dating back to Warburg's classic experiments (Warburg, 1956, 1969), that in cancer a cellular state of anaerobiosis has come to substitute for aerobic metabolism. Consequently the cancerous cell may minimize its endogenous production of radical and other activated derivatives of oxygen. The abnormally low levels of detectable free radical and lipid peroxidation activity observed in cancer (reviewed in Floyd, 1982 and McBrien and Slater, 1982) are attributable to cancerous tissue being in an anaerobic state.

The state of anaerobiosis which we suggest is characteristic of cancerous or precancerous cells must (at least in the initial stages) coexist with oxidative damage to the genetic material from carcinogen exposure. Later this oxidative damage can become fixed into the cellular genotype, resulting in DNA sequence alterations and somatic mutations, gene deletions and rearrangements, and eventually in chromosomal alterations detectable by sophisticated cytogenetic techniques (Yunis, 1983). We suggest that the nature and scope of such genetic aberrations, and their effects on the efforts of the mutated cells to homeostatically regulate their redox state, will determine whether the progeny cell lines become irreversibly growth-transformed and invasive (i.e., malignant), or (eventually) regain some form of aerobic, differentiated existence.

We have proposed elsewhere (Levine and Kidd, 1985) that free radical-mediated oxidative damage is a common pathway by which all stressors contribute to tissue and cell damage. Thus in addition to oxidant chemicals (Levine and Reinhardt, 1983), factors as diverse as irradiation (Greenstock, 1981); inflammation and wound irritation (Halliwell, 1982); emotional stress mediated by oxidative breakdown of epinephrine, norepinephrine and dopamine (Schenkman et al., 1979); physical trauma (Demopoulos et al., 1982); and certain physiological and behavioral states (Tache et al., 1979; de la Pena, 1983) all appear to be capable of initiating or exacerbating oxidative stress at the level of the cell, thereby endangering redox homeostasis and favoring chemical redox homeostasis from toxic xenobiotic overload (Levine and Kidd, 1985) The existence of a reducing cellular redox

state resulting from anaerobic metabolism is a pivotal feature of our hypothesis. We propose that the contribution of hypoxia to cancer causation is best understood by viewing disease in the broadest sense: as a result of cumulative damage to our body's redox homeostasis (our localized and systemic antioxidant defenses), with the consequent increased "friction" from our normal biological functions exacerbating endogenous free radical production and accelerating wear and tear on the body. The circulatory system and the lungs become progressively less efficient, and oxygen delivery to the tissues becomes progressively more impaired. A chronic ischemic/hypoxic state ensues in susceptible tissues, creating an environment which favors cell populations sufficiently adaptable to survive without a steady supply of oxygen. The genetically-hypermutable, functionally-impaired, nutrient- and energy-deficient cell population becomes subject to powerful selective pressures for the eventual predominance of anaerobic cell clones.

Clonal evolution is another key feature of our hypothesis. It has been thoroughly documented for prokaryotic microorganisms, than *an increased mutation rate favors survival in hostile and changing environments* (Ames et al., 1975). An ischemic/hypoxic tissue state would certainly constitute a hostile environment for normal, aerobic eu-karyotic cells. By analogy with prokaryotes, it would seem logical that the mutated state of the precancerous cell population would combine with antioxidant impairments and poor oxygenation status to create a rigorous clonal selection process. Only the most hearty of these multiply-damaged cells would be expected to survive and reproduce under such heavy selection pressure. Continued reproductive success of a few such clones (or even just one!) could eventually result in a malignant tumor.

Earlier Free Radical Hypotheses for Carcinogenesis. **The** idea that cancer may result from exposure(s) to free radicals dates back more than two decades. Szent-Gyorgyi and collaborators put forward an electron transfer hypothesis of chemical carcinogenesis in 1960. Floyd (1982) in his Preface stressed that cancer development is a highly complex process which evidently proceeds through a number of steps, some of which are more likely to be causally related to free radical exposure(s) than are others. That free radicals can cause cancer is established beyond doubt, due to the proximate role of free radicals in radiation carcinogenesis (Ny-gaard and Simic, 1983). Moreover, both chemical carcinogens and hyperbaric oxygen can act in synergy with radiation, indicating that both these categories of toxins share with radiation a common mechanism for carcinogenesis. Both are in fact well established as exacerbating endogenous free radical production. Nevertheless, the key issue remains: have free radicals been satisfactorily proven to initiate, or significantly affect, the progression of chemical carcinogenesis? We believe the answer to this question is a resounding YES.

Earlier free radical approaches to unraveling carcinogenesis were reviewed in a historical context by Swartz (1979), who evaluated the "high" points and the "low" points in the accumulation of supportive data and concluded that the key issue of causality had not yet been answered satisfactorily. More recently, he has elaborated on the various free radical theories of cancer causation and how each might be proven or disproven (Swartz, 1982). The maturation of electron spin resonance (ESR) and allied methods for the precise detection and study of free radicals has served to confirm earlier reports that malignant tissue has abnormally lower levels of detectable free radical activity — Swartz attributed this finding to reduced free radical generation by the cancer cell mitochondria. Other longstanding observations — that "antioxidant" activity in cancerous tissue is abnormally elevated, and that lipid peroxidation rates are abnormally low (Duchesne, 1977; Swartz, 1982) — may seem paradoxical if cancer is seen as arising from elevated free radical oxidative stress to the cell. Nevertheless these findings are logically consistent with a partial or complete shutdown of mitochondrial oxidative respiration associated with anaerobic metabolism. Our hypothesis attempts to incorporate the classic data on cancer metabolism with the voluminous data currently being generated, to construct a harmonious, testable model for chemical carcinogenesis.

The "Natural History" of Chemical Carcinogenesis. We have chosen to restrict our model to cancer resulting from chemical exposure, which appears to account for at least 80 percent of all cancer (Heidelberger, 1975; Cimino and Demopoulos, 1980; Chowka, 1981; Smuckler, 1983). The two other major causes of cancer are irradiation and viruses. Currently, the evidence that any virus can cause human cancer in the absence of a co-carcinogen is less than convincing. On the other hand, irradiation is firmly established as a cause of human cancer (Nygaard and Simic, 1983) although the frequency of radiation carcinogenesis is markedly lower than that of chemical carcinogenesis. Radiation nevertheless stands as a useful model agent against which to compare chemical carcinogens, since the two agents often act synergistically in experimental carcinogenesis. Evidence from other sources also strongly indicates that chemical carcinogens, like radiation, act primarily through free radical mechanisms to generate somatic mutations, deplete cellular antioxidant defenses, and initiate cancer.

Mammalian cell lines are thought to attain the cancerous state in *vivo* by a multistage process (Miller and Miller, 1981; Slaga, 1984; Farber, 1984). The cancerous state is characterized by 1) unregulated cellular proliferation; 2) untypical cellular differentiation; and 3) abnormal growth patterns (malignancy). There occurs expansion of the tumor tissue into surrounding normal tissue (invasiveness), and the release of cells able to generate new proliferations at distant sites (metastasis). Slaga (1984) has referred to the major stages of chemical carcinogenesis as "initiation," "promotion," and "progression." "Initiation" is seen as the event of genetic damage to the target cell population by the toxic chemical insult. "Promotion" then fixes these genetic alterations into the genotype of the cell line, through encouraging cellular proliferation. Once set into motion, the proliferative process usually continues for many cellular generations ("progression"), culminating with the differentiative and growth abnormalities which collectively typify neoplastic transformation.

Attainment of the neoplastic state signals commitment of the cell population to a pathological course, in that no further direct or indirect exogenous intervention is necessary to maintain the growth-transformed state. The neoplastic tumor is characterized by uncontrolled growth and by deranged differentiation, features often reminiscent of embryonic tissue. Nevertheless, it continues to resemble the differentiated tissue from which it was originally derived. Some neoplasms regress spontaneously to a state of normal, regulated growth. Some proceed only as far as a "benign" state, wherein the tumor remains locally contained and noninvasive. Alternatively, the neoplasm can progress further — via further selection of one or more cell clones — all the way to a life-threatening state, that of malignancy.

Malignant tumors characteristically consist of one or a few cell clones which proceed *to* invade the surrounding nonneoplastic tissue and metastasize, i.e., colonize new tissue sites in more distant areas. It appears that by a process of selection in the host organism, clones emerge which manifest greater autonomy from the host tissue, coupled with invasiveness and metastasis (Nowell, 1976; National Cancer Advisory Board, 1977). This phase has been termed clonal expansion by Farber (1984). We therefore visualize four clearly-definable stages in chemical carcinogenesis: initiation, promotion, transformation, and clonal expansion. We will explore our hypothesis within this mechanistic framework.

Limitations of the "Initiation-Promotion" Paradigm. Following the emergence of the "two-stage" paradigm of chemical carcinogenesis initially for the mouse skin system (Berenblum and Shubik, 1947), and later in other "model" systems (Berenblum, 1974), it had become widely accepted that "initiation" events must be coupled with "promotion" events which then lead to transformation. Typically, a brief (usually single) treatment with an "initiator" compound had to be followed with several applications of a "promoter" compound over a more extended period. Initiator compounds were generally seen as "strong" carcinogens, which nevertheless required complementation by promoter agents (some of which were "weak" carcinogens) to achieve carcinogenicity. From the vast number of studies which have

been carried out since the inception of this "model" experimental system for chemical carcinogenesis, current understanding suggests that the two-stage paradigm can be challenged for its oversimplicity (Iversen and Astrup, 1984).

Challenges to the "two-stage" paradigm stem from findings that several "strong" chemical carcinogens can act both as initiators and as promoters, i.e., as "complete" carcinogens (Miller and Miller, 1981). Also, almost all promoters can act as weak or even strong initiators under the right conditions. One widely-used promoter, phenobarbital, even can act as an anti-initiator. Thus the strict assignment of carcinogenic chemicals to the category of "cancer initiator" or "cancer promoter" has become progressively more questionable (Iversen and Astrup, 1984). Nevertheless, *the data suggest very clearly that the overwhelming majority of chemical carcinogens, whether classed as initiators or promoters, are carcinogenic through free radical mechanisms.*

Cancer Initiation from Free Radical Damage to DNA

We suggest that the initiation event in carcinogenesis is the culmination of two sets of cellular alterations which may proceed simultaneously or sequentially. One class of cellular alterations is the depletion of antioxidant factors due to free radical-mediated chemical oxidant attack, which causes the cell to suffer unavoidable impairment of its antioxidant defenses. Conceivably either a single exposure to a strong chemical carcinogen or chronic, "low-level" exposure to a weaker chemical carcinogen could eventually overwhelm the antioxidant defenses of a healthy cell. The second class of alteration is the increasing susceptibility of the cell's genetic material (DNA) to oxidative modification, which would be heightened by any impairment of the cell's antioxidant defenses. In the case of alkylating carcinogens, the genetic damage which is the result of this increasing susceptibility appears to be mediated by the very same electrophilic ("electron-loving"), free radical species that deplete protective antioxidant factors and thereby impair cellular antioxidant defense mechanisms (Johnson, 1979, 1982, 1983; Demopoulos et al., 1980; Miller and Miller, 1981). The subsequent "fixation" of the damaged DNA into heritable mutations, as the DNA replicates at cell division, would constitute an irreversible step in the initiation process. Efficiency of DNA repair could be a critical factor at this stage (Riley, 1982).

Many Initiator Carcinogens are Free Radical Oxidants. Chemical carcinogens can be grouped into six major classes: halogenated hydrocarbons, aromatic hydrocarbons, fused or conjugated amines, nit-rosamines, mycotoxins, and heterocyclic compounds (Johnson, 1982, 1983; Demo-poulos et al., 1980). Carcinogens of all these classes can be metabolically converted to reactive derivatives within the cell by the Mixed-Function Oxidase enzymes of the endoplasmic reticulum, and by a variety of other metabolic pathways (Mason, 1982). The metabolism of xenobiotic compounds (i.e., compounds foreign to the cell) invariably increases the frequency of electron transfer reactions within cellular membranes to generate highly reactive, electrophilic and radical molecular species*.

Some of these derivatives are comparatively long-lived. Those which are aromatic often are stabilized by resonance and often are the most toxic, for example the benzene semi-quinone described from cigarette tars which has a half-life on the order of days (Pryor, 1982). Many can alter DNA directly, adding to DNA bases such as guanine to generate covalent molecular adducts (Margison, 1980). Others undergo "redox cycling" in the cell: in the presence of molecular oxygen they cyclically generate reactive oxygen derivates. Some can form adduts with DNA which redox cycle *in situ* (refer to Stier et al., and Lesko et al., in McBrien and Slater, 1982).

Those derivatives of chemical carcinogen compounds which are free radical in character and detectable by electron spin resonance (ESR) techniques appear in each case to be the active carcinogenic species (Nagata et al., 1982). Usually the "procarcinogen" (the less-reactive parent compound) can be-

* Recall the definition of free radicals as molecular species with unpaired electrons.

come activated to several reactive intermediates; those which generate an ESR free radical signal are much more likely to be carcinogenic than those which are not free radicals by ESR. Also, the degree of covalent binding of a radical intermediate to DNA in the target tissue often correlates closely with its measurable free radical concentration (Nagata et al., 1982). It has been stated that *"all known carcinogens are easily convertible to free radicals." (Johnson*, 1982, p. 120). Demopoulos et al. (1980), in their cogent review of chemical carcinogenesis, also embraced the Radical Oxidant interpretation, stating:

"Many carcinogens are either free radicals, or are converted to active free radicals *in vivo*, or stimulate the production of free radicals, or are products of biological free radical reactions" (Demopoulos et al., 1980, p. 277).

The findings that the active derivatives of the "strong" carcinogen initiators (i.e., the "ultimate carcinogens") are free radicals *in vivo* is not at all new. Some initiator carcinogens such as the nitroso compounds appear able to act as carcinogens without enzymatic activation (Nagata et al., 1982). Miller and Miller (1970, 1981) were the first to suggest that the ultimate initiator carcinogens were reactive electrophiles derived metabol-ically from the parent compounds. As Johnson has pointed out, the Reactive Electrophile interpretation does not explain synergy with radiation carcinogenesis, or the blocking of initiation by antioxidant compounds, as well as does the Radical Oxidant interpretation.

Since free radicals are electrophiles, and many electrophiles are free radicals, these two hypotheses can be nicely integrated. Some investigators, including Tso et al. (1977), Johnson (1982, 1983) and Demopoulos et al. (1980), maintain that it is the free radical nature of these reactive electrophilic forms of initiator compounds which most satisfactorily accounts for their carcinogenicity, and evidence continues to accumulate in favour of this interpretation (Pryor, 1982; Ames, 1983, 1984; Slaga, 1984; Borek, 1983).

The proven efficacy of both synthetic and natural antioxidant compounds for inhibiting the initiation event strongly supports the Radical Oxidant interpretation for the under-lying mechanism. Slaga (1984) reported that the synthetic antioxidant BHA (butylated hydroxyanisole), flavone antioxidants (7,8-benzoflavine and 5,6 benzoflavone), and the antioxidant vitamins C and E, all inhibited initiation in the mouse skin tumor system, simultaneously with decreasing the covalent binding of the initiator compound to the DNA of the skin basal cells (the stem cell population. Experimental *in vivo* and *in vitro* findings from many other laboratories also support the efficacy of antioxidants in protecting against the somatic mutation event(s) -of initiation and promotion (Little et al., 1983; Borek, 1983; boutwell, 1983; Griffin, 1982; Want and Howell, 1982; Wattenberg, 1982).

In addition to the proven efficacy of anti-oxidants in blocking experimental carcinogenesis, epidemiological findings strongly indicate that diets high in antioxidant nutrients, especially beta-carotene (provitamin A), ascorbate (vitamin C), tocopherols (vitamin E), and selenium, are protective against human cancer (Schrauzer, 1979; Newberne and Suphakarn, 1983; Peto et al., 1981; Kummet et al., 1983). Taken together, these findings from cell culture studies, experiments with laboratory animals, and human cancer occurrence all support the assertion by Ames (1984) that *antioxidants protect against cancer, and the natural antioxidant substances are the natural anticancer agents.* They argue strongly in favor of a causal involvement of free radicals in chemical carcinogenesis.

Oxidative Stressors as Initiator Agents. To the well-founded assertions of Johnson (1982, 1983) and Demopoulos et al. (1980) that the somatic mutation effects of chemical carcinogens are free radical-mediated, we wish to add an Expanded Oxidative Stress perspective: that the category of cancer initiators includes, in addition to the oxidant chemical carcinogen compounds, any or all cellular physiological states that can directly or indirectly lead to depletion, damage, or overload of cellular antioxidant defense capabilities and subsequently mediate mutational events in structural or regulatory genes. We suspect that one important initiating process from this broadened oxidative stress category is an inflammatory tissue state (Halliwell, 1982; Parke, 1982).

Oxygen and activated oxygen derivatives have been shown to be mutagenic, as has the crosslinking compounds malondialdehyde derived from the breakdown of lipid peroxides. A state of inflammation may be mutagenic *per se,* by way of reactive oxygen derivatives and other oxidant species generated from activation of immune phagocytes at the inflammation site (DiGuiseppi and Fridovich, 1984). Activation of the respiratory burst of of granulocytes in *vitro* is associated with damage to the DNA of cells in close proximity to them; this can be protected against by adding the antioxidant enzyme superoxide dismutase to the culture medium (Emerit and Cerrutti, 1981).

From our expanded perspective that oxidative stress (whatever its proximate form) can contribute significantly to the initiation of cancer, it follows that *the effectiveness of an initiator carcinogen should vary with the antioxidant status of its target tissue.* Thus cellular susceptibility to oxidant carcinogen-sis likely to be determined by an often delicate balance between conditions in the cell which promote oxidation, and those which inhibit oxidation, i.e., the localized redox-balance (Chow, 1979; Levine and Reinhardt, 1983; Borek, 1983; Levine and Kidd, 1985). There are many indications in the literature of pathological consequences occasioned by shifts in the redox balance. A few examples follow.

Glutathione is a peptide antioxidant and antitoxin which is found in relatively high concentrations in mammalian tissues and has proven anticancer efficacy (Novi et al., 1982; Brada and Bulba, 1982). Treatments which deplete tissue glutathione levels in experimental animals have been shown to render target tissues more susceptible to the adverse effects of carcinogens and other toxic chemicals (Miranda et al., 1981; Parke, 1982).

Hypoxic conditions increase the endogenous generation of oxygen radicals in aerobic cells (Freeman and Crapo, 1982). The imposition of hypoxic conditions on cultured liver hepatocytes results in a shift of the toxic effects of halocarbons (i.e., carbon tetrachloride and halothane), towards increased tissue damage at lower doses — (Trudell, 1984). Similarly, hypoxic tissues are also hypersensitized to the carcinogenic and other toxic effects of radiation (Green-stock, 1981).

Redox-active compounds are efficacious in restoring the NADPH/NADP+ balance in experimental rheumatoid arthritis and hypoglycemia (Chayen, 1982).

The existence of a delicate balance between pro-oxidative tendencies and anti-oxidative tendencies in mammalian cells and tissue may explain the existence of carcinogen thresholds. Initiators generally act in a dose-dependent manner: as more of the initiator is used, more neoplastic growths result. However, many potent initiators do not produce an effect in small quantities — there seems to be a threshold for their action. We speculate that small amounts of initiators sometimes may fail to produce a statistically-significant incidence of cancer — precisely because the tissue antioxidant defenses have remained intact to a sufficient degree. The quantities of reactive derivatives generated in the tissue may have failed to shift the redox balance sufficiently to generate detectable genetic damage to the tissue. Nevertheless, the localized antioxidant defenses may still have been significantly challenged by this oncologically-insignificant oxidant exposure and may be impaired as a result.

Net Result of Initiation: Oxidative DNA Damage. In chemical carcinogenesis, at the conclusion of the initiation event the cell's genetic material (i.e., the nuclear DNA and possibly also the mitochondrial DNA) has become oxidatively modified. These alterations may have occurred by relatively direct routes, such as the electrophilic binding of activated "ultimate" carcinogens to DNA bases (Margison, 1980; Miller and Miller, 1981), histones, nucleolar proteins, or ribonucleoprotein particles. Alternatively, DNA damage may have occurred by less direct routes, such as oxidative attack mediated by activated oxygen species generated from carcinogens by redox cycling (Bachur et al., 1979a; Greenstock, 1981; Kappus and ies, 1981; Oberley, 1982). Much of the initial DNA damage may be reversible due to the activity of DNA repair enzymes (Riley, 1982), but the residual damage is likely to become fixed into the genotype as the DNA replicates at cell division. Since initiator carcinogens

invariably cause tissue killing (histologic "necrosis") in their target organ (Smuckler, 1983), we presume that a substantial proportion of the "initiated" cells die prematurely from their incurred oxidative damage, or simply fail to divide further due to oxidative damage to their genetic material.

There is a high probability that the initiation event would damage cellular DNA sequences essential for aerobic functioning. A *great* many diverse enzyme systems make up the complex machineries of aerobic metabolism and cellular antioxidant defenses (Lehninger, 1982; Forman and Fisher, 1981; DiGuiseppi and Fridovich, 1984). Gene regions on the nuclear (or mitochondrial) DNA which code for these enzyme systems, and other genes which regulate these, are likely to become damaged in some proportion to the lengths of DNA required to code for the many proteins involved. Alternatively, specific zones on chromosomes or in the primary DNA sequence may be particularly susceptible to oxidative damage (Yunis, 1983). Impairment of mitochondrial antioxidant defenses by free radical attack during the initiation period could render the mitochondrial DNA abnormally susceptible to oxidative damage. The likelihood of this occurrence deserves to be explored, particularly since it could account for the consistent abnormal lowering of the mitochondrial MnSOD enzyme in cancer cells (Oberley, 1982). This enzyme has close prokaryotic affinities, so (according to the endosymbiont theory of origin of the mitochondria) is probably coded for by the mitochondrial DNA (DiGuiseppi and Fridovich, 1984).

As an alternative or adjunct to oxidative damage to DNA sequences, the oxidative impairment of enzymes associated with DNA synthesis, transcription, or repair could enhance cellular susceptibility to oxidative damage. Hereditary impairment of DNA repair enzymes in the human disease xeroderma pigmentosum renders skin cells more susceptible to radiation carcinogenesis (Rob-bins, 1979). Demopoulos et al. (1980) have reviewed the evidence that damage to the genetic material by free radicals could be initiated through peroxidative damage to the nuclear envelope, by reactive xenobiotic derivatives generated at the outer nuclear membrane. The chromosomes of the eukaryotic cell are attached to the nuclear envelope at or near the nuclear pores, where inner and outer nuclear membranes meet. Moreover, the outer nuclear membrane is functionally continuous with the endoplasmic reticulum, and carries Mixed-Function Oxidase and other electron-transferring (reductase) activity (Bachur et al., 1979b). Thus inactive parent carcinogens are subject to metabolic activation by the outer nuclear membrane in close proximity to the DNA-membrane attachment points.

At the conclusion of the initiation process, two major types of heritable changes are likely to have occurred in the target tissue, and in its stem cell population in particular (Slaga, 1984). One is likely to be the depletion of antioxidant nutrients (and hence a reduction of antioxidant defenses), as a result of heightened oxidative stress to the cell. Though not a genetic change *per se*, such a nutrient-depleted state in a parent cell could be passed on to its progeny in a manner resembling classical cytoplasmic inheritance. Inevitably, progeny of a nutrient-depleted parent cell will also be nutrient-depleted if their supply of nutrients via the circulation is not augmented. The second heritable change is damage to the nuclear (and perhaps also the mitochondrial) DNA, as the result of free radical oxidant attack on the cell in the absence of adequate antioxidant protection.

Cancer Promotion: Cell Proliferation Triggered by Oxidative Stress

The promotion step appears to be necessary in carcinogenesis to "fix" within the cell's genotype the DNA damage which has occurred at the initiation step, thereby initiating somatic mutations or other forms of repression or derepression of genes involved in carcinogenesis (Birnboim, 1983). It seems essential for "two-step" carcinogenesis that the damage event(s) of initiation become incorporated into the DNA double helix as it replicates prior to cell division. Thus *the key quality of promoter agents seems to be their ability to trigger cellular proliferation* (Slaga, 1984; Farber, 1984). It has been suggested that this effect is mediated preferentially through their effects on the subpopulation of poorly-differentiated cells in the target tissue. Such "stem cells" usually have a greater

propensity towards proliferation, and therefore are likely to be more "promotable" than their differentiated counterparts in the same tissue (Slaga, 1984).

Cancer Promoters Deplete Cellular Antioxidant Defenses. The cumulative evidence suggests that the promoter agents, like the initiator carcinogens, operate through free radical mechanisms. Almost any agent which acts to generate free radicals in the target tissue can act as a promoter. Slaga et al. (1983) have listed a number of free radical-generating compounds that are active as mouse skin tumor promoters, including benzoyl peroxide which is commonly employed in the treatment of acne. Organochlorine compounds, which are metabolically activated to free radical intermediates in the liver, are potent promoters of liver cancer. Their promoting activity is effectively protected against by the antioxidant vitamins C and E and the synthetic antioxidant DPPD (Johnson, 1979, 1982). The promoting action of certain "model" promoters, the phorbol esters, is enhanced by added superoxide anion radical and is inhibited by the antioxidant enzyme superoxide dismutase, which detoxifies ("dismutates") the superoxide anion* (Borek and Troll, 1983). Superoxide dismutase (SOD) also inhibits the "clastogenic" (chromosome-breaking) effects of the phorbol ester TPA *in vitro* (Little et al., 1983), Thus some of the most common promoter compounds act through free radical-mediated oxidative mechanisms to damage the cell's genetic material, and very likely also to impair cellular antioxidant protective factors such as the enzymes SOD and glutathione peroxidase (Slaga et al., 1983).

Phorbol esters are in widespread experimental use as skin tumor promoters. There is a good deal of evidence that phorbol esters accomplish promotion through stimulating a skin inflammatory response. Activated granulocytes are mutagenic in the Ames test (Weitzman and Stossel, 1981) and those phorbol esters which are successful as promoters elicit the respiratory burst from granulocytes in the skin, whereas non-promoter phorbol esters fail to elicit the respiratory burst (DiGuiseppi and Fridovich, 1984). Viaje et al. (1977) showed that phenol ester promoter activity could be blocked by the antiinflammatory corticosteroids, with a specificity which closely matched their antioxidant radical-scavenging ability. When the promotion event in mouse skin was inhibited by retinoids (antioxidants related to vitamin A) there was coordinate inhibition of superoxide production in the tissue (Witz et al., 1980).

Certain known major promoters of cancer in humans also may act through indirectly accelerating tissue free radical production. Archer (1979) proposed that the carcinogenicity of certain films and fibers implanted into a target tissue (in particular, asbestos fibers) can be explained by a "frustrated phagocytosis" phenomenon. Neutrophils or other phagocytes, in their futile attempts to engulf a highly elongate, flexible fiber by "exocytosis," tend to spread over the surface of the fiber and undergo their respiratory burst. In their repeated attempts to overpower this source of irritation, they release excessive amounts of superoxide and other toxic "phagocytic artillery." If the fiber is not removed as a source of chronic irritation, continued futile attempts by phagocytes to engulf it could initiate foci of chronic inflammation, eventually induce somatic mutations, and culminate in tumor development. Archer proposed that pulmonary fibrosis by inhaled foreign bodies also could be attributed to such a mechanism. Ruff and Pert (1984) have suggested a related mechanism for promotion by chronic exposure to cigarette smoke, and Konstantinidis et al. (1982) reported that a single dose of the carcinogen nitrosome-thylurea preferentially resulted in tumors at sites of chronic inflammation in rodents.

A free radical mechanism of action for the promotion event in carcinogenesis may explain why most promoters are also initiators under the right circumstances (Iversen and Astrup, 1984). The few compounds which are purely promoters, if any really do exist, must not be radical-formers and therefore incapable of acting as complete carcinogens.

*The synthetic copper salicylates, anti-inflammatory compounds which mimic SOD, block the promoting effects of phorbol esters in mouse skin (Walker, 1983). Salicylates also block DMBA tumor initiation and the mutagenicity of 6-thioguanine in the mouse (reviewed in Bland, 1984).

Such compounds may act to stimulate cellular proliferation by non-radical means.

Promoter Mitogenesis may be Free Radical-Mediated. Agents which "promote" chemical carcinogenesis invariably are mitogenic - they stimulate the "initiated" cell population to proliferate (Slaga et al., 1983; Slaga, 1984). If the initiated cell can divide successfully in the face of the oxidative damage which it has sustained from the initiator agent (as well as from exposure to the promoting agent), its progeny may proceed to the next stage of carcinogenesis: neoplastic transformation. An "initiated" cell population usually must go through a number of cell replication cycles for the phenotypic characteristics of neoplastic transformation to emerge. The common promoters appear to trigger cell division through their effects on the membranes of initiated cells (Demo-poulos et al., 1980). *Promoter agents may mediate oxidative damage to the plasma membrane which in turn affects the cell's cyclic nucleotide pools and associated "internal messenger" systems, thereby stimulating the cell to divide.*

Current understanding of the cell cycle suggests that a cellular shift from quiescence to mitosis requires a marked lowering of cytosolic cyclic AMP (cAMP) levels (Boynton and Whitfield, 1983). Usually cyclic GMP (cGMP) levels increase. The adenylate cyclase enzyme which generates cAMP is an "integral" plasma membrane protein which is phospholipid-dependent, and therefore highly susceptible to free radical attack on the lipids in the plasma membrane (Demo-poulos et al., 1980). Thus cAMP production could become impaired as a consequence of free radical attack on the cell via the action of promoters (or initiators, or both). The guan-ylate cyclase enzyme which generates cGMP apparently requires oxidation to become active (de Robertus and Craven, 1982). Thus oxygen radicals produced due to the action of promoters on cellular membranes may inhibit intracellular cAMP production and stimulate cGMP production, thereby triggering mitosis in initiated cells.

It has been suggested that a shift in the intracellular cyclic nucleotide balance towards cGMP will trigger cell division (Oberley et al., 1982), but this hypothesis currently remains unproven (deRobertus and Craven, 1982). It seems, nevertheless, that (a) cAMP levels must fall before the cell can enter a division cycle; (b) elevated intracellular cGMP could serve as a positive signal for cell growth and transformation. The levels of cGMP have been found to be elevated in a number of tumors and in several rapidly-proliferating cell lines.

Any change in cGMP that plays a primary role in the initiation of cancer would be expected to occur soon after exposure of the target cells to a carcinogenic stimulus. Recent studies have demonstrated that several chemical carcinogens, including N-nitroso compounds, hydrazine, and butadiene epoxide, cause marked and rapid activation of the guanylate cyclase enzyme following exposure (de Robertus and Craven, 1982). As eluc-icated by a new radioimmunoassay for cGMP, stimulation of guanylate cyclase by chemical carcinogens or their activated metabolites appears to proceed via a unique free radical pathway.

Mechanism of Activation of Guanylate Cyclase by Free Radicals. Guanylate cyclase is present in all mammalian tissues. This enzyme can become "spontaneously" activated by exposure to air or oxygen, or become inactivated by -S-S (disulfide group) reducing agents. The enzyme appears to become activated by the oxidation of contiguous sulfhydryl groups in the molecule to a disulfide bond (2 - SH to -S-S-). Thus a variety of oxidant agents, including hydrogen peroxide and superoxide anion (Mittal and Murad, 1977), as well as fatty acid hydroperoxides and prostaglandin endoperoxides (Goldberg et al., 1978) activate the enzyme. Conversely, a variety of sulfhydryl compounds and antioxidant agents inhibit its activation (de Robertus and Craven, 1982). Thus cysteine and glutathione are effective inhibitors, as are the antioxidants ascorbate (vitamin C), BHA, BHT, and retinoids (vitamin A analogs), all of which have anticarcinogenic activity. *Thus it is likely that this enzyme is modulated in vivo by the redox state of its environment* (Mittal and Murad, 1977; Goldberg et al., 1978).

Both cGMP and cAMP affect the cyclic nucleotide-dependent protein kinases, which

enzymatically activate other cell enzymes in a cascade of reactions. Cyclic GMP has been reported to stimulate the phosphorylation both of cell membranes and of nucleopro-teins, and to increase plasma membrane permeability by a similar phosphorylation mechanism (reviewed in Oberley et al., 1981). These may be the major pathways by which cGMP regulates cell growth and cell division. We suggest that by increasing cellular oxidative stress cancer promoters can activate the guanylate cyclase system in the "initiated" target cell, thereby helping shift the cell cycle into a proliferative mode.

We suggest that promoter agents, by acting directly or indirectly to impair antioxidant enzymes and/or deplete antioxidant nutrients in the target tissue (Slaga et al., 1983), very likely contribute to an elevation of the pro-oxidant/antioxidant ratio, thereby exacerbating the oxidative stress caused by the initiation event. The outcome of the promotion event may be further impairment of the cell's respiratory metabolism beyond the damage effected by the initiation event, as a result of further damage to the DNA coding for the critical enzymes involved (Birnboim, 1983), combined with impairment of cellular antioxidant defenses.

The tissue response to the localized injury incurred from the effects of initiators and promoters may initiate a "vicious cycle" leading to further deterioration. The oxidative damage to target tissue which results from exposure to carcinogen(s) could initiate inflammatory events that deplete oxygen supplies as they further accelerate free radical production in the tissues, such as the generation of prostaglandins and leuko-trienes and stimulation of the respiratory burst in phagocytic cells. Aromatic carcinogens often undergo redox cycling in the cell, generating superoxide anion and depleting cellular oxygen levels (Kappus and Sies, 1980; Mason, 1983). Novi et al. (1982) suggested that certain of these could be retained in the cell and become sources of chronic toxicity. Lipid peroxidation and other processes resulting from cell injury also utilize oxygen (Yagi, 1982). Hence *following the promotion event two pathological processes could ensue, more or less in parallel: impairment of antioxidant defenses and reduction in oxygen availability.* These occurrences would presumably put the aerobic mutagenized cell at great selective disadvantage in terms of its ability to synthesize ATP, conserve its genetic material, retain sufficient oxygen for metabolic functions, and protect its lipid-dependent membrane enzymes involved in nutrient uptake and aerobic respiration. Conversely, a mutagenized cell line which successfully becomes anaerobic should enjoy some selective advantage from its unique metabolism.

Anaerobiosis and Hypoxia in Malignant Transformation

So far we have hypothesized that *cancer initiators*, by initiating free radical attack on susceptible tissues, impair cellular antioxidant defenses and damage the DNA of target cells. *Cancer promoters* cause further damage to the initiated cell population by free radical mechanisms, acting to "fix" this initial DNA damage into the genotype by stimulating the cell population to proliferate. Promoters seem also to contribute to a chronic inflammatory tissue state, which in its turn may favor cellular proliferation and hyper-mutability. Multiple mutations in the resultant proliferative cell line could generate cell clones with severely incapacitated respiratory metabolism, thereby setting the stage for the clonal evolution of glycolytic-anaero-bic cell lines which do not require oxygen for growth.

Following the promotion event, the next key event in carcinogenesis may be the emergence (from an originally aerobic, genetically-altered target cell population) of anaerobic cell clones which rely on the more primitive form of glycolytic metabolism ("fermentation"). Mutational damage to the structural or regulatory genes for antioxidant enzymes (glutathione peroxidase, cat-alase, and especially superoxide dismutase), or to genes coding for other proteins involved in aerobic metabolism, is likely to result in a marked impairment of the cellular aerobic lifestyle during or immediately following the promotion event. There are biochemical indications that the preneoplastic cells make attempts to adapt metabolically to initiator and promoter exposures (Farber, 1984). However, some form of "differentiative

regression" of the cell line to an anaerobic state (perhaps reminiscent of the embryonic tissue lifestyle) appears to be inevitable if these cell lines are to survive. The major antioxidant enzymes are indeed lowered in activity in malignant tissues — *catalase* and *glutathione peroxidase axe* often decreased and *superoxide dismutase* (particularly its mitochondrial form) is consistently decreased (Oberley, 1982).

Loss of the Mitochondrial Superoxide Dismutase in Cancer. The manganese-dependent superoxide dismutase (MnSOD) constitutes the first enzymatic defense in the mitochondria against superoxide anion radical generated from electron leakage during aerobic respiration (Freeman and Crapo, 1983). This radical species is generated in amounts of 2 to 5 percent of all oxygen consumed, and by some reports as much as 95 percent of the total superoxide anion generated in the aerobic cell is generated in the mitochondria (Boveris and Chance, 1973; Forman and Boveris, 1982). *The activity of the mitochondrial SOD is, without exception, decreased in all cancers so far examined.*

This marked reduction of MnSOD activity in cancer has been established for spontaneous, transplanted, virally induced, chemically induced, in *vitro, in vivo* tumors in human, rat, and mouse (Oberley, 1982). The causes of the loss are unknown, but could be impaired synthesis, increased degradation, presence of an inhibitor, production of an inactive protein, or dysregulation (the MnSOD is known to be substrate-inducible). There are, of course, numerous other enzymatic abnormalities that can be measured in cancerous cells. Is there evidence that the loss of MnSOD is more important than other enzyme changes characteristic of the malignant state?

The MnSOD enzyme is believed to be present in all oxygen-metabolizing cells. It is lacking in obligate anaerobes but is present in aerotolerant anaerobes, presumably because its major (if not its sole) physiological function is to protect against the toxic effects of superoxide anion radical (McCord et al., 1971; DiGuiseppi and Fridovich, 1984). The MnSOD of the mitochondrial matrix seems to be the only mitochondrial enzyme capable of detoxifying the superoxide anion radical; in its absence oxidative damage should soon ensue to the respiratory enzyme assemblies of the inner mitochondrial membrane, to the mitochondrial DNA, and possibly to other organelles of the cell.

Since it is likely that the mitochondrial MnSOD is coded for by DNA in the mitochondrion, synthesis of the enzyme would very likely be impaired by damage to the DNA of this organelle. Mutants of £. *coli* with MnSOD defects behave like obligate anaerobes (DiGuiseppi and Fridovich, 1982). The consistent lowering of MnSOD activity in cancer may be linked with the mitochondrial abnormalities observed in cancer cells by electron microscopy (Springer et al., 1980), and possibly with the abnormal hesitancy of mitochondria isolated from malignant tissue to respire aerobically (Oberley et al., 1981).

Many enzymes of aerobic metabolism appear to be particularly susceptible to oxidative damage, due to dependence on phospholipids and/or sulfhydryl groups for their biological activity. Demopoulos et al. (1980)-have compiled a list of such enzymes. Many of the Krebs Cycle and respiratory electron transfer enzymes situated in the mitochondrial membranes are on this list. Oxidative damage *to* these enzymes or to proteins or genes associated with their regulation could cripple the aerobic respiration of the cell; it would follow that the progeny of such cells must become obligate anaerobes or fail to survive. We believe that *many malignant cell lines may have become obligatorily anaerobic. Lacking the mitochondrial MnSOD, they have undergone a critical redox shift beyond the bounds of antioxidant biochemical adaptation either they no longer need, or are incapable of, antioxidant adaptive responses.*

There indeed appears to be a reciprocal relationship between the degree of anaerob-iosis in malignant cells and their MnSOD activity, as exemplified by the Morris hepatomas (Bize et al., 1980; Oberley, 1982). These in *vitro* tumors range in phenotype from fast growing, undifferentiated to slow growing, well-differentiated tumors, and all have abnormally-lowered MnSOD activity. The lowest MnSOD activity was manifested by the fastest-growing and least-differentiated

tumor. This correlation has been confirmed by other laboratories and for other tumors. Also, the Morris hepatomas appear to generate superoxide anion radical at different rates. The fast-growing, undifferentiated strain generated only a third as much superoxide as did the slow-growing, well-differentiated Morris hepatomas (Bize et al., 1980). This finding would be predicted from our hypothesis. It indicates that (at least for some cancers) the attainment of the differentiated state is closest metabolically to the "normal" state, in that those tumors which grow the slowest and are the most differentiated have SOD activity closest to normal, and generate the most superoxide. The corollary is that those tumors which are the fastest-growing are the least differentiated, generate the least superoxide, and have the lowest MnSOD activity.

Superoxide dismutase is known to be inducible by conditions of increased oxidative stress (DiGuiseppi and Fridovich, 1984); therefore SOD activity in solid cancerous tumors may correlate with the degree of oxidative stress hypoxia in each. In one chemically-induced cancer that was tested (rat mammary carcinomas initiated by the potent carcinogen DMBA), the SOD content correlated inversely with the degree of hypoxia in the tumor tissue (Petkau et al., 1977). The SOD concentration at the centre of the carcinoma (the most hypoxic zone) was approximately half that at the edge of the tumor. Experimental imposition of hyp-eroxic conditions significantly increased the SOD concentrations in both zones. Thus tissue SOD levels in this cancer may have been subject to down-regulation or repression by hypoxia (implying anaerobiosis) and inductive increase by hyperoxia (implying a greater degree of aerobic respiration in the presence of higher tissue concentrations of oxygen). We conclude that tissue content or activity of SOD in cancer may reflect the degree of oxygenation which prevails locally and the abilities of cancerous cells to function anaerobically.

Mitochondria isolated from some types of tumors are able to generate superoxide when provided with substrate, but nevertheless exhibit metabolic abnormalities and may not generate normal (non-tumor) levels of superoxide *in vivo* (Oberley, 1982). Cells (whether cancerous or non-cancerous) which are metabolizing anaerobically should generate fewer endogenous free radicals since they are no longer transferring electrons to oxygen, and indeed the level of measurable free radical activity has been consistently found to be lowered in cancer cell populations (reviewed in Swartz, 1982). Those proliferative, genetically-altered, hypermutable cells which survive in hypoxic cellular environments may well be those clones with mutations which best confer selective advantage on the hypoxic cell.

Cancer cells which are quantitatively anaerobic compared to normal, differentiated cells, correspond to "facultative anaerobes" as suggested by Warburg (1969). Hypoxic tissue states may well play a key role in encouraging or facilitating the process of cellular transformation to malignancy. Those mutation events which favor survival of the hypoxic and hypermutable clones may be simultaneously those which confer malignant phenotype(s). The phenotypic changes required for anaerobic survival may be incongruous with the normal homeostasis of the host, and an invasive, malignant tumor may result.

Central Role for Hypoxia in Malignant Transformation. We suggest that hypoxia plays a pivotal role in the causation of most cancer. Here we build on hypotheses originally put forward several decades ago by the great scientists Otto Warburg and Albert Szent-Gyorgyi. During the periods when Warburg and Szent-Gyorgyi formulated their hypotheses, the free radical contribution to carcinogenesis was not known, since the sophisticated techniques for identifying and quantifying free radicals were not yet in use. Nevertheless, these earlier theories linking anaerobiosis with cancer causation continue to be well supported by the experimental data. We suggest that as more is learned about carcinogenesis from the field of free radical biochemistry, significant attention will be directed back to the ideas of these scientists regarding the causes of cancer.

Our assertions concerning a central role for hypoxia in neoplastic transformation and malignancy rests largely on the work of

Warburg*, who wrote extensively on what he thought to be the prime causes of cancer and options for its prevention (Warburg, 1956, 1969). Warburg was apparently the first to suggest that anaerobiosis is that property of cancer cells that uniquely distinguishes them from all normal body cells. According to Warburg, oxygen gas, the primary electron acceptor in plants and animals, is dethroned in cancer cells and replaced by an energy-yielding mechanism typical of the more primitive prokaryotic cells: the fermentation of glucose in the absence of oxygen (glycolysis). His assertions currently are well-supported by a large number of confirmatory observations (reviewed in Smith and Kenyon, 1973), and have led to cancer therapies based on the glycolysis rate differential between normal and cancerous tissue (Suppan, 1979). Holt (1983) has suggested that "the basic cause of cancer is failure of aerobic glucose metabolism to control anaerobic glycolysis," akin to an "uncoupling" of these metabolic pathways for energy production in the affected cell.

Warburg studied the effects of hypoxia on cultures of embryonic mouse cells, and discovered that lowered oxygen availability could result in their irreversible metabolic transformation to an anaerobic state. In a suitable culture medium with adequate oxygenation, these cells grew normally. If during such normal growth the oxygen tension was reduced, within 48 hours the population had become "fermenting", i.e., glycolytic-anaer-obic. If the cells so transformed were then returned to an environment of normal oxygen tension, they retained their anaerobic state; Warburg concluded that the metabolic transformation was irreversible. He found that a reduction in aerobic respiration of as little as 35 percent (achieved by lowering the ambient oxygen tension) was sufficient to trigger this irreversible shift to anaerobic respiration, and suggested these cells were "facultative anaerobes." These findings of Warburg's, subsequently repeated in other laboratories lead us to conclude that *hypoxic conditions initiated an irreversible metabolic transformation from a predominantly aerobic to a predominantly anaerobic existence in cultured mammalian cells.*

Warburg also succeeded in initiating cancer in *vivo*, simply by implanting solid discs of relatively inert substances under the skin of rats (summarized in Warburg, 1969). The discs soon became surrounded by capsules of living tissue. Normal cells became malignantly transformed in the region of the capsules — sarcomas developed. It was immaterial whether the solid discs were made of plastic, gold or ivory; what did seem to matter was their effective reduction of bloodborne oxygen to the capsular cells — which triggered their malignant transformation. Warburg concluded that sufficiency of oxygen allowed cells to maintain their most efficient levels of energetic metabolism, consequently to overcome thermodynamic limitations and differentiate normally, whereas lack of oxygen could not support the sophisticated energetics essential for differentiation and therefore the cell population reverted to the more primitive, more thermodynam-ically-favorable, anaerobic state.

Warburg may also have been the first to grasp the significance of the relationship between "fermentation" (glycolysis, anaerobiosis) and malignancy. In his 1966 Lindau Lecture to Nobelists (Warburg, 1969) he discussed the experiments of Burk and Woods, which were the first to quantitatively demonstrate the relationship between fermentation (anaerobiosis) and growth rate in the Morris hepatomas. From this example we conclude that, at least in some cancers, the greater the "degree" of malignancy the greater the degree of cellular anaerobic functioning.

These characteristics of malignant cells are reminiscent of the pre-oxygen, lesser-differentiated state of our anaerobic ancestors, which has been termed by Szent-

* Professor Otto Warburg won the Nobel Prize in Medicine in 1931 for his discovery of the oxygen-transferring enzyme of cell respiration and was voted a second Nobel Prize in 1944 for his work on the hydrogen-transferring enzymes. "Many universities, including Harvard, Oxford, and Heidelberg, have offered him honorary degrees. His main interests are Chemistry and Physics of Life. In both fields no scientist has been more successful." (Dean Burk, Editor, in Warburg, 1969, p. 6.) Warburg favored an "Orthomolecular" approach to cancer therapy: supplementation with "active groups", i.e., vitamins, minerals, coenzymes) after operations to remove tumor masses, in combination with avoidance of toxins. Even at that time (1956) Warburg could state that chemicals cause at least 80 percent of all cancers.

Gyorgyi the *alpha state.* Szent-Gyorgyi (1979) viewed cancer as originating from an insufficient availability of oxygen for accepting electrons*. We propose that a state of chronic hypoxia which does not sacrifice the precancerous cell outright could influence the cell's differentiative course and contribute to malignancy by selecting for those cell clones best suited for anaerobic existence in the aerobic organism. It is likely that the multiple mutational events which damage the enzymatic apparatus of aerobic metabolism, when combined with pathological oxygen consumption patterns which encourage the development of a functionally hypoxic state in the oxidatively-stressed cell, will in many cases damage the cell sufficiently to cause its death (Kappus and Sies, 1981; Yagi, 1982; Mason, 1983). However if the cell survives and can generate enough ATP to survive in the presence of limited oxygen concentrations (i.e., via anaerobic metabolism) a regressive, precancerous cell line may result.

Hypoxic States Mediate Cell Injury.

Hypoxic/ischemic tissue states may play a determinant role in a variety of degenerative diseases. According to Robbins and Cotran in their authoritative *text The Pathological Basis of Disease, "Hypoxia is probably the most common cause of cell injury and may also be the ultimate mechanism of damage initiated by a variety of physical, biological and chemical agents." (p. 24)* Under optimal conditions of oxygen availability, superoxide is generated in the respiratory electron transfer chain as single electrons become shunted to molecular oxygen (Forman and Boveris, 1982); this potentially-damaging situation is normally offset by protective antioxidant defenses in the cell. However, when oxygen tension is not regulated within normal limits, such electron leakage becomes intensified (Freman and Crapo, 1982; Levine and Kidd, 1985). Factors which precipitate hypoxia *in vivo* include:

- *Vessel Occlusion and Traumatic Injuries.* Impairment of blood flow (ischemia) and consequent tissue hypoxia often result from blood vessel occlusion by atherosclerotic plaques or thrombi, and from vessel spasm. Ischemic hypoxia also plays a key role in death from traumatic injury.

Cold and thermal shock may induce vasoconstriction and subsequent tissue ischemia.

- *Vascular Disruption.* Blood vessel endothelial linings can be disrupted by antigen-antibody reactions occurring in the vicinity of the vascular endothelium such as the Arthus reaction (Robbins and Cotran, 1979), and probably also from granulocyte discharge and platelet aggregation (del Maestro et al., 1982). Experimentally, peroxidized lipids injected into animals also can disrupt vessel endothelia.parti-cularly those of the aorta and pulmonary artery (refer to Hirai and to Goto in Yagi, 1982).
- *Hemorrhages from Traumatic Damage.* Blood carrying cell fragments and other debris as a result of physical trauma can introduce into the tissues significant quantities of redox-active metals, particularly iron and copper, capable of catalysing free radical generation locally. Thus rapidly-proliferating free radical reactions catalyzed by redox-active metals mediate experimental ischemic trauma to the central nervous system (Demopoulos, 1982).
- *Decreased Blood Oxygen-Carrying Capacity.* Any decrease in hemoglobin concentration in the blood, or in its oxygen-carrying efficiency, results in a decrease in blood oxygen-carrying potential (Robbins and Cotran, 1979).
- *Inefficient Respiration.* Any impairment in lung function may result in a chronic hypoxic state. Forced expiratory volume (FEV) and vital capacity, both standard measures of lung function, are positively correlated with lifespan (Cullen et al., 1983; Menotti et al., 1983). The strength of this correlation was much greater than those between blood cholesterol or cigarette smoking and longevity, suggesting that hypoxia is a determinant factor in a variety of human disease states (Lipinski, 1983).
- *Hyperoxia Increases Electron Leakage.* Hyperoxia (arising from exposure to

* Szent-Gyorgyi believed that the very creation of life must have demanded "electron donors" and "electron acceptors". The elements with fewer available electrons became electron acceptors, whereas electron-rich molecules became electron donors.

hyperbaric oxygen) paradoxically may mimic hypoxia by also exacerbating the "electron leakage" phenomenon (Freeman and Crapo, 1982). The presence of elevated concentration of oxygen in the inner mitochondrial membrane may abnormally "speed up" electron transfer, thereby generating abnormally high levels of superoxide anion.

Clearly dysfunctions of oxygen acquisition and utilization are deleterious to cells and underlie many common pathologies. The question then arises: *does the toxicity of activated oxygen species consistently underlie hypoxic injury?* We suggest that the answer to this question is "yes". Surely an aerobic cell subjected to hypoxia cannot maintain itself indefinitely. As less NADH (and other electron carriers) is utilized for "injecting" electrons into the electron transfer process under hypoxic conditions, the ratio of reduced carriers to oxidized carriers (usually measured as the NAD(P)H/NAD-(P)+ ratio) should rapidly build up and trigger a cellular metabolic shift to the glycolytic mode (the "reverse" Pasteur Effect — Suppan, 1979; Holt, 1983)* This metabolic energy-yielding mode is anaerobic since it does not require oxygen to generate ATP from ADP. Cancer cells, being anaerobic, may carry a higher intracellular NAD(P)H/-NAD(P)+ ratio, and exist in an abnormally reduced redox state.

Hypermutability and Hypoxia Favor Malignant Transformation. We suggested earlier that precancerous cells are likely to be hypermutable. Much as a nutrient-depleted, unstable environment is hostile to the pro-karyotic microorganism (Ames et al., 1975), so must the ischemic/hypoxic tissue environment also be hostile to the aerobic euk-aryotic cell of higher organisms, since availability of oxygen (and also nutrient uptake) is substantially reduced. Ionic equilibria and transmembrane electrical potentials, to which are linked a variety of cellular metabolic and regulatory functions (Alberts et al., 1983), will suffer as membrane transport proteins become inactivated from sulfhydryl group inactivation and/or peroxidative damage to membrane lipids. ATP production falls as aerobic respiration is compromised. Furthermore, since Na/K ATPase pump activity, which requires a chunk of the cell's aerobic production, is linked to cellular transport systems for the absorption of amino acids and glucose (Alberts et al., 1983), these nutrients also may become limiting to the hypoxic cell. Thus hypermutability combined with a hostile ischemic/hypoxic environment could greatly enhance selective evolution of a cell population able to withstand these environmental rigors.

Clonal Selection for Malignant Traits

The supposition that clonal selection is involved in the cellular progression from neoplastic transformation to malignancy is implicit in the cancer literature. Farber (1984) has formulated it as "clonal expansion," "differential inhibition, " or "differential stimulation," and attributes some of the initial phenotypic changes of neoplastic transformation to a cellular response to chemical carcinogens. Smuckler (1983) has advanced a scheme for chemical carcinogenesis which relies on clonal selection for explaining many of the features of malignancy.

Evidence for Clonal Selection in Malignancy. Hypotheses that describe neoplastic progression in terms of sequential selection of mutant cell subpopulations (so-called "tumor stemlines") date back to the 1950's, and currently there is considerable evidence to support a clonal origin for most malignant neoplasms (Nowell, 1976; Yunis, 1983). In some cancers all the cells of a given tumor show the same abnormal chromosome pattern, suggesting a common cell origin. Studies of glucose-6-phosphate dehydrogenase (G6PD) activity in a variety of neoplastic tumors from heterozygous women have shown that typically the same member of the X-chromosome pair is functional in all cells of a given tumor, indicating descent from a single precursor cell. Also, in lym-phoproliferative neoplasms the immunoglo-

* The Pasteur Effect first described by Pasteur in the 1860's, is inhibition of glycolysis (fermentation) by oxygen.

bulins produced typically display biochemical homogeneity characteristic of a single clone. Malignant cells of most neoplasias carry chromosomal abnormalities which tend to be characteristic of the tumor type. Cytogenetically-discernible alterations in chromosome structure (i.e., band deletions, reciprocal translocations, trisomies, and others) have recently become more clearly definable in neoplasias due to methodological improvements (Yunis, 1983). Instances in which the first neoplastic event is visible at the chromosomal level are becoming increasingly more common — as with the "marker" Philadelphia chromosome of chronic granulocytic leukemia, monosomy of chromosome 22 in meningeal tumors, and an aberrant chromosome 14 in certain lymphopro-liferative disorders. Further experimental investigations of the mechanisms which underlie chromosomal breakage and rearrangement, point mutations, and the roles of oncogenes inherent in or acquired by the host genome (Guerrero et al., 1984; Yunis, 1983) should facilitate more precise clonal definition of malignant neoplasms.

Progression Past the Transformed State

The most significant biological characteristic of the post-neoplastic tumor is its capacity for producing cells able to invade the surrounding tissue and also to metastasize to distant sites. We suggest that this progression represents the cumulative effects of genetic instability in the neoplastic cell population as a result of free radical damage, coupled with the sequential emergence of aberrant subpopulations under the selective pressures of hypermutability and hypoxia. The data suggest that each cell division of a neoplastic cell carries an increased risk of genetic variation, and that (for human solid tumors at least) genetic instability becomes more pronounced as the neoplasm evolves (Nowell, 1976; Kovacs, 1980).

The key point here is that major genetic events do occur in tumor cell populations, with sufficient frequency to permit the sequential selection of mutant subpopulations able to successfully conform with the biochemical challenges to these sublethally-damaged cell lines. The carcinogenic action

of many popular chemo- and radiation "therapies" (see, for example, Wang and Howell, 1983) may serve to accelerate the appearance of new sublines within the tumor, or result in the emergence of other malignancy at some later date, months or years subsequent.

ClonalSelection for Malignancy. Within each neoplastic or preneoplastic cellular aggregate, it is usual for a wide variety of phenotypic combinations to be expressed by the progenies of individual cells, possibly in response to selective pressures from (a) the persistent effects of xenobiotic derivatives on their metabolism (Farber, 1984); (b) hypoxia due to poor vascularization, a situation common at the cores of solid tumors (Petkau et al., 1977); (c) tissue ischemic states resulting from chronic, non-malignant disease, or any of a host of possible in *vivo* stressors. It is thought that most neoplastic aggregates never attain a state of malignancy, rather that they redifferentiate to a quasi-normal phenotype (Farber, 1984). One or a few cellular aggregates ("nodules") may persist to become benign (non-invasive) tumors. Others totally escape the growth control of the host and become malignant tumors (Smuckler, 1983). The degree of hypoxia to which these neoplastic aggregates are subject, and the status of their antioxidant defenses, may determine their differentiative course subsequent.

Protection Against Malignant Progression by Antioxidants. *There* is a great deal of evidence that nutrient-derived and synthetic antioxidant factors can successfully intervene to halt the progression of chemical carcinogenesis at any point. Various antioxidants protect against the actions of initiator carcinogens in the "model" skin-tumor system (Slaga, 1984). Antioxidant compounds also can block the promotion process (Demopoulos et al., 1980; Slaga, 1984). Progression past promotion to neoplasia can also be protected against by antioxidants. Vitamin A and its related retinoid derivatives are widely recognized as tissue growth regulators and have also been shown to protect by antioxidant mechanisms (Bollag and Matter, 1981).

Dietary antioxidant factors can be potent inhibitors of malignant progression. Exposure to all-trans-retinoic acid (a synthetic vitamin A analog) can effectively block further progression of preneoplastic skin growths, skin (basal cell) carcinoma, and bladder papillomas (Witz et al., 1980; Boutwell, 1983). Other retinoids caused the regression of chemically-induced mesenchymal neoplasias and radiation-induced pulmonary adenomas (Bollag and Matter, 1981). *Glutathione, the tripeptide nucleophilic antioxidant, may cause the regession of established malignant tumors.* Novi reported in *Science* (Novi, 1981) that dietary supplementation with reduced glutathione (GSH) caused the regression of hepatocellular carcinomas induced in rodents by the highly potent carcinogen aflatoxin. Two subsequent studies failed to confirm this finding (Neal and Legg, 1983; Cook et al., 1984). Novi et al. (1982) cautioned that the route by which glutathione is administered is important; a second group (Brada and Bulba, 1982) has reported that reduced glutathione causes hepatocellular carcinomas to regress.

Antioxidant Nutrients Stimulate Immunity. Immune processes must depend critically on nutrient antioxidants, since supplementation of the "normal" diets of laboratory animals with antioxidants commonly enhances their immune resistance (Baum-gartner, 1979; Beisel, 1982). It is also evident that the antioxidant nutrients (and factors which resemble them chemically) are many of the same substances that are most important for host immune function (Beisel et al., 1981). A partial list of such substances would include retinols (vitamin A), ascorbate (vitamin C), selenium, alpha-tocopherol (vitamin E), glutathione, and zinc. The simple sulfhydryl compound mercaptoethanol can stimulate virtually every known category of cell-mediated immune response *in vitro,* as can a number of other sulfhydryl compounds (Baumgartner, 1979). According to Baum-gartner, both sulfhydryl compounds and alpha-tocopherol (vitamin E) can substitute in culture as mitogenic factors for antibody-forming cells, and some of these effects have also been observed in *vivo.*

It appears that the key physiological function of antioxidants in cellular immunity is that they protect immune phagocytic cells against inadvertent suicide from their phagocytic "respiratory burst" (Babior, 1982). Adequate availability of nutrient-derived antioxidants seems particularly critical for the continued functioning of these cells. Serfass and Ganther (1975) reported that deficiency in dietary selenium reduced the levels of the selenium-dependent enzyme glutathione peroxidase in rat neutrophils, as well as their ability to kill ingested bacteria, though having no effect on their ability to phagocytize their prey. Sulfhydryl groups are among the most vulnerable of the moieties located at the phagocyte cell surface, since they are located at the active sites of so many enzymes (including transport proteins). For lymphocytes to become activated, they must approach into close spatial association with macrophages, yet lymphocytes are exquisitely sensitive to the oxidants produced by the macrophage respiratory burst — lipid hydroperoxides, superoxide anion radical, singlet oxygen, hydrogen peroxides, hydroxyl radicals, and hypochlorite anion — whereas macrophages are considerably more resistant (Baumgartner, 1979). Hence the essentiality of antioxidants for cellular immunity.

The wide-ranging involvement of antioxidants in immunity is best exemplified by ascorbic acid (vitamin C). Table 2 in Beisel (1982) lists a number of consequences of *vitamin C deficiency* for the immune system, among them increased host susceptibility to infection; lowered T-cell/B-cell ratio; prolonged allograft survival; impairment of neutrophil motility and chemotaxis, along with reduced metabolic activity; and reduced macrophage motility concomitant with increased fragility. Similar findings are reported for the other well-investigated antioxidants such as alpha-tocopherol (vitamin E), and the retinoids. Table 4 in Beisel (1982) lists some possible effects of *dietary vitamin A supplementation,* among them enhanced host resistance to infection; shortened allograft survival; enhanced antibody production and systemic plaque-forming ability following immunization; and increased lymphocyte responsiveness to antigens.

Hence, from epidemiological studies, from controlled studies on laboratory animals,

and from in vitro cell culture research, we see that a host of antioxidant substances provide a major defense against neoplastic transformation and metastasis. Various differing research approaches lead to the very same conclusion: antioxidants are anti-inflammatory, are immune stimulants, and are the natural anticancer agents.

Recapitulation

According *to* the hypothesis presented here, chemical carcinogenesis is a multistage process featuring on the one hand, environmental and endogenous free radical oxidative stress; and on the other hand, the cellular antioxidants which protect against them. Following oxidative damage to their DNA, resulting either directly from electrophilic radical carcinogens or less directly from activated oxygen species generated by redox cycling, damaged cells in a target tissue initially attempt to adapt biochemically in an effort to restore optimal oxidant-antioxidant balance, but eventually (having suffered such crippling damage to their aerobic enzymatic apparatus) they become unable to adjust and proceed past a point of no return. These cells proceed to a metabolic state "Beyond Adaptation", an anaerobic-glycolytic state in which they are less vulnerable to oxidant attack. In aerobic tissues, exposure to chemical carcinogens most often exacerbates endogenous oxygen radical production, and may (in the case of redox-active carcinogens) also deplete cellular oxygen availability. This metabolic situation is likely to favor the selection of cell clones best able to survive and proliferate under anaerobic conditions. Stem cells are more likely to give rise to such harder clones, and the long hiatus between exposure to the carcinogen(s) and the emergence of cancer in humans and experimental animals can be attributed to the many generations of cellular proliferation typically required for the selection process to generate detectable malignant tumors.

Thus cancer is likely to be derived from genetically damaged cells, hypermutable due to impaired antioxidant defenses, and able to proliferate under hypoxic conditions) The conditions most critical for optimal functioning of the eukaryotic cell are a sufficient supply of oxygen (to ensure efficient burning of energy-yielding substrates) and the proper kinds and concentrations of natural antioxidants (to protect against the unrestrained reactivity of the oxygen molecules to which the cell is invariably exposed). The cancer cell has been deprived of these — yet has managed to survive and multiply.

Critical areas of support for our hypothesis include the highly persuasive evidence on the free radical nature of carcinogens and the effectiveness of antioxidants in blocking every stage in the carcinogenesis process; the consistent findings on the loss of MnSOD activity in cancer tissue coupled with an-aerobiosis as the malignant cellular lifestyle; the preferential susceptibility of cancer cells to redox-active drugs, attributable to their impaired antioxidant defenses and altered redox balance from glycolytic metabolism; and the evidence supporting somatic mutations and a clonal origin for most cancers. Taken together, these diverse lines of evidence serve to unify Warburg's early hypothesis that cancer originates from irreversible injury of aerobic respiration (and related conceptions by Szent-Gyorgyi) with more recent revelations that chemical carcinogenesis is mediated, directly or indirectly, by radical-electrophiles or activated oxygen species. These damage the antioxidant defenses of target tissues and mutate their cellular genetic material.

Judging from earlier critiques (reviewed in Swartz, 1982), any free radical hypothesis of cancer causation would have to satisfy several seemingly-anomalous findings: that free radicals are detected at abnormally low, rather than abnormally high, levels in cancerous tissue; that detectable lipid peroxidation also is abnormally low; and that cancer cells have been reported to have higher, rather than lower, antioxidant reserves. The incorporation into our hypothesis of the concept that cancer cells are very likely functioning in an anaerobic, rather than an aerobic, state would rationally resolve these potential objections. An anaerobic cell is likely to generate fewer free radicals endogenously, since by far the greatest amounts of these are normally generated in the mitochondria from aerobic respiration, It follows that as a consequence of lowered

endogenous free radical generation in the anaerobic cancer cell, ongoing lipid peroxidation should also be abnormally low.

The observations of elevated "antioxidant reserves" in cancer tissue really arose from crude estimates of their sulfhydryl content as "reducing power" (Duchesne, 1977), and are attributable to the extra-reduced state into which the cancer cell appears to take itself as a consequence of its shift to glycolytic metabolism. In the cancer cell the major mode of ATP generation appears to be non-mitochondrial, i.e., glycolytic. Cancer cells respiring glycolytically may encounter a major limiting factor in their capacity to generate ATP by this means: a relative glut in the reduced electron carriers NADH and NADPH, and a relative deficiency in their corresponding oxidized forms NADP+ and NAD+. These occur as a result of the impaired cellular ability to utilize these electron carriers in electron transfer to the ultimate electron acceptor — oxygen. Hence an extra-reduced cellular state has developed, i.e., a state *beyond antioxidant adaptation.* The cell in this state has markedly lower reliance on oxygen for metabolism, and fewer oxygen radicals are available to present an oxidizing challenge to the cell. The drawback for the cancer cell is that it now lacks a full complement of antioxidant enzymes and the flexibility to mount an effective antioxidant response to oxidant attack. In such a state of "redox delicateness," the intake of relatively high quantities of redox-active substances by the cell could lead to its incapacitation and subsequent destruction. The apparent abnormal redox state of cancerous cells is mirrored in their differential sensitivity to superoxide anion generated from the antineoplastic antibiotics by a redox cycling mechanism. Redox cycling, by which electrons are cyclically donated to molecular oxygen to generate superoxide and other activated oxygen species, is the mechanism of action of major anticancer drugs such as adriamycin and daunomycin (anthracyclic quinones); carminomycin, rubidazone, no-glamycin, aclacinomycin A, and steffimycin (benzanthraquinones); mitomycin C and streptonigrin (N-heterocyclic quinones); and lapachol (naphthoquinone) (Bachur et al., 1979a; Oberley, 1982). Their semiquinone intermediates may be selectively toxic to

cancer cells both directly, by their ability to generate superoxide anion preferentially, and/or indirectly, by inducing a cellular hypoxic state due to the increased oxygen consumption from redox cycling.

The major limitation of the redox-active antibiotics in cancer chemotherapy is their lack of tissue specificity. Adriamycin, the best-studied, is highly toxic to heart muscle cells, which are to some degree anaerobic, and can even induce cancer in the mammary gland, which is known to have comparatively low antioxidant defenses (Wang and Howell, 1982). Thus the impaired antioxidant flexibility and altered oxygen tension of cancerous tissues appear to be the primary parameters which determine their sensitivity to the toxic effects of oxygen radicals generated from redox cycling. We speculate that nutrient-derived, redox-active antioxidants are likely to be more effective against cancer as "redox drugs" (Chayen, 1982) than the synthetic compounds presently used.

Ascorbate, alpha-tocopherol, and glutathione may prove to be extremely valuable against malignant tissues as their partially-oxidized, free-radical intermediates (the ascorbyl radical, the tocopheryl radical, and the glutathionyl radical), which can be reconverted to the fully-reduced nonradical forms by normal tissues, but very likely not by cancerous tissues. Tsao and Salimi (1984) have summarized the findings from several studies published in Japan, that *oxidized* derivatives of ascorbic acid were more effective against cancer in mice than (reduced) ascorbic acid, and that the enzyme ascorbate oxidase had antitumor activity. Also, Kimoto et al. (1983) showed that this antitumor activity of ascorbate in mice was enhanced when ascorbate was injected with copper (a redox-active metal). We interpret these results to mean that *it is not so much the redox state of cancer cells which renders them susceptible* to the toxic species produced from redox cycling (i.e., from antibiotics and in this case from a natural antioxidant compound also); *rather, it is their lack of redox-buffering capacity* due to the repressed state or non-inducibility of their antioxidant defenses*. Whether or not cancer cells have a higher steady-state level of antioxidant activity from existing on the reduced side of

normalcy, their antioxidant "bounce" (flexibility, adaptability, depth) has been severely compromised due to their lowered reserves of SOD and other antioxidant enzyme activity.

Acknowledgements

We acknowledge contributions to this thesis from Jeffrey Reinhardt, and the assistance of David Brownell in typing and editing the text.

References

ALBERTS, B., BRAY, D., LEWIS, J., RAFF, M.,-ROBERTS, K., and WATSON, J.D.: Molecular Biology of the Cell. Garland Publishing, New York, 1983.

AMES, B.N: Dietary Carcinogens and Anticarcino-gens. Oxygen Radicals and Degenerative Diseases. Science 221: 1256-1264 (Sep. 23, 1983); letters to Science, 224: 659-760 (May 18, 1984).

AMES, B.N., McCANN, J., and YAMASACHI, E.: Methods for Detecting Carcinogens and Mutagens with the Salmonella/Mammalian Microsome Mutagenicity Test. Mutation Res. 31: 347-364, 1975.

ARCHER, V.E.: Carcinogenicity of Fibers and Films: a Theory. Med. Hypoth. 5: 1257-1262, 1979.

BABIOR, B.M.: The Role of Active Oxygen in Microbial Killing by Phagocytes. In: Pathology of Oxygen, pp. 45-58. Ed. by A. P. Autor. Academic Press, New York, 1982.

BACHUR, N.R. et al.: NADPH-Cytochrome P-450 Reductase Activation of Quinone Anticancer Agents to Free Radicals. Proc. Natl. Acad. Sci. USA 76: 954-957, 1979a.

BACHUR, N.R., FRIEDMAN, R.D., and GEE, M.V.: Nuclear Catalyzed Antibiotic Free Radical Formation. Proc. Am. Assoc. Cancer Res. 20: 128, 1979b.

BAUMGARTNER, W.A.: Antioxidants, Cancer, and the Immune Response. In: Trace Metals in Health and Disease. Ed. by N. Kharasch. Raven Press, New York, 1979.

BEISEL, W.R.: Single Nutrients and Immunity. Am. J. Clin. Nutr. 35 (Feb. Suppl.), 417-468, 1982.

BEISEL, W. R., EDELMAN, R., NAUSS, K., and SUSKIND, R.M.: Single-nutrient Effects on Immunologic Functions (Report of a Workshop). J. Am. Med. Assoc. 245: 53-58, 1981.

BERENBLUM, I. and SHUBIK, P.: A New Quantitative Approach to the Study of the Stages of Chemical Carcinogenesis in Mouse Skin. Brit. J. Cancer 1: 383-391, 1947.

BIRNBOIM, H.C.: Importance of DNA Strand-break Damage in Tumor Promotion. In: Radioprotectors and Anticarcinogens, pp. 539-556. Ed. by O. F. Nygaard and M. G. Simic. Academic Press, New York, 1983.

BIZE, I. B., OBERLEY, L. W., and MORRIS, H.P.: Superoxide Dismutase and Superoxide Radical in Morris Hepatomas. Cancer Res. 40: 3686-3693, 1980.

BLAND, J.: Copper Salicylates and Complexes in Molecular Medicine. Int. Clin. Nutr. Rev. 4:130-134, 1984.

BOLLAG, W. and MATTER, A.: From Vitamin A to Retinoids in Experimental and Clinical Oncology: Achievements, Failures, and Outlook. In: Modulation of Cellular Interaction by Vitamin A and Derivatives (Retinoids). Ed. by L.M. de Luca and S.S. Shapiro. New York Academy of Sciences, Annals, Vol. 359: 9-20, 1981.

BOREK, C: Nutritional, Hormonal and Enzymatic Factors as Modulators of Radiation and Chemical Oncogenesis in uifro.In: Radioprotectors and Anticarcinogens, pp. 495-513. Ed. by O.F. Nygaard and M. G. Simic. Academic Press, New York, 1983.

BOREK, B. and TROLL, W.: Modifiers of Free Radicals Inhibit in *vitro* the Oncogenic Actions of X-rays, Bleomycin, and Tumor Promoter TPA. PNAS (U.S.) 80: 1304-1307, 1983.

BOUTWELL, R. K.: Retinoids and Prostaglandin Synthesis Inhibitors as Protective Agents Against Chemical Carcinogenesis and Tumor Promotion. In: Radioprotectors and Anticarcinogens, pp. 557-566. Ed. by O.F. Nygaard and M.G. Simic. Academic Press, New York, 1983.

BOVERIS, A. and CHANCE, B.: The Mitochondrial Generation of Hydrogen Peroxide. General Properties and Effect of Hyperbaric Oxygen. Biochem. J. 134: 707-716, 1973.

BOYNTON, A.L. and WHITFIELD, J.F.: The Role of Cyclic AMP in Cell Proliferation: A Critical Assessment of the Evidence. In: Advances in Cyclic Nucleotide Research, Vol. 15, 193-272. Ed. by P. Greengard and G.A. Robison. Raven Press, New York, 1983.

BR AD A, Z. and BULBA, S.: The Regression of dl-Ethionine Induced Hepatocellular Carcinoma in Rats by Glutathione Treatment. Presented at the Fifth Annual Seminar of Cancer Researchers in Florida, at Tampa, February 6, 1982.

CHAYEN, J.: Editorial: Concerning the Possibility of Redox Drugs. Agents and Actions 12: 530-535, 1982.

CHOW, C.K.: Nutritional Influences on Cellular Antioxidant Defense Systems. Am. J. Clin, Nutrit. 32: 1066-1081, 1979.

CHOWKA, P. B.: Cancer Research: The $20 Billion Failure. East-West Journal, 13-19, March 1981.

CIMINO, J.A. and DEMOPOULOS, H.B.: Introduction: Determinants of Cancer Relevant to Prevention, in the War on Cancer. J. Env. Pathol. Toxicol. 3: 1-10, 1980. COOK, J. R. et al.: Assessment of the Anti-tumor Potential of Glutathione. Cancer Lett. 21: 277-283, 1984.

CULLEN, K. et al.: Multiple Regression Analysis of

* Since antioxidant enzymes are induced by substrate elevation, and since the MnSOD response appears to be impaired in malignant tissues, then the glutathione peroxidase activity which is thought to be highly inducible (Levine and Kidd, 1984) may not be exposed to increased levels of hydrogen peroxide or lipid peroxides as substrates, hence would not become augmented.

Risk Factors for Cardiovascular Disease and Cancer Mortality in Busselton, Western Australia — 13-year Study. J. Chron. Dis. 36: 371-377, 1983.

DEL MAESTRO, R.F., BJORK, J., and ARFORS, K.E. : Free Radicals and Microvascular Permeability. In: Pathology of Oxygen, 157-173. Ed. by A. P. Autor. Academic Press, New York, 1982.

DEMOPOULOS, H.B.: The Basis of Free Radical Pathology. Fed. Proc. 32: 1859-1861 (Symposium Issue), 1973.

DEMOPOULOS, H.B., PETRONIGRO, D.D., FLAMM, E.S., andSEUGMAN, M.L.: ThePossible Role of Free Radical Reactions in Carcinogenesis. J. Env. Pathol. Toxicol, 3:272-303. 1980.

DEMOPOULOS, H.B., FLAMM, E.S., SELIGMAN, M.L., and PETRONIGRO, D.D.: Oxygen Free Radicals in Central Nervous System Ischemia and Trauma. In: Pathology of Oxygen, 127-155. Ed. by A.P. Author. Academic Press, New York, 1982.

DE LA PENA, AM.: The Psychobiology of Cancer Automization and Foredom in Health and Disease. Praeger Publishers, New York, 1983.

DE ROBERTUS, F.R. and CRAVEN, P.A.: Activation of Guanylate Cyclase by N-nitroso Carcinogens and Related Agonists: Evidence for a Free Radical Mechanism. In: Free Radicals and Cancer, pp. 201-244 (Chapter 7). Ed. by R.A. Floyd. Marcl Dekker, New York, 1982.

DIGUISEPPI, J. and FRIDOVICH, I.: The Toxicology of Molecular Oxygen. CRC Crit. Rev. Toxicol. 12: 315-342,1984.

DICHESNE, J.: A Unifying Biochemical Theory of Cancer, Senescence and Maximal Life Span. J. Theor. Biol. 66: 137-145,1977.

EMERIT, I. and CERUTTI, P.A.: Tumor Promoter Phorbol-12-myristate-13-acetate Induces Chromosomal Damage via Indirect Action. Nature 293,144-146, 1981.

FARBER, E.: Chemical Carcinogenesis: A Current Biological Perspective. Carcinogenesis 5:1-5,1984.

FLOYD, R.A. (Ed.): Free Radicals and Cancer. Marcl Dekker, New York, 1982.

FORMAN, H. J. and BOVERIS, A.: Superoxide Radical and Hydrogen Peroxide in Mitochondria. In: Free Radicals in Biology, Vo. 5, pp. 65-89, Ed. by W.A. Pryor. Academic Press, New York, 1982.

FORMAN, H. J. and FISHER, A.B.: Antioxidant De fenses. In: Oxygen and Living Processes, pp. 235-272. Ed. by D. L. Gilbert. Springer-Verlag, New York, 1981.

FREEMAN, B. A. and CRAPO, J.D.: Biology of Disease: Free Radicals and Tissue Injury. Lab. Invest. 47: 412-426, 1982.

FRIDOVICH, I.: Superoxide Dismutase in Biology and Medicine. In: Pathology of Oxygen, pp. 1-19. Ed by A. P. Autor. Academic Press, New York, 1982.

GERSCHMAN, R., Gilbert, D.L., NYE, SW., DVVYER, P., and FENN, W.O.: Oxygen Poisoning and X-irradiation: A Mechanism in Common. Science 119, 623-626, 1954.

GREENSTOCK, C.L.: Redox Processes in Radiation Biology and Cancer. Rad. Res. 86: 196-211, 1981.

GOLDBERG, N.D., GRAFF, G., HADDOX, M.K., STEPHENSON, J.H., GLASS, D.B., and MOSER, M.E.: Redox Modulation of Splenic Cell Soluble Guanylate Cyclase Activity: Activation by Hydrophilic and Hydrophobic Oxidants Represented by Ascorbic and Dehydroascorbic Acids, Fatty Acid Hydroperoxides, and Prostaglandin Endoper-oxides. In: Advances in Cyclic Nucleotide Research, Vol. 9, 101-130. Ed. by W. J. George and L. J. Ignarro. Raven Press, New York, 1978.

GRIFFIN, A.C.: The Chemopreventive Role of Selenium in Carcinogenesis. In: Molecular Interrelations in Nutrition and Cancer, pp. 401-408. Ed. by M.S. Arnott et al. Raven Press, New York, 1982.

GUERRERO, I., VILLASANTE, A., D'EUSTACHIO, P., and PELLICER, A.: Isolation, Characterization and Chromosome Assignment of Mouse N-ras Gene from Carcinogen-Induced Thymic Lymphoma. Science 225: 1041-1043 (7 September 1984).

HAFEMAN, D.G. and HOEKSTRA, W.G.: Protection Against Carbon Tetrachloride-induced Lipid Per oxidation in the Rat by Dietary Vitamin E, Selenium, and Methionine as Measured by Ethane Evolution. J. Nutr. 107: 656-665, 1977.

HALLIWELL, B.: Production of Superoxide, Hydrogen Peroxide and Hydroxyl Radicals by Phagocytic Cells: a Cause of Chronic Inflammatory Disease? Cell Biol. Int. Repts. 6: 529-542, 1982.

HAUGAARD, N.: Cellular Mechanisms of Oxygen Toxicity. Physiol. Rev. 48: 311-373, 1968. HEIDELBERGER, C: Chemical Carcinogenesis. Ann. Rev. Biochem. 44: 79-121, 1975.

HOLT, J.A.G.: Cancer, a Disease of Defective Glucose Metabolism. Med. Hyp. 10: 135-150, 1983.

IVERSEN, O.H. and ASTRUP, E.G.: The Paradigm of Two-stage Carcinogenesis: a Critical Attitude. Cancer Invest. 2: 51-60,1984.

JOHNSON, F.C.: The Antioxidant Vitamins. CRC. Crit. Rev. Food Sci. Nutr. 11: 217-309, 1979.

JOHNSON, F.C.: Editorial. Carcinogenesis, Vascular Disease, and the Free Radical Reaction. Nutrition and Cancer 3: 117-121, 1982.

JOHNSON, F.C.: Editorial. Cancer Promoters and Initiators, and the Free Radical Theory of Car cinogenesis. Ibid., 4: 169-170, 1983.

KAPPUS, H. and SIES, H.: Toxic Drug Effects Assoc iated with Oxygen Metabolism: Redox Cycling and Lipid Peroxidation. Experientia 37:1233-1241,1981.

KIMOTO, E., TANAKA, H., GYOTOKU, J., MOR-ISHIGE, F., and PAULING, L: Enhancement of Antitumor Activity of Ascorbate Against Ehrlich Ascites Tumor Cells by the Copper Glycylglycylhist-idine Complex. Cancer Res. 43: 824-828, 1983

KONSTANTINIDIS, A., SMULOW, J.B., and SON-NENSCHEIN, C: Tumorigenesis at a Predetermined Oral Site After One Intraperitoneal Injection of N-Nitroso-N-Methylurea. Science 216: 1235-1237, 1982; letters to Science, 225: 352-353, (19 October 1984).

KOVACS, G.: Cytogenetic Evidence of Clonal Evolution in Human Solid Malignant Tumors. In: Biology of the Cancer Cell, pp. 377-381. Ed. By K. Let -nansky. Kugler Publications, Amsterdam, 1980.

KUMMET, T., MOON, T.E., and MEYSKENS, F.L.: Vitamin A: Evidence for its Preventive Role in Human Cancer. Nutr. Cane. 5: 96-06,1983.

LEHNINGER, A.L.: Principles of Biochemistry. Worth Publishers, New York, 1982.

LEVINE, S.A. and KIDD, P.M.: Antioxidant Adaptation: a Unified Disease Theory. J. Orthomol. Psy-

chiatry 14(i): 19-38, 1984.
LEVINE, S.A. with KIDD, P.M.: Antioxidant Adaptation: Its Role in Free Radical Pathology, 1985 (382 pp.). Allergy Research Group, 400 Preda Street, San Leandro, CA 94577.
LEVINE, S.A. and REINHARDT, J.H.: Biochemical Pathology Initiated by Free Radicals, Oxidant Chemicals, and Therapeutic Drugs in the Etiology of Chemical Hypersensitivity Disease. J. Orthomol. Psychiatry 12: 166-183, 1983.
LIPINSKI, B.: Rationale for Treatment of Cancer with Ozone. Unpublished manuscript. Boston Cardi ovascular Health Center, 1 Washington Street, Wellesley MA 02181, 1983.
LITTLE, J.B., KENNEDY, A.R., and NAGASAWA, H.: Involvement of Free Radical Intermediates in Oncogenic Transformation and Tumor Promotion in *vitro*. In: Radioprotectors and Anticarcinogens, pp. 487-493. Ed. by O.F. Nygaard and M.G. Simic. Academic Press, New York, 1983.
MARGISON, G.P.: Carcinogenicity of Alkylating Agents . In: Biology of the Cancer Cell, pp. 3-9. Ed. by K. Letnansky. Kugler Publications, Amsterdam, 1980.
MASON, R.P.: Free Radical Intermediates in the Met abolism of Toxic Chemicals. In: Free Radicals in Biology, Vol. 5, 163-221. Ed. by W. A. Pryor. Academ ic Press, New York, 1983. McBRIEN, D.C.H. and SLATER, T.F.: Free Radicals, Lipid Peroxidation, and Cancer. Academic Press, New York, 1982.
McCORD, J.M., KEELE, B.B., and FRIDOVICH, I.: An Enzyme Based Theory of Obligate Anaerobiosis: The Physiological Function of Superoxide Dismutase. Proc. Natl. Acad. Sci. U. S.A. 68: 1024-1027, 1971.
MENOTTI, A., CONTI, S., DIMO, F., GAIMPOLI, B.G., RUMI, A., SECCARECCIO, F., and SIGN-ORETTI, P.: Prediction of All Causes of Death as a Function of Some Factors Commonly Measured in Cardiovascular Population Surveys. Prevent. Med. 12: 318, 1983. MILLER, J.A.: Carcinogenesis by Chemicals — ah Overview. Cancer Res. 30: 559-576, 1970.
MILLER, E.C. and MILLER, J. A.: Mechanisms of Chemical Carcinogenesis. Cancer 47: 1055-064,1981.
MIRANDA, C.L., CARPENTER, H.M., CHEEKE, P.R., and BUHLER, D.R.:Effect of Ethoxyquin on the Toxicity of the Pyrrolizidine Alkaloid Monocrotaline and on Hepatic Drug Metabolism in Mice. Chem.-Biol. Interact. 37: 95-107, 1981.
MITTAL, C.K. and MURAD, F.: Activation of Guanylate Cyclase by Superoxide Dismutase and Hydroxyl Radical: A Physiological Regulator of Guanosine 3', 5'-monophosphate Formation. Proc. Natl. Acad. Sci. USA, 74 (10): 4360-4363, 1977.
NAGATA, C, KODAMA, M., IOKI, Y,, and KIMURA, T.: Free Radicals Produced from Chemical Car cinogens and their Significance in Carcinogenesis. In: Free Radicals and Cancer, pp. 1-62 (Chapter 1). Ed By R. A. Floyd. Marcel Dekker, New York, 1982. NATIONAL CANCER ADVISORY BOARD, SUBCOMMITTEE ON ENVIRONMENTAL CARCINOGENESIS: General Criteria for Assessing the Evidence for Carcinogenicity of Chemical Substances. J. Natl. Cancer Inst. 58: 461-463, 1977.
NEAL, G.E. and LEGG, R.F.: The Ineffectiveness of Reduced Glutathione in Preventing the Development of Liver Tumors from Aflatoxin-induced Pre-neo-plastic Liver Lesions. Cancer Lett. 21: 83-87, 1983. NEWBERNE, P.M. and SUPHAKARN, V.: Nutrition and Cancer: a Review, with Emphasis on the Role of Vitamins C and E and Selenium. Nutr. Cane. 5: 107-119, 1983.
NORONHA-DUTRA, A.A. and STEEN, E.M.: Lipid Peroxidation as a Mechanism of Injury in Cardiac Myocytes. Lab. Invest. 47: 346-353, 1982.
NOVI, A.M.: Regression of Aflatoxin Bl-Induced Hep atocellular Carcinomas by Reduced Glutathione. Science 212: 541-542, 1981.
NOVI, A.M., FLOKKE, R., and STUKENKEMPER, M.: Glutathione and Aflatoxin Bl-Induced Liver Tumors: Requirement for an Intact Glutathione Molecule for Regression of Malignancy. In: Cell Proliferation, Cancer, and Cancer Therapy: A Conference in Honor of Anna Goldfeder, Ed. by R. Baserga. New York Academy of Sciences, Annals 397:62-71,1982. NOWELL, P.C.: The Clonal Evolution of Tumor Cell Populations. Science 194: 23-28, 1976.
NYGAARD, O.F. and SIMIC, M.G. (eds): Radiopro tectors and Anticarcinogens (First Conference on Radioprotectors and Anticarcinogens, National Bureau of Standards, 1982). Academic Press, New York, 1983.
OBERLEY, L.W., OBERLEY, T.D., and BUETTNER, G . R.: Cell Division in Normal and Transformed Cells: The Possible Role of Superoxide and Hydrogen Peroxide. Med. Hypoth. 7: 21-42, 1981.
OBERLEY, L.W.: Superoxide Dismutase and Cancer. In: Superoxide Dismutase (Volume II), 127-166 (Chapter 6). Ed. by L. W. Oberley. CRC Press, Boca Raton, Florida, 1982.
PARKE, D.V.: Mechanisms of Chemical Toxicity — a Unifying Hypothesis. Regul. Pharmacol. Toxicol. 2: 267-286. 1982.
PETKAU, A., MONASTERSKI, L.G., KELLY, K., and FRIESEN, H.G.: Modification of Superoxide Dismutase in Rat Mammary Carcinoma. Res. Comm. Chem. Pathol. Pharmacol. 17: 125-132, 1977.
PETO, R., DOLL, R., BUCKLEY, J.D., and SPORN, M.B.: Can Dietary Beta-carotene Materially Reduce Human Cancer Rates? Nature 290: 201-208, 1981. PRYOR, W.A.: Free Radical Biology: Xenobiotics, Cancer and Aging. In: Vitamin E: Biochemical, Hematological, and Clinical Aspects, 1-22. Ed. by B. Lubin and L. J. Machlin. New York Academy of Sciences, Annals vol. 393, 1982.
RILEY, P.A.: Is the Establishment of a Clone Exhibiting Defective DNA Repair the Initial Stage of Car cinogenesis? Med. Hypoth. 9: 163-168, 1982. ROBBINS, S.L., and COTRAN, R.S.: The Pathologic Basis of Disease, (Chapter 7: Genetic Disorders). W. B. Saunders, New York, 1979. RUFF, M.R., and PERT, C.B.: Small Cell Carcinoma of the Lung: Macrophage-Specific Antigens Suggest Hemopoietic Stem Cell Origin. Science 225: 1034-1036 (7 September 1984).
SCHENKMAN, J.B., JANSSON, I., POWIS, G. and KAPPUS, H.: Active Oxygen in Liver Microsomes: Mechanism of Epinephrine Oxidation. Mol. Phar macol. 15: 428-438, 1979.
SERFASS, R.E. and GANTHER, H.E.: Defective Micro bicidal Activity in Glutathione Peroxidase-Deficient

Neutrophils of Selenium Deficient Rats. Nature 255: 640-641, 1975.

SLAGA, T.J., SOLANKI, V., and LOGANI, M.: Studies on the Mechanism of Action of Antitumor Promoting Agents: Suggestive Evidence for the Involvement of Free Radicals in Promotion. In: Radioprotectors and Anticarcinogens, 471-485. Ed. by O.F. Nygaard and MG. Simic. Academic Press, New York, 1983.

SLAGA, T.J.: Potential for Preventing Cancer by Chemical Inhibitors. Cane. Bull. 36: 61-64, 1984.

SCHRAUZER, G.N.: Trace Elements in Carcinogenesis. Adv. Nutr. Res. 2: 219-244, 1979.

SMITH, A.E. and KENYON, D.H.: A Modifying Concept of Carcinogenesis and its Therapeutic Implications. Oncology 27: 459-479, 1973.

SMUCKLER, E.A.: Chemicals, Cancer and Cancer Biology (Medical Progress). West J. Med. 139:55-74, 1983.

SPALLHOLZ, J.E.: Selenium: What role in Immunity and Immune Cytotoxicity? In: Selenium in Biology and Medicine (Proceedings Second International Symposium, 1980), 103-117. Ed. by J. E. Spallhoz, J. L. Martin, and H. E. Ganther. Westport, Connecticut: AVI Publishing, 1981.

SPRINGER, E.L.: Comparative Study of the Cytoplasmic Organelles of Epithelial Cell Lines Derived from Human Carcinomas and Nonmalignant Tissues. Cancer Res. 40: 803-817,1980.

SUPPAN, P.: Glycolysis-dependent Antimetabolites: Possible Improvement in Antineoplastic Activity. Med. Hyp. 5: 103-108, 1979.

SWARTZ, H.M.: Free Radicals in Cancer. In: Sub-molecular Biology and Cancer (Ciba Foundation Symposium 67 (new series) in honor of A. Szent-Gyorgyi), pp. 107-130. Ed. by G.E.W. Wolstenholme et al. Excerpta Medica, New York, 1979.

SWARTZ, H.M.: Electron Spin Resonance Studies of Cancer Using Complex Biological Systems. In: Free Radicals and Cancer, pp. 81-116 (Chapter 3). Ed. by R.A. Floyd. Marcel Dekker, New York, 1982.

SZENT-GYORGYI, A., ISENBERG, I., and BAIRD, S.: On the Electron Donating Properties of Carcinogens. Proc. Natl. Acad. Sci. (U.S.) 46: 1444-1449, 1960.

SZENT-GYORGYI, A.: The Living State and Cancer. In: Submolecular Biology and Cancer (Ciba Foundation Symposium 67). Ed. by G. E. W. Wolstenholme et al. New York: Excerpta Medica, 1979.

TAPPEL, A L.: Measurement of and Protection from in vivo Lipid Peroxidation. In: Free Radicals in Biology, Vol. 4, 2-47. Ed. by W. A. Pryor. Academic Press, New York, 1980.

TRUDELL, J.: Reported at the Workshop on Biomedical Implications of Oxygen Toxicity. University of California, Berkeley, March 1984. TSAO, C.S. and

SALIMI, S.L.: Evidence of Rebound Effect with Ascorbic Acid. Med. Hyp. 13: 303-320, 1984.

TS'O P.O.P., CASPARY, W.J., and LORENTZEN, R.J.: The Involvement of Free Radicals in Chemical Carcinogenesis. In: Free Radicals in Biology, Vol. 3, 251-305. Ed. by W. A . Pryor. Academic Press, New York, 1977.,

VESELY, D.L., WATSON, B., and LEVEY, G.S.: Activation of Liver Guanylate Cyclase by Paraquat:Possible Role of Superoxide Anion. J. Pharmacol. Exp. Therap. 209: 162-164,1979.

VIAJE, A., SLAGA, T.J., WIGLER, M., and WEIN-STEIN, I.B.: Effects of Antiinflammatory Agents on Mouse Skin Tumor Promotion, Epidermal DNA Synthesis, Phorbol Ester-Induced Cellular Proliferation, and Production of Plasminogen Activator. Cancer Res. 37: 1530-1536, 1977.

VITHAYATHIL, A., TERNBERG, J.L., and COMMONER, B.: Changes in Electron Spin Resonance Signals of Rat Liver During Chemical Carcinogenesis. Nature 207: 1246-1249, 1965.

WALKER, D.H.: Inhibition of Tumor Promotion by a Biomimetic Superoxide Dismutase. Science 221:75-77, 1983.

WANG, Y.-M. and HOWELL, S.K.: Alpha-tocopherol as a Potential Modifier of Daunorubicin-induced Mammary Tumors in Rats. In: Vitamin E: Biochemical, Hematological, and Clinical Aspects, 1-22. Ed. by B. Lubin and L. J. Machlin. New York Academy of Sciences, Annals vol. 393, 1982.

WARBURG, O.: On the Origin of Cancer Cells. Science 123: 309-314, 1956.

WARBURG, O.: The Prime Cause and Prevention of Cancer (1966 Lindau Lecture, English Edition by D. Burk). Wurzburg, Germany: K. Triltsch, 1969.

WATTENBERG, L.M.: Inhibition of Chemical Carcinogens by Minor Dietary Components. In: Molecular Interrelations of Nutrition and Cancer, pp. 43-56. Ed. by M. S. Arnott et al. Raven Press, New York, 1982.

WEITZMAN, S.A. and STOSSEL, T.P.: Mutation Caused by Human Phagocytes. Science 212: 546-547,1981.

WITZ, G., GOLDSTEIN, B.D., AMORUSO, M., STONE, D.S., and TROLL, W.: Retinoid Inhibition of Superoxide Anion Radical Production by Human Polymorphonuclear Leukocytes Stimulated with Tumor Promoters. Biochem. Biophys. Res. Comm. 97: 883-888, 1980.

YAGI, K. (ed): Lipid Peroxides in Biology and Medicine. New York: Academic Press, 1982.

YUNIS, J.J.: The Chromosomal Basis of Human Neoplasia. Science 221: 227-236, 15 July 1983.