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HOST BIOCHEMICAL DEFENSE MECHANISMS AGAINST PROOXIDANTS

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INTRODUCTION

All forms of aerobic life are constantly subjected to oxidant pressure from molecular oxygen (O_2) and reactive oxygen metabolites (ROMs)[1] produced both during the biochemical utilization of O_2 and by prooxidant stimulation of O_2 metabolism. Due to the intrinsic reactivity of ROMs, their production in biological systems and their effects upon normal physiological processes were largely matters of conjecture until McCord & Fridovich described an enzyme whose catalytic activity is directed towards the destruction of the one electron–reduced metabolite of O_2, the superoxide anion radical ($O_2^{\cdot+}$) (1). Since the discovery of superoxide dismutase (SOD), many other endogenous principles have been defined whose functions are to control the existence of ROMs in biological systems.

The direct analysis of ROMs in biological material is difficult because of their instrinsic reactivity. Thus, much of the evidence coupling their production with normal physiological processes and abnormal pathophysiological events has come from studying the disposition of the host defense mechanisms themselves. Thus, in a manner analogous to the original discovery of

[1]Abbreviations: ROM, reactive oxygen metabolite; SOD, superoxide dismutase; MPO, myeloperoxidase; PMN, polymorphonuclear leucocyte; GSH, glutathione (reduced form); GSSG, glutathione disulfide; PrSH, protein thiol; SeGSHpx, selenium-dependent glutathione peroxidase; DEM, diethylmaleate; NAC, N-acetylcysteine, BCNU, bis-(chloroethyl)-1-nitrosourea; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene.

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SOD, these studies "mirror" the potential involvement of ROMs in normal and abnormal metabolism from the test tube to the clinic. By briefly detailing the nature, reactivity, and biological effects of relevant ROMs and the multiplicity of host antioxidant defense mechanisms, we hope to suggest the complexity of their relationships at the molecular and cellular levels. Additionally, the medical ramifications of studying the disposition of host antioxidant defenses during normal and abnormal metabolism are discussed. Due to the limitations of space, selected examples of current literature are used throughout.

BIOLOGICALLY RELEVANT REACTIVE OXYGEN METABOLITES

The production of ROMs in biological systems and their chemical reactivities have been the subject of recent extensive reviews (2, 3); these issues are dealt with very briefly here.

Central to the ROM "cascade" is the one-electron reduction of O_2 to $O_2^{\cdot-}$. This is catalyzed by the activity of a variety of enzymes such as xanthine oxidase and NADPH-cytochrome P-450 reductase and hemoproteins such as hemoglobin and cytochromes P-450 (2–4). Additionally, $O_2^{\cdot-}$ is produced during mitochondrial electron transport and in the autooxidation of endogenous small molecules such as catecholamines (2, 4). Accelerated $O_2^{\cdot-}$ production can result from the interaction of prooxidant species, such as quinones (5), with biological material. This process is termed redox cycling and is considered shortly.

Once formed within the biological milieu, $O_2^{\cdot-}$ can undergo a variety of chemical and metabolic reactions yielding other ROMs. These reactions include dismutation to hydrogen peroxide (H_2O_2) and protonation to form the hydroperoxy radical ($HO_2^{\cdot}$). H_2O_2 may also arise directly from O_2 by the two-electron reduction catalyzed by a variety of enzymes such as monoamine oxidase (4). Hydrogen peroxide may then undergo a Fenton reaction with metal ions such as Fe^{2+} and Cu^{+}, yielding the hydroxyl radical ($\cdot OH$) (2–4). Hypohalous acids are also produced from H_2O_2 by the action of myeloperoxidase (MPO) in phagocytosing cells such as polymorphonuclear leucocytes (PMNs) (3). Hydroxyl radicals may generate further ROMs and organic radicals by interaction with biological macromolecules. Such species are produced during lipid peroxidation of biological membranes and include lipid peroxy radicals ($ROO\cdot$) and lipid hydroperoxides (ROOH) (6, 7). Lipid hydroperoxides can undergo further metal-catalyzed reactions, yielding lipid alkoxy radicals ($RO\cdot$) and, eventually, aldehyde species. Lipid peroxidation is also associated with the production of the first excited state of O_2, singlet oxygen $^1O_2$, and other energized species such as excited carbonyls. Singlet oxygen may also arise both enzymatically in cells through the activity of a

variety of peroxidases such as prostaglandin hydroperoxidase (8) and non-enzymatically through the photosensitization of endogenous compounds such as retinal (9).

Chemically, ROMs possess differing reactivity and biological half-lives. Species such as ·OH, $^1O_2$, and RO· are so reactive that their half-lives approach diffusion-controlled limits (10^{-9} sec) (2). These species may potentially react with any biological molecule (2, 10). $O_2^{\cdot}$ is a relatively unreactive radical and possesses poor nucleophilicity and lipid solubility. However, the widespread occurrence of SOD makes estimation of the half-life of $O_2^{\cdot}$ in biological systems difficult. Similarly, although hydroperoxides such as H_2O_2 are weak oxidizing agents, estimates of their biological half-lives are made complicated by the existence of specific detoxication mechanisms and Fenton reactions with trace metals. H_2O_2 is, however, more lipid soluble than $O_2^{\cdot}$, and this allows it to move more readily between biological compartments.

At higher levels of biological organization, such as the intact cell, the reaction of ROMs with biological molecules can elicit a number of secondary events with great bearing on cellular functionality. These events may be conveniently illustrated in model studies where the intracellular production of ROMs is greatly accelerated through redox cycling. Various quinones such as menadione and doxorubicin (adriamycin®) and the bipyridilium compounds paraquat and diquat are frequently used in studies of the effects of stimulated ROM production on different cell functions.

Exposure of hepatocytes to toxic levels of menadione causes acute cytotoxicity (5) preceded by the oxidation of intracellular glutathione (GSH), NADPH, and protein thiols (PrSHs) (11). It is increasingly clear that such alterations may be interrelated and that they can interfere with vital cell functions. Protein thiol groups are essential to the activity of many enzymes and the function of structural proteins (12). The redox balance of PrSHs is influenced both by chemical reactions (thiol-disulfide exchange) and by various oxidoreductases (13). Thus, under conditions where ROM "pressure" is increased within cells, enhanced formation of mixed disulfides between low molecular weight thiols and PrSHs may occur (11, 13), interfering with the normal activity of proteins. One key family of enzymes which may be affected are the transport ATPases which regulate intracellular ion compartmentation. For example, menadione metabolism elicits a sustained increase in cytosolic Ca^{2+} concentration in hepatocytes which appears to be related to a modification of critical thiol group(s) in the plasma membrane Ca^{2+} "pump," causing inhibition of Ca^{2+} extrusion from the cells (14). As intracellular Ca^{2+} plays a critical role in cellular metabolism (15), this nonphysiological rise in cytosolic Ca^{2+} is thought to have multiple effects on normal metabolism, including the activation of various Ca^{2+}-dependent catabolic enzymes (phospholipases, proteases, endonucleases), which are thought to contribute to acute cytotoxicity (14).

In addition to these elicited effects, the metabolism of the bipyridilium herbicides paraquat and diquat have been shown to induce lipid peroxidation in isolated hepatocytes. Although a critical role for lipid peroxidation in the ultimate cytotoxicity of these agents has not yet been established, it is clear that it may contribute to the propagation of cell damage (16).

Apart from their acute consequences, the production of ROMs in biological systems may have chronic effects through the alteration of the viability of the cellular genome, altered gene expression, and resultant cellular transformation (17).

HOST ANTIOXIDANT DEFENSE SYSTEMS

We now consider the biochemical defense mechanisms evolved by biological systems to balance the equation of aerobic life. For the sake of clarity, these are dealt with individually; however, many interrelationships exist between them and they should be regarded as an integrated "network."

Primary Defense: Enzymes

SUPEROXIDE DISMUTASES The SODs are a family of metalloenzymes that catalyze the dismutation of $O_2^{\cdot-}$ (1, 17):

$$2O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2^{\cdot-}$$

They operate extremely efficiently with rate constants approaching $2 \times 10^9 M^{-1} \cdot s^{-1}$ (4); the reaction mechanism has been reviewed previously (4, 18). The SODs have a variety of prosthetic groups, allowing their classification into several distinct groups. The prevalent enzyme is the CuZnSOD, a stable, dimeric protein (32,000 daltons), which has been detected in nearly all eukaryotic cells (4). The Cu-atom is essential to the catalytic activity of the enzyme whilst the Zn-atom imparts stability (18). This SOD is mostly localized within the cell cytosol but may also be present in the nucleus (19). Early work on the human tissue distribution of CuZnSOD demonstrated considerable tissue heterogeneity in autopsy material; enzyme levels were highest in the liver, certain brain areas, and testis, but extremely low in erythrocytes, thyroid gland, pancreas, and lung tissue (20).

A second enzyme containing manganese has been identified in prokaryotes but also in eukaryotes (4, 18). Eukaryotic MnSOD, mainly a tetramer, is predominantly localized within the mitochondrion, protecting it against $O_2^{\cdot-}$ produced during electron transport. In higher primates (4) and rodents (19), MnSOD has been demonstrated in liver cytosol also.

An iron-containing SOD has been isolated from a variety of prokaryotic

and plant cell sources, but it is absent from all animal cells thus far investigated (4, 18).

A fourth enzyme has been identified in most animal tissues tested. This Cu-containing enzyme, a tetrameric, hydrophobic glycoprotein (135,000 daltons), has been found heterogeneously distributed among most human tissues, but it is highest in blood plasma. Its distribution bears no relationship to that of CuZnSOD. This enzyme is also termed extracellular SOD (21).

The possession of SOD activities clearly is conducive to the function of most forms of aerobic life. This may be illustrated by their wide distribution, highly conserved structures (22), and their inducibility in response to a variety of stimuli, some of which we discuss shortly.

CATALASE This mainly tetrameric hemoprotein present in most aerobic cells tested (a few exceptions are discussed later), catalyzes the reduction of H_2O_2 to H_2O and O_2. The mechanism of the catalase reaction has been discussed fully elsewhere (23) and involves the formation of an enzyme-substrate intermediate, Compound I. Catalase is a relatively active enzyme with second order rate constants in the order of $10^7 M^{-1}s^{-1}$; it has a high capacity for the reaction but a relatively low affinity for its substrate (23).

The catalase activity of eukaryotic cells is localized within the peroxisomes, organelles which contain many of the H_2O_2-generating enzymes present in aerobic cells (4, 23). As with SOD, human catalase levels show great tissue heterogeneity; they are highest in liver and erythrocytes and lower in brain, skeletal muscle, pancreas, and lung tissue (20, 24). Tissue catalase activity is also affected by a variety of stimuli which we discuss shortly.

SELENIUM-DEPENDENT GLUTATHIONE PEROXIDASE (SeGSHPx) This protein is a member of a family of peroxidases that catalyze the reduction of hydroperoxides by GSH:

$$ROOH + 2GSH \rightarrow GSSG + ROH.$$

SeGSHpx is a tetrameric protein (85,000 daltons) containing four atoms of selenium bound as selenocysteine moieties which confer the catalytic activity. The enzyme will reduce both H_2O_2 and free organic hydroperoxides, but it has an absolute requirement for GSH as cosubstrate. In contrast to catalase, SeGSHpx has high affinity for its substrates but low capacity for the reaction. Other aspects of the catalytic mechanism have been summarized previously (25). This enzyme is mainly located within the cytosol of eukaryotic cells but may also occur intramitochondrially (25). SeGSHpx also shows considerable distributional heterogeneity in human tissues. Like SOD and catalase, SeGSHpx activity is highest in liver but, unlike catalase, it is low in erythro-

cytes (24). The activity of this enzyme is also modulated by a variety of environmental factors, especially the dietary supply of selenium (25).

As the GSH/GSSG ratio of cells is generally kept high, the metabolism of hydroperoxides by SeGSHpx gives an absolute requirement for the re-reduction of GSSG. This function is fulfilled by NADPH-linked glutathione reductase, a dimeric protein (105,000 daltons) which catalyzes the reduction of GSSG:

$$GSSG + 2NADPH \rightarrow 2GSH + 2NADP^{+}.$$

In addition to this reaction, the enzyme catalyzes the reduction of other low molecular weight disulfides but not mixed disulfides (26). The tissue distribution of this cytosolic enzyme seems tightly coupled to that of SeGSHpx (25). As NADPH is critical to a large number of cellular reductive processes, the activities of SeGSHpx and glutathione reductase are further coupled to the production of NADPH via the pentose phosphate shunt (27).

OTHER PEROXIDASES A variety of other peroxidases with affinity for H_2O_2 have been identified in biological systems. These may play a protective role, especially in tissues otherwise lacking catalase and/or GSHpx. These include, cytochrome *c*-, NADPH-, chloro-, horseradish-, and ascorbate peroxidases found predominantly in prokaryotes and lower eukaryotes as well as lacto-, and myeloperoxidases, prostaglandin synthetase, and hemoglobin found predominantly in higher eukaryotic cells (28). Caution should be taken in determining a host protective role for these peroxidases as they are known to be able to activate xenobiotics to prooxidants and electrophilic, tissue-binding metabolites by co-oxidation reactions (29).

MISCELLANEOUS ENZYME ACTIVITIES Enzymes that prevent the formation and/or metabolism of prooxidant species themselves in biological systems may play a role in host defense against ROMs. One such example is NADPH: quinone oxidoreductase (DT-diaphorase) which catalyzes the two-electron reduction of many quinones (30). Evidence also exists for an enzyme activity that catalyzes the one-electron reduction of semiquinone free radicals in tumor cells (31). Similarly, enzymes that react with epoxide species, which may be produced during lipid peroxidation (6), may play a role in host defense. These include the epoxide hydrolases demonstrated in a wide variety of cells (32). These enzymes are not further considered here.

Primary Defense: Small Molecules

GLUTATHIONE The biosynthesis, intra- and intertissue transport, and biochemical functions of this thiol-containing tripeptide have been reviewed

previously (33–37). Here we only consider those characteristics of GSH that *directly* involve it in host defense against ROMs.

GSH is present at high concentrations in most pro- and eukaryotic cells. Accumulation is facilitated by its γ-glutamyl peptide bond which is insensitive to the action of normal peptidases. Body fluids such as bile, glomerular filtrate, blood plasma, and epithelial cell lining also contain GSH. As a typical nucleophilic thiol, GSH can react chemically with ROMs in a number of ways. First, it may act as a reductant, reducing species such as H_2O_2 directly to water with the formation of GSSG (38). As we have seen, this reaction is catalyzed by SeGSHpx in most cells. Second, it may react directly with free radicals such as $O_2^{\cdot-}$, ·OH, and RO· by a radical transfer process, yielding the thiyl radical of GSH, GS·, and eventually, GSSG (39). Third, although not directly involved in the detoxication of those reactive agents we are considering here, GSH may react with electrophiles to form covalent adducts. These reactions are catalyzed by a group of enzymes, the glutathione transferases (40), which we will further consider only in terms of the GSHpx activity of certain isoenzymes.

VITAMIN C (ASCORBATE) This water soluble molecule is found both intra- and extracellularly in most biological systems. The biosynthesis and functions in vitamin C have been previously reviewed (41, 42). Here we discuss its redox chemistry which is of most interest in terms of its role in host defense.

Like GSH, ascorbate may directly reduce free radical ROMs with the concurrent formation of dehydroascorbate via the semidehydroascorbate free radical. The metabolism of ascorbate and GSH are linked also in other respects. There is evidence to suggest that GSH may reduce the semidehydroascorbate free radical with the formation of GS· (42). Additionally, as dehydroascorbate metabolism yields oxalate, a potent cytotoxin, many cells contain GSH-dependent dehydroascorbate reductase which yields ascorbate and GSSG. Other enzymes that may directly reduce the semidehydroascorbate radical have been noted in a variety of tissues (31, 43).

When considering ascorbate as a host protective agent it should be noted that it will react with trace metal ions such as Fe^{2+} and Cu^{+} to yield ROMs.

URIC ACID Urate is produced in most animal cells by the catabolism of purines. In higher primates urate is accumulated, particularly in blood plasma, due to a deficiency in uricase. At concentrations normally occurring in human plasma (44), urate has been shown to directly interact with ROMs such as ·OH (45). Additionally, urate has recently been shown to protect human blood plasma ascorbate from oxidation (44). Thus, it may be that this metabolic "waste product" has some host protective function.

TAURINE This β-amino acid has been identified in most eukaryotic cells and is also found extracellularly in a variety of body fluids. Due to its nonavailability for incorporation into proteins, taurine accumulates to high intracellular concentrations, particularly in those cells normally associated with high rates of generation of ROMs or in cells rich in membranes (46). In addition to its established role in xenobiotic conjugation reactions (47) this amino acid may also have a host protective role against ROMs. Taurine reacts directly with ROMs such as HOCl, to form less reactive species (48).

Primary Defense: Miscellaneous Proteins

METAL ION CHELATORS The existence of ROMs in biological material may be greatly affected by different aspects of metal metabolism. Because of the risk for metal-catalyzed Fenton-type reactions, biological systems possess a variety of mechanisms which minimize the levels of free metal ions such as Fe^{2+} and Cu^{+} and which may therefore contribute to host defense. Central to these are a number of binding proteins that act as intracellular storage sites and intercellular transport vectors. Transferrin and lactoferrin are Fe-binding glycoproteins which serve as transport vectors for iron in the circulation. Iron is then passed to the intracellular protein ferritin, which possesses 24 subunits and ca 4500 metal-binding sites (49). Similarly, copper is bound in blood plasma both to albumin and to the specific Cu-binding protein, caeruloplasmin, a glycoprotein with 6–7 metal-binding sites (50).

MUCOPOLYPEPTIDES Recent reports have suggested that the high molecular weight mucopolypeptide glycoproteins present in tracheobronchial and gastrointestinal mucus may have some host protective functions in these extracellular spaces. Such proteins would almost certainly reduce ROMs such as ·OH by nonspecific mechanisms (51).

Secondary Defense

Here we briefly discuss mechanisms that may combat those secondary processes elicited by ROM-mediated damage to biological molecules.

ANTI-LIPID PEROXIDATION SYSTEMS This discussion is limited to those principles that have been demonstrated within biological membranes and whose activity either directly interrupts initiation and/or propagation of the lipid peroxidation process, or repairs and/or removes peroxidized membrane components.

GSH peroxidases In addition to SeGSHpx most cells possess nonSeGSHpx activity. This activity has been clearly demonstrated for several glutathione transferases (40) which, unlike the selenium-dependent enzyme, do not

metabolize H_2O_2 but show specificity only for low molecular weight organic hydroperoxides. As lipid hydroperoxides themselves are not substrates for either enzyme, the protective activities of these enzymes are dependent upon release of "bound" hydroperoxides through the activity of enzymes such as phospholipase A_2, which preferentially hydrolyze peroxidized fatty acids in membrane phospholipids (52).

In addition to the peroxidase activity of the glutathione transferases, recent reports demonstrate the presence of a further peroxidase activity in mammalian tissue. This small Se-containing protein (23,000 daltons), originally referred to as "peroxidation inhibitory protein" (PIP), catalyzes the direct reduction of lipid hydroperoxides without the need for phospholipase A_2 activity. This protein is now termed phospholipid hydroperoxide GSH peroxidase (53).

Tocopherols The tocopherols are a family of naturally occurring chroman derivatives found within biological membranes. The most commonly encountered tocopherol is α-tocopherol, vitamin E, which has been found in the membranes of most cells, particularly those exposed to high oxygen partial pressure (54). Vitamin E is also present extracellularly in body fluids such as blood plasma, which functions as a transport vector for the vitamin (55). Vitamin E reacts directly (on the chroman "head" group) with ROMs such as ROO· (55) yielding lipid hydroperoxides which can then be removed by the activity of the phospholipase-GSHpx systems. This is thought to interrupt the radical chain-reaction processes that propagate the peroxidation of membranes. Thus, vitamin E is often termed a "chain-breaking" antioxidant.

Vitamin E uptake and metabolism are closely linked to those of selenium. Primarily, a variety of selenoenzymes may affect the metabolic disposition of the vitamin (56). Additionally, some evidence suggests that the vitamin E radical produced during lipid peroxidation may be directly reduced by the ascorbate-GSH redox couple present in the cytosol of cells (42).

β-Carotene This carotenoid, a metabolic precursor to vitamin A, is accumulated to high concentrations in the membranes of certain tissues such as the occular retina. β-carotene is known both to quench excited species such as $^1O_2$ (57) and to react directly with free radicals such as ROO·, a reaction which operates maximally at low oxygen tension and may provide some synergism to vitamin E which reacts most efficiently at higher oxygen concentrations (58).

Bilirubin This lipid soluble product of hemoprotein catabolism, generally considered as a tissue toxin if accumulated to high concentrations, has recently been proposed as a chain-breaking antioxidant of physiological

relevance (59). Under conditions of physiological oxygen tension, bilirubin reacts directly with ROMs such as ROO· and thus may supplement the activity of β-carotene in many tissues.

PROTEIN-SPECIFIC OXIDOREDUCTASES The critical requirement to control and coordinate reversible protein S-thiolation has led to the development of a variety of cellular enzymes which catalyze PrSH redox reactions. Enzymes catalyzing the formation of mixed disulfides between PrSH and low molecular weight disulfides include a cytosolic thiol transferase and a membrane bound PrSH oxidoreductase present in a variety of tissues. The reverse reaction is catalyzed by enzymes such as the thioredoxin-thioredoxin reducatase couple and glutaredoxin. The characteristics and mechanisms of activity of these proteins have been summarized elsewhere (60).

It is not clear whether such enzymes may function directly to maintain PrSH redox balance by re-reduction of ROM-stimulated mixed disulfides. However, under conditions of "mild oxidative insult" the cell may activate salvage pathways through rapid PrSH redox reactions (61). For instance, critical enzymes in glycolysis and gluconeogenesis may be deactivated in order to channel energy equivalents into the synthesis of NADPH via the pentose phosphate shunt. Clearly, when we discover more of the physiological functions of these enzymes it may become apparent that they contribute in other ways to host defense.

In addition to these PrSH-specific proteins, an enzyme catalyzing the re-reduction of oxidized methionine groups in cellular protein has been described in a variety of cells and tissues (62). This may help to reactivate methionine-dependent enzymes after reaction with ROMs.

DNA REPAIR MECHANISMS The eukaryotic cell nucleus possesses a variety of enzymes whose functions are to maintain the accurate flow of the DNA sequence into the nuclear division process. These enzymes probably play a critical role in the protection of cells from stimulated hydrolysis of DNA caused by reaction with ROMs such as ·OH. As the "excision-repair" system of eukaryotic cells has been extensively reviewed elsewhere (63), we only mention those enzymes involved. These include DNA glycosylases and various endonucleases, which recognize the sites of strand breakage and cleave out the flanking bases; synthetase enzymes which re-insert the correct bases; and ligases which anneal the strand.

In addition to these cell salvage functions, DNA repair processes may protect the greater integrity of the organism by actually stimulating acute cytotoxicity. When DNA damage is sustained, a nuclear enzyme (protein-ADP ribosyl transferase) ribosylates chromatin, causing relaxation of its structure and allowing excision-repair to occur. When the genome sustains

excessive damage this enzyme may greatly deplete cellular NAD^+ levels and initiate cytotoxicity. This would ensure that such cells would not survive to replicate and "fix" mutations (64).

PROOXIDANTS VS ANTIOXIDANTS: A DYNAMIC BALANCE IN VIVO

The multiplicity of antioxidant defense mechanisms and their often overlapping specificity, indicate that tight control of redox balance is critical to normal cell function. Due to the intrinsic reactivity of most ROMs in biological material and the lack of suitable, noninvasive analytical techniques (with the exception of chemiluminescence measurements; 6), it is not possible to monitor directly the disposition of this side of the redox equation in most cases. Thus, considerable attention has been given to assay for elicited effects of ROMs and "reflexes" in the antioxidant defenses themselves. These criteria have been used to "mirror" the producton of ROMs in biological systems from the test-tube to the clinic.

Much of this research has been performed in model systems suffering oxidative stress through prooxidant stimulation of ROM production, assuming that this approach can mimic the development of pathophysiological changes and disease states in humans. Indeed, monitoring reflexes in antioxidant defense in relevant model systems is beginning to link prooxidant-derived ROMs to such pathophysiological changes. Several representative examples of potential oxidative stress situations are considered here. These all have clinical relevance and special attention is paid to the disposition of the antioxidant systems in affected human tissue and attempts to modulate their host protective functions pharmacologically.

Xenobiotic Prooxidants

DOXORUBICIN (ADRIAMYCIN®) This quinonoid agent is used widely in the chemotherapy of human tumors. Its usage, however, is limited by the induction of cardiomyophathy and other side effects in many patients (65). The production of ROMs via redox cycling has been implicated as a mechanism of doxorubicin cardiotoxocity. At the cellular level, doxorubicin metabolism is associated with GSH depletion, lipid peroxidation, and the inactivation of enzymes (65, 66). Its cytotoxicity can be greatly potentiated by depletion of intracellular GSH with diethylmaleate (DEM) (67). Recent studies using isolated doxorubicin-treated sarcosomes have directly monitored the production of ·OH using electron spin resonance spectroscopy (66). Conversely, depletion of cardiac GSH levels with DEM does not potentiate the in vivo cardiotoxicity of doxorubicin in rodents (67), whereas the administration of large parenteral doses of GSH protects cardiac GSH levels and prevents

doxorubicin-induced histopathological changes (68). In view of the protective effect of GSH administration in vivo it is interesting to note that N-acetylcysteine (NAC), a drug of clinical relevance (69), has provided little clear adjunctive benefit in doxorubicin treatment of patients (70). NAC can react directly as a reductant and also serves as a precursor to systemic GSH biosynthesis (69).

As doxorubicin-induced cardiomyopathy shows similarity with pathophysiological changes seen in the hearts of vitamin E-deficient animals, it has been suggested that this vitamin may have a critical antioxidant role (71). The administration of relatively large quantities of vitamin E to rodents inhibits both myocardial lipid peroxidation and histopathological changes, and it improves the survival response to doxorubicin. However, this finding could not be reproduced conclusively in human trials (71). Recent work shows that rodents are protected from the cardiotoxicity of doxorubicin by elevation of the activities of SOD, catalase, and SeGSHpx in plasma, heart, and liver by physical exercise (72).

In addition to cardiotoxicity, doxorubicin causes interstitial pulmonary edema in treated patients. This effect is blocked in rodents by the co-administraton of SOD (73). No trial of this kind has been reported in humans.

PARAQUAT AND DIQUAT The bipyridilium herbicides, paraquat and diquat, present a human health problem either through contact during crop spraying or by their misuse. These agents are thought to cause lung, kidney, and liver toxicity through the production of ROMs by intracellular redox cycling (74). Thus, paraquat induces acute cytotoxicity in association with lipid peroxidation and oxidation of GSH to GSSG and mixed disulfides both in isolated cells and in the isolated, perfused rat liver (75). Similar effects occur in isolated, perfused lungs (76), a system more relevant in terms of the in vivo toxicity of paraquat (74). In addition, the depletion of lung SeGSHpx activity by feeding animals with a selenium-deficient diet, greatly potentiates the toxic effects in the isolated, perfused lungs, further supporting a key role for the GSH system in combating paraquat toxicity (77). Similarly, selenium-deficient chickens are more sensitive to the toxicity of paraquat, presumably due to depressed levels of SeGSHpx in the lungs. This assumption is further supported by the demonstation that SeGSHpx inhibitors potentiate paraquat toxicity in this species (78).

SOD may also play a critical role in the protection against paraquat toxicity in vivo. Weenling rats exposed to hyperbaric O_2 for short periods are more resistant to the lethal effects of paraquat than are older rats similarly treated (79). It is known that weenling rats induce lung SOD to a greater extent than older ones, in response to O_2.

Diquat causes acute hepatocellular toxicity in conjunction with GSH deple-

tion and lipid peroxidation. In isolated hepatocytes these effects are critically dependent upon the inhibition of normal glutathione reductase activity by bis-(chloroethyl)-1-nitrosourea (BCNU) (16, 80). These studies also show that supplementation of cellular incubations with various antioxidants yields cytoprotective effects. The iron chelator desferrioxamine effectively inhibits lipid peroxidation and prevents cytotoxicity (16, 80). Only lipid peroxidation is inhibited by the vitamin E analogue Trolox C, with the onset of toxicity occurring as normal (80). Very recent studies show that the selenoorganic heterocycle Ebselen protects cells against the toxic effects of diquat (81). Ebselen is a unique agent possessing GSHpx-like activity with various thiols, including NAC (81), anti-lipid peroxidation activity (81, 82), and other pharmacological effects which together give it antiinflammatory properties (83). However, the potential of these antioxidant agents in the clinical management of bipyridilium intoxication has yet to be explored.

TOBACCO SMOKE The chemical composition of cigarette smoke is complex, with many free radical species, aldehydes, peroxides, epoxides, and other prooxidants being present. Overwhelming epidemiological data correlates long-term cigarette smoking with a variety of chronic obstructive and degenerative lung diseases, including bronchitis (84) and emphysema (85). Although a prooxidant mechanism has been implicated in the development of these disease states, evidence for this is still circumstantial.

Cigarette smoking has been found to cause α-1-antiprotease inactivation in vivo, which is thought to allow proteases such as elastase to function freely within lung connective tissue (85). In this respect, a recently developed, pharmacologically active antiprotease substitute, Eglin C, demonstrates antiemphysemic activity in animal models, presumably by its inhibition of endogenous elastase (86). Due to the complex chemical nature of cigarette smoke it is not clear which oxidants are responsible for this inhibitory effect. Tobacco smoke inhibits a variety of other, mainly thiol-dependent enzymes (87) and causes acute cytotoxicity in isolated cell systems (88, 89), often in association with GSH depletion (88). Inhalation of cigarette smoke by rats was similarly shown to deplete both intra- and extracellular pulmonary GSH pools (90). NAC has been found to provide considerable protection against the effects of cigarette smoke in isolated cells (88) but has not yet been explored in vivo.

Rats deficient in dietary vitamin E suffer premature mortality when exposed chronically to cigarette smoke. Resupply of dietary vitamin E provides resistance to this effect (91). Thus, it is interesting to note that in asymptomatic human smokers the level of vitamin E in bronchial lavage is depleted as compared to control subjects (92). Attempts to restore vitamin E levels through dietary supplementation have been only partially successful (92). In

smokers, erythrocyte catalase and GSH, but not SeGSHpx, levels are elevated as compared to controls (93). These changes may represent some attempt to increase systemic antioxidant protection, the absolute level of which may be critical in the development of human lung diseases, particularly those related to α-1-antiprotease deficiency (94).

Naturally Occurring Prooxidants

MOLECULAR OXYGEN We have now seen how xenobiotic prooxidants may induce acute and chronic pathophysiological changes in biological systems by the activation of O_2 to ROMs. However, it may be that under normobaric O_2 concentrations, a steady stream of ROMs is produced also in "nonstressed" systems. This may be critical to the process of normal tissue aging and responsible for the eventual failure of aerobic life. Similarly, when tissues are exposed to hyperbaric O_2, the production of ROMs may be increased by these same mechanisms.

Newborn animals experience a marked change in environment, including a change from the slightly hyperbaric maternal O_2 supply to the normobaric atmospheric O_2 (45). Is this reflected in an altered oxidative burden on the lungs? The SOD (95) and GSH (96) levels in the rat lung fall significantly from gestation through the postnatal period. Conversely, hepatic glutathione synthetase, glutathione reductase, and SeGSHpx activities increase in rats after birth (97). Despite the difficulty in obtaining perinatal human tissue, lung explants from premature and post-term infants show declining glucose-6-phosphate dehydrogenase and GSHpx activities after birth, while the activity of glutathione reductase remains unaltered (96). Similarly, the non-SeGSHpx activity has been shown to remain unaltered following birth (98). In a recent comparison of mature rodent and human lung tissues, similar levels of SOD were detected, but SeGSHpx activity was much lower in human lung than in rat (99).

When the normal oxygenation of a particular tissue is interrupted by occlusion of the blood flow, a state of ischemia is produced. On resupply of blood flow and tissue oxygenation, acute damage may occur. This reperfusion injury may be due to the generation of ROMs associated with the rapid metabolism of accumulated hypoxanthine. The hypoxanthine is derived from the utilization of ATP during ischemia and further metabolized via xanthine oxidase upon reperfusion (100), although other mechanisms may also contribute (101). The danger of reperfusion injury presents clinical problems, especially in patients who have suffered traumatic injuries, undergone major surgical treatments (including organ transplantation), or suffered infarctions.

Reperfusion of the isolated, ischemic dog heart causes depletion of GSH and catalase activity in the myocardium, effects which are blocked by allopur-

inol, an inhibitor of xanthine oxidase (102). Reperfusion of the isolated, ischemic rabbit heart produces similar effects without altering the activities of glutathione reducatase or GSHpx (103). Reperfusion of the isolated, ischemic pig heart in the presence of SOD and catalase accelerates myocardial ATP resynthesis, decreases myocardial injury, and improves coronary blood flow (104). In this case and in other equivalent systems, the protective effect of exogenous SOD is only partial, emphasizing the multifactorial nature of reperfusion injury. For instance, one must also consider the role of altered Ca^{2+} homeostasis in the induction of myocardial ischemia-reperfusion damage. Ca^{2+} antagonists such as nifedipine and verapamil successfully block reperfusion injury in model systems (105), possibly by interfering with the Ca^{2+}-dependent proteolytic conversion of xanthine dehydrogenase to xanthine oxidase. Similarly, if myocardial ischemia is maintained for extended periods, there comes a critical point where SOD provides no protection (106). Similar protective effects of SOD have been noted in ischemic kidneys (107) and liver (108). In addition, vitamin E depletion and lipid peroxidation produced in the ischemic liver upon reperfusion is partially blocked by co-perfusion with vitamin E (109). To date, no reports of clinical trials have appeared using SOD or vitamin E in these contexts.

Now let us consider some effects of high oxygen concentrations in biological systems. The lethality of prolonged exposure of animals to hyperbaric O_2 is well established (110). However, under milder conditions animals can respond to elevated O_2 tension by the induction of host defense mechanisms, particularly within the lungs. Rats exposed to sustained hyperbaric O_2 respond by induction of lung SOD, catalase, glucose-6-phosphate dehydrogenase, SeGSHpx, and glutathione reductase activities. This inductive response is linked to the maturity of the animals, with young animals being more responsive than mature ones. It is also species-specific, and hamsters are totally unresponsive (111). Thus, exposure to short periods of hyperoxia actually protects rats against severe hyperoxic stress through such induction (112). Similar effects have been shown in Chinese hamster ovary cells which adapt to survival in hyperbaric oxygen, probably through the induction of SOD, catalase, the GSHpx activities (113). A key protective role for lung GSH is indicated, as depletion of lung GSH with DEM greatly potentiates hyperbaric O_2 toxicity (114) and pulmonary GSH levels are elevated by sustained hyperoxia (115). Additionally, NAC reportedly provides protection against hyperbaric O_2 toxicity in rodents (116). Deficiency in vitamin E has also been shown to potentiate O_2 toxicity in rodents, an effect reversed by dietary supply of the vitamin (117). Little work has been performed with human tissues in these respects, although it is clearly of interest to make antioxidant therapy available to patients receiving O_2 therapy.

Specific Disease States

CANCER Studies of the disposition of host antioxidant defense systems and effects of their modulation have suggested a fundamental role for ROM-mediated oxidative stress in several stages of the carcinogenesis process. As these aspects have been the subject of extensive recent reviews (17, 118), we limit the present discussion to some general principles.

Endogenous antioxidant defense may be depressed during tumor promotion. In a recent study, using a model for two-stage carcinogenesis, exposure to tumor promotors such as phorbol esters caused a transient decrease in mouse epidermal cell GSH and GSHpx levels (119). Administration of selenium, vitamin E, and GSH to the animals prophylactically reversed these effects and inhibited promotion (120). This and other studies using antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and SOD, (17, 118), indicate potential anticarcinogenic properties for antioxidants, particularly those occurring naturally in the diet (118).

Depression of antioxidant defense has also been noted in a variety of human tumors. The catalase, GSHpx, and GSH levels of human hepatoma tissue are all lower than those found in normal liver. However, unaffected tissue from livers containing tumor foci also shows decreased GSHpx but not catalase activity (121). Human hepatoma cells have greatly reduced levels of SOD and catalase, compared to normal cells, and are deficient in SeGSHpx and glutathione transferase activities. These cells also have extreme iron overload (122). However, caution must be expressed in generalizing these findings, as human breast cancer tissue has been found actually to contain higher levels of GSHpx and glutathione reductase than does normal breast tissue (123). Clearly, more analysis of human tissue is necessary in order to establish these relationships further.

In addition to the promotion stage of tumorigenesis, ROMs can play a role in tumor progression (17, 118). A recent report suggests that the production of O_2^- in the extracellular environment can greatly enhance the invasive potential of tumor cells in vitro, an effect blocked by SOD (124). These studies also illustrate one facet of the relationship between inflammation and tumorigenesis (118), as activated PMNs could be a potential source for such effects in vivo.

INFLAMMATION The bioactivation of PMNs clearly results in the release of ROMs and other tissue damaging products, and PMNs accumulate at sites of tissue injury as part of the normal inflammatory response (3, 125). Although a detailed review is not applicable here, we discuss some of the evidence for the involvement of ROMs in inflammatory events gained from the study of antioxidant systems.

Acute inflammation is a complex process characterized by vascular permeability changes with interstitial edema and PMN infiltration of the tissue. SOD has antiedemic and antiinflammatory effects in a variety of animal models of acute inflammation. These include rodent polyarthritis and nonspecific interstitial edema (126), nephritis (127), and pancreatitis (128). Most of these effects are dependent upon the type of SOD used, and recombinant human SODs are not always effective. The mechanism of action of SOD in these respects is uncertain. However, the enzyme may scavenge ROMs which otherwise might elicit changes in, and damage to, the vascular endothelium. In this respect note that human vascular endothelial cells have some impairment in antioxidant defense (129). An antiedemic property of desferrioxamine in some models has also been reported (130).

SOD preparations for clinical usage are being tested in patients presenting symptoms associated with tissue-specific PMN infiltration. Preliminary results demonstrate considerable antiinflammatory properties for SOD in diseases ranging from rheumatoid arthritis to Crohns' disease (131). It appears that these effects are not due to an inhibitory effect of SOD on PMN function (132).

Despite these effects few attempts have been made to monitor the disposition of antioxidant systems during normal acute inflammatory events. Similar information is lacking in chronic inflammation, due mainly to the lack of suitable models. Thus, there is little appreciation of the role of ROM-mediated oxidative stress in the generation of pathophysiological changes (e.g. fibrosis and hyperplasia) which are often associated with chronic inflammation in humans (125).

INHERITED DISEASES Perhaps the most direct evidence for the involvement of oxidative stress in pathophysiological changes gained through study of antioxidant defense systems has come from the identification of genetic variants in the human population which lack particular host protective enzymes. Cases of severe hemolytic anemia have been reported in association with deficiencies in enzymes of GSH metabolism. These include rare cases of GSH synthetase deficiency which may also be coupled to deficiencies in glutathione transferases (133). Hereditary glucose-6-phosphate dehydrogenase deficiency, which is common in some ethnic groups, is a well-studied case in which hemolytic anemia may occur due to prooxidant-induced oxidative stress on erythrocyte GSH pools. This defect probably survives within the gene pool at such high levels because it may confer resistance to malarial infections in affected subjects (134). Similarly, rare cases of hereditary glutathione reductase deficiency are known, and these also manifest hemolytic anemia (135).

Acatalasemia is a rare inherited disease associated with a deficiency in catalase; it presents with severe infections of mucus membranes, perhaps due to unimpeded autoinhibition of PMN function by H_2O_2 during localized inflammation (136). Hereditary deficiencies in metal chelator proteins have also been noted. Wilson's disease is associated with a deficiency in caeruloplasmin and the deposition of copper in the tissues. This is thought to cause the severe, degenerative neurological symptoms of the disease via the generation of ROMs such as ·OH by a Fenton-type reaction. This disease is effectively treated with chelators such as penicillamine (44). Finally, an inherited deficiency in α-1-antiprotease activity has been noted in some patients developing emphysema (84).

CONCLUDING REMARKS

Biological systems clearly possess an impressive array of antioxidant defense mechanisms, whose study is beginning to establish the existence of ROMs at various levels of biological organization and their role in an increasing variety of prooxidant-related toxicities. Despite our current knowledge, several areas of this research lack the information necessary for a full understanding of these relationships. First, for model in vitro studies we should consider host antioxidant systems as a dynamic network of defense. Simultaneous analysis of many components must occur in order to appreciate fully how individual parts may overlap in activity and compensate for each other. This is important as it has not proven possible to generalize from prooxidant to prooxidant and from tissue to tissue which components are critical to host defense. Coupled to this, more specific "tools" are needed to modulate individual components of the network and to gain knowledge of the biochemical mechanisms underlying the inductive processes elicited by environmental stimuli. Secondly, at higher levels of biological organization, the intra- and intertissue heterogeneity of components of the network need to be probed more fully, particularly with regard to potential homeostatic control of the supply of metabolic precursors to individual antioxidants. More work should be performed with human tissue in these respects. Lastly, a closer relationship is needed between basic and applied research in this area. It is clear that prooxidant-mediated oxidative stress may play a role in the development of an increasing number of human disease states. Thus, it is critical that potential antioxidant therapies be selected on the basis of sound observations made in model systems. Additionally, clinical trials should be conducted with the appropriate controls, which often is not done (137).

Literature Cited

1. McCord, J. M., Fridovich, I. 1969. Superoxide dismutase, an enzyme function for erythrocuprein (hemocuprein). *J. Biol. Chem.* 344:6049–6055
2. Pryor, W. A. 1986. Oxy-radicals and related species: Their formation, lifetimes, and reactions. *Ann. Rev. Physiol.* 48:657–67
3. Weiss, S. J., Lobuglio, A. F. 1982. Biology of disease. Phagocyte-generated oxygen metabolites and cellular injury. *Lab. Invest.* 47:5–18
4. Fridovich, I. 1983. Superoxide radical: An endogenous toxicant. *Ann. Rev. Pharmacol. Toxicol.* 23:239–57
5. Thor, H., Smith, M. T., Hartzell, P., Bellomo, G., Jewell, S. A., et al. 1982. The metabolism of menadione (2-methyl-1,4-naphthoquinone) by isolated hepatocytes. A study of the implications of oxidative stress in intact cells. *J. Biol. Chem.* 257:12419–12425
6. Kappus, H. 1985. Lipid peroxidation: Mechanisms, analysis, enzymology, and biological relevance. In *Oxidative Stress,* ed. H. Sies, pp. 273–310. New York: Academic
7. Porter, N. A. 1984. Chemistry of lipid peroxidation. *Methods Enzymol.* 105: 273–82
8. Cadenas, E., Sies, H., Nastainczyk, W., Ullrich, V. 1983. Formation of singlet oxygen detected by low-level chemiluminescence during enzymatic reduction of prostaglandin G_2 to H_2. *Hoppe-Seylers Z. Physiol. Chem.* 364:519–28
9. Ziegler, J. S., Goosey, J. D. 1981. Photosensitized oxidation in the occular lens. Evidence for photosensitisers endogenous to the human lens. *Photochem. Photobiol.* 33:869–76
10. Brown, K., Fridovich, I. 1981. DNA strand scission by enzymatically generated oxygen radicals. *Arch. Biochem. Biophys.* 206:414–19
11. Di Monte, D., Ross, D., Bellomo, G., Eklöw, L., Orrenius, S. 1984. Alterations in intracellular thiol homeostasis during the metabolism of menadione by isolated rat hepatocytes. *Arch. Biochem. Biophys.* 235:334–42
12. Torchinsky, Y. M. 1981. In *Sulfur in Proteins,* ed. D. Metzler, Oxford: Pergamon
13. Ziegler, D. M. 1985. Role of reversible oxidation-reduction of enzyme thiol-disulfides in metabolic regulation. *Ann. Rev. Biochem.* 54:305–29
14. Nicotera, P., Hartzell, P., Baldi, C., Svensson, S. Å., Bellomo, G., et al. 1986. Cystamine induces toxicity in hepatocytes through the elevation of cytosolic Ca^{2+} and the stimulation of a non-lysozomal proteolytic system. *J. Biol. Chem.* 261:14628–35
15. Weeds, A. 1982. Actin-binding proteins - regulators of cell architeture and motility. *Nature* 296:811–16
16. Sandy, M. S., Moldéus, P., Ross, D., Smith, M. T. 1986. Role of redox cycling and lipid peroxidation in bipyridyl herbicide cytotoxicity. Studies with a compromised isolated hepatocyte model system. *Biochem. Pharmacol.* 35:3095–3101
17. Cerutti, P. A. 1985. Pro-oxidant states in tumor promotion. *Science* 227:375–81
18. Fridovich, I. 1975. Superoxide dismutases. *Ann. Rev. Biochem.* 44:147–59
19. Slot, J. W., Genze, H. J., Freeman, B. A., Crapo, J. D. 1986. Intracellular localisation of copper-zinc and manganase superoxide dismutase in rat liver parenchymal cells. *Lab. Invest.* 55:363–71
20. Hartz, J. W., Funakoshi, S., Deutsch, H. F. 1973. The levels of superoxide dismutase and catalase in human tissues determined immunochemically. *Clin. Chem. Acta* 41:125–32
21. Marklund, S. L. 1984. Extracellular superoxide dismutase in human tissue and human cell lines. *J. Clin. Invest.* 74: 1398–1403
22. Marklund, S. L., Beckman, G., Stigbrand, J. 1976. A comparison between the common type and rare type genetic variant of human cupro-zinc superoxide dismutase. *Eur. J. Biochem.* 65:415–21
23. Chance, B., Sies, H., Boveris, A. 1979. Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.* 59:527–605
24. Marklund, S. L., Westman, N. G., Lundgren, E., Roos, G. 1982. Copper- and zinc-containing superoxide dismutase, manganese-containing superoxide dismutase, catalase and glutathione peroxidase in normal and neoplastic cell lines and normal human tissues. *Cancer Res.* 42:1955–61
25. Wendel, A. 1980. Glutathione peroxidase. In *Enzymatic Basis of Detoxication,* ed. W. B. Jakoby, J. R. Bend, J. Caldwell, pp. 333–48. New York: Academic
26. Griffith, O. W., Meister, A. 1980. Ex-

cretion of cysteine and γ-glutamyl cysteine moieties in humans and experimental animals with γ-glutamyl transpeptidase deficiency. *Proc. Natl. Acad. Sci. USA* 77:3384–87
27. Reed, D. J. 1986. Regulation of reductive processes by glutathione. *Biochem. Pharmacol.* 35:7–13
28. Halliwell, B., Gutteridge, J. M. C., eds. 1985. *Free Radicals in Biology and Medicine,* pp. 78–84. Oxford: Clarendon
29. Moldéus, P., Larsson, R., Ross, D. 1985. Involvement of prostaglandin synthase in the metabolic activation of acetaminophen and phenacetin. In *Arachidonic Acid Metabolism and Tumor Initiation,* ed. L. J. Marnett, pp. 171–98. Boston: Nijhoff
30. Lind, C., Hochstein, P., Ernster, L. 1982. DT-Diaphorase as a quinone reductase: A cellular control device against simiquinone and superoxide radical formation. *Arch. Biochem. Biophys.* 216:178–85
31. Pethig, R., Gascoyne, P. R. C., McLaughlin, J. A., Szent-Györgyi, A. 1985. Enzyme-controlled scavenging of ascorbyl and 2,6-dimethylsemiquinone free radicals in Ehrlich ascites tumor cells. *Proc. Natl. Acad. Sci. USA* 82: 1439–42
32. Moody, D. E., Silvra, M. H., Hammock, B. D. 1986. Epoxide hydrolysis in the cytosol of rat liver, kidney and testis. Measurement in the presence of glutathione and the effect of dietary clofibrate. *Biochem. Pharmacol.* 35: 2073–2080
33. Kosower, N. S., Kosower, E. M. 1978. The glutathione status of cells. *Int. Rev. Cytol.* 54:109–60
34. Meister, A., Anderson, M. E. 1983. Glutathione. *Ann. Rev. Biochem.* 52: 711–60
35. Meister, A. 1985. Methods for the selective modification of glutathione metabolism and study of glutathione transport. *Methods Enzymol.* 113:571–85
36. Larsson, A., Orrenius, S., Holmgren, A., Mannervik, B., eds. 1983. *Functions of Glutathione, Biochemical, Physiological, Toxicological and Clinical Aspects,* New York: Raven
37. Ross, D., Cotgreave, I. A., Moldéus, P. 1985. The interaction of reduced glutathione with active oxygen species generated by xanthine—oxidase-catalysed metabolism of xanthine. *Biochem. Biophys. Acta* 841:278–82
38. Ross, D., Norbeck, K., Moldéus, P. 1985. The generation and subsequent fate of glutathionyl radicals in biological systems. *J. Biol. Chem.* 260:15028–15032
39. Ketterer, B. 1986. Detoxication reactions of glutathione and glutathione transferases. *Xenobiotica* 16:957–73
40. Orrenius, S., Moldéus, P. 1984. The multiple roles of glutathione in drug metabolism. *Trends Pharm. Sci.* 5:432–35
41. Bielski, B. H. J. 1982. Chemistry of ascorbic acid radicals. *Adv. Chem. Ser.* 1982(200):81–100
42. McCay, P. B. 1985. Vitamin E: Interactions with free radicals and ascorbate. *Ann. Rev. Nutr.* 5:323–40
43. Dilberto, E. J., Dean, G., Coster, V., Allen, P. L. 1982. Tissue, subcellular and submitochondrial distibutions of semidehydroascorbate reductase: possible role of semidehydroascobate reductase in cofactor regeneration. *J. Neurochem.* 39:563–68
44. Sevanian, A., Davies, K. J. A., Hochstein, P. A. 1985. Conservation of vitamin C by uric acid in blood. *J. Free Radicals Biol. Med.* 1:117–24
45. Ames, B. N., Cathcart, R., Schwiers, E., Hochstein, P. 1981. Uric acid provides an antioxidant defence in humans against oxidant and radical-caused ageing and cancer. (An hypothesis.) *Proc. Natl. Acad. Sci. USA* 78:6858–62
46. Wright, C. E., Tallan, H. H., Lin, Y. Y. 1986. Taurine: Biological update. *Ann. Rev. Biochem.* 55:427–53
47. Emudianughe, T. S., Caldwell, J., Smith, R. L. 1983. The utilization of exogenous taurine for the conjugation of xenobiotic acids in the ferret. *Xenobiotica* 13:133–38
48. Pasantes-Morales, H., Wright, C. E., Gaull, G. E. 1985. Taurine protection of lymphoblastoid cells from iron-ascorbate induced damage. *Biochem. Pharmacol.* 34:2205–2207
49. Aisen, P., Liskowsky, S. 1980. Iron transport and storage proteins. *Ann. Rev. Biochem.* 49:357–93
50. Gutteridge, J. M. C., Stocks, J. 1981. Caeruloplasmin: Physiological and pathological perspectives. *C.R.C. Crit. Rev. Clin. Lab. Sci.* 14:257–329
51. Cross, C. E., Halliwell, B., Allen, A. 1984. Antioxidant protection: A function of tracheobronchial and gastrointestinal mucus. *Lancet* 1:1328–1329
52. Van Kuijk, F. J. G. M., Handelman, G. J., Dratz, E. A. 1986. Consecutive action of phospholipase A_2 and glutathione peroxidase is required for reduction of phospholipid phydroperoxides and provides a convenient method to determine

peroxide values in membranes. *J. Free Radicals Med. Biol.* 1:421–27
53. Ursini, F., Maiorino, M., Gregolin, C. 1986. Phospholipid hydroperoxide glutathione peroxidase. *Int. J. Tissue React.* 8:99–103
54. Bieri, J. G., Corasin, L., Hubbard, V. S. 1983. Medical uses of vitamin E. *N. Engl. J. Med.* 18:1063–1071
55. Burton, G. W., Joyce, A., Ingold, K. U. 1982. First proof that vitamin E is the major lipid-soluble, chain-breaking antioxidant in human plasma. *Lancet* 2: 327
56. Burk, R. F. 1983. Biological activity of selenium. *Ann. Rev. Nutr.* 3:53–70
57. Foote, C. S., Denny, R. W. 1968. Chemistry of singlet oxygen VII. Quenching by β-carotene. *J. Am. Chem. Soc.* 90:6233–35
58. Burton, G. W., Ingold, K. U. 1984. β-Carotene: An unusual type of lipid antioxidant. *Science* 224:569–73
59. Stocker, R., Yamamoto, Y., McDonagh, A. F., Glazer, A. N. Ames, B. N. 1987. Bilirubin is an antioxidant of possible physiological importance. *Science* 235:1043–1045
60. Freedman, R. B. 1979. How many distinct enzymes are responsible for several cellular processes involving thiol:disulfide interchange? *FEBS. Lett.* 97:201–10
61. Brigelius, R. 1985. Mixed disulfides: Biological functions and increase in oxidative stress. See Ref. 6, pp. 243–71
62. Brot, N., Weissbach, H. 1983. Biochemistry and physiological role of methionine sulfoxide residues in proteins. *Arch. Biochem. Biophys.* 223: 271–81
63. Lindahl, T. 1982. DNA repair enzymes. *Ann. Rev. Biochem.* 51:61–87
64. Berger, N. A. 1985. Poly (ADP-ribose) in the cellular responses to DNA damage. *Radiat. Res.* 101:4–15
65. Unverferth, D. V., Magorien, R., Leir, C. V., Balcerzak, S. 1982. Doxorubicin cardiotoxicity. *Cancer Treat. Rev.* 9:149–64
66. Thornally, P. J., Dodd, J. F. 1985. Free radical production from normal and adriamycin-treated rat cardiac microsomes. *Biochem. Pharmacol.* 34:669–74
67. Paraideathathu, T., Combs, A. B., Kehrer, J. P. 1985. In vivo effects of 1,3-bis (chloroethyl)-1-nitrosourea and doxorubicin on cardiac and hepatic glutathione systems. *Toxicology* 35:113–24
68. Yoda, Y., Nakazama, M., Abe, T., Kawakami, K. 1986. Prevention of doxorubicin myocardial toxicity in mice by reduced glutathoine. *Cancer Res.* 46: 2551–56
69. Moldéus, P., Cotgreave, I. A., Berggren, M. 1986. Lung Protection by a thiol-containing antioxidant: N-Acetylcysteine. *Respiration* 50(Suppl. 1):31–42
70. Myers, C., Bonow, R., Palmer, I., Jenkins, J., Corden, B., et al. 1983. A randomized controlled trial assessing prevention of doxorubicin cardiomyopathy by N-acetylcysteine. *Semin. Oncology* 10(Suppl. 1):53–55
71. Legha, S. S., Wang, Y. M., Mackay, B., Ewer, M., Hortobagyi, G. N., et al. 1982. Clinical and pharmacological investigations of the effects of α-tochopherol on adriamycin cardiotoxicity. *Ann. NY Acad. Sci.* 393:411–18
72. Kanter, M. M., Hamlin, R. L., Unverferth, D. V., Davis, H. W., Merola, A. J. 1985. Effect of excercise training on antioxidant enzymes and cardiotoxicity of doxorubicin. *J. Appl. Physiol.* 59:1298–1303
73. Jadot, G., Michelson, A. M., Pruget, K., Baret, A. 1986. Anti-inflammatory activity of superoxide dismutases. Inhibition of adriamycin-induced edema in rats. *Free Radicals Res. Commun.* 2:19–26
74. Smith, L. L., Rose, M. S., Wyatt, I. 1978. In *Oxygen Free Radicals and Tissue Damage. CIBA Found. Symp.*, ed. D. W. Fitzsimons, 65:321–41. Amsterdam: Excerpta Medica
75. Brigelius, R., Lenzen, R., Sies, H. 1982. Increase in hepatic mixed disulfides and glutathione disulfide by paraquat. *Biochem. Pharmacol.* 31:1637–41
76. Dunbar, J. R., De Lucia, A. J., Bryant, L. R. 1984. Glutathione status of isolated rabbit lungs. Effects of nitrofurantoin and paraquat perfusion with normoxic and hyperoxic ventilation. *Biochem. Pharmacol.* 33:1343–48
77. Glen, M., Sutherland, M. W., Forman, H. J., Fisher, A. B. 1985. Selenium deficiency potentiates paraquat-induced lipid peroxidation in isolated perfused rat lung. *J. Appl. Physiol.* 59:619–22
78. Mercurio, S. D., Combs, G. F. 1986. Selenium-dependent glutathione peroxidase inhibitors increase the toxicity of pro-oxidants in chicks. *J. Nutr.* 116:1726–34
79. Petrović, V, M., Spacić, M., Milić, B., Saicić, C. 1986. Age-dependent resistance to the toxic effects of paraquat in relation to superoxide dismutase activity in the lung. *Free Radicals Res. Commun.* 1:305–9
80. Eklöw-Låstbom, L., Rossi, L., Thor,

H., Orrenius, S. 1986. Effects of oxidative stress caused by hyperoxia and diquat. A study in isolated hepatocytes. *Free Rad. Res. Comms.* 2:57–68

81. Cotgreave, I. A., Sandy, M. S., Berggren, M., Moldéus, P., Smith, M. T. 1987. N-Acetylcysteine and glutathione-dependent protective effect of Pz51 (Ebselen) against diquat-induced cytotoxicity in isolated hepatocytes. *Biochem. Pharmacol.* 36:2899–904
82. Müller, A., Cadenas, R., Graf, P., Sies, H. 1984. A novel biologically active seleno-organic compound I. Glutathione peroxidase activity in vitro and antioxidant capacity of PZ51 (Ebselen). *Biochem. Pharmacol.* 33:3235–40
83. Cotgreave, I. A., Johansson, U., Moldéus, P., Brattsand, R. 1987. The anti-inflammatory activity of ebselen but not thiols in experimental alveolitis and bronchiolitis. *Agents Actions*. In press
84. Carp, H., Miller, F., Hoidal, R., Janoff, A. 1982. A potential mechanism for emphysema α-1-antiprotease recovered from the lung of cigarette smokers contains oxidised methionine and has decreased elastase-inhibitory activity. *Proc. Natl. Acad. Sci. USA* 779:2041–2045
85. Rogers, D. F., Jeffrey, P. K. 1986. Inhibition by oral N-acetyl cysteine of cigarette smoke-induced "bronchitis" in the rat. *Exp. Lung Res.* 10:267–83
86. Schnebli, H. P., Seemüller, U., Fritz, H., Maschler, R., Liersch, M., et al. 1985. Eglin C, a pharmacologically active elastase inhibitor. *Eur. J. Respir. Dis.* 66(Suppl. 139):66–70
87. Roth, W. J., Chang, S. I., Janoff, A. 1986. Inactivation of alveolar macrophage transglutaminase by oxidants in cigarette smoke. *J. Leucocyte Biol.* 39:629–44
88. Moldéus, P., Berggren, M., Grafström, R. 1985. N-acetylcyteine protection against the toxicity of cigarette smoke and cigarette smoke condensates in various tissues and cells in vitro. *Eur. J. Respir. Dis. 66*(Suppl. 139):123–29
89. Green, G. M. 1985. Mechanism of tobacco smoke toxicity on pulmonary macrophage cells. *Eur. J. Respir. Dis.* 66(Suppl.):82–85
90. Cotgreave, I. A., Johansson, U., Moldéus, P., Brattsand, R. 1987. The effect of acute cigarette smoke inhalation on lung and systemic glutathione and cysteine redox states in the rat. *Toxicology* 45:203–12
91. Chow, C. K., Chen, L. H., Thackar, R. R., Griffith, R. B. 1984. Dietary vitamin E and pulmonary biochemical responses of rats to cigarette smoking. *Environ. Res.* 34:8–17
92. Pacht, E. R., Kaseki, H., Mohammed, J. R., Cornwall, D. G., Davis, W. B. 1986. Deficiency of vitamin E in the alveolar fluid of cigarette smokers. Influence on alveolar macrophage cytotoxicity. *J. Clin Invest.* 77:789–96
93. Toth, K. M., Berger, E. M., Beehler, C. J., Repine, J. E. 1986. Erythrocytes from cigarette smokers contain more glutathione and catalase and protect endothelial cells from hydrogen peroxide better than do erythrocytes from nonsmokers. *Am. Rev. Respir. Dis.* 134:201–84
94. Taylor, J. C., Madison, R., Kosinska, D. 1986. Is antioxidant deficiency related to chronic obstructive pulmonary disease? *Am. Rev. Respir. Dis.* 134: 285–89
95. Pakkar, P., Jaffrey, F. N., Viswanathan, P. N. 1986. Neonatal development pattern of superoxide dismutase and aniline hydroxylase in the rat lung. *Environ. Res.* 41:302–8
96. Warshaw, J. B., Willson, C. W., Saito, K., Prough, R. 1985. The responses of glutathione and antioxidant enzymes to hyperoxia in developing lung. *Pediat. Res.* 19:819–23
97. Diilio, C., Del Boccio, G., Casalone, E., Aceto, A., Sacchetta, P. 1986. Activities of enzymes associated with metabolism of glutathione in fetal rat liver and placenta. *Biol. Neonate* 49:96–101
98. Fryer, A. A., Hume, R., Strange, R. C. 1986. The development of glutathione-S-transferase and glutathione peroxidase activities in human lung. *Biochim. Biophys. Acta* 883:448–53
99. Jenkinson, S. J., Lawrence, R. A., Tucker, W. Y. 1984. Glutathione peroxidase, superoxide dismutase and glutathione-S-transferase activities in human lung. *Am. Rev. Respir. Dis.* 130: 302–4
100. Granger, D. N., Rutili, G., McCord, J. M. 1981. Superoxide radicals in feline intestinal ischaemia. *Gastroenterology* 81:122-29
101. Kehrer, J. P., Piper, M. H., Sies, H. 1987. Xanthine oxidase is not responsible for re-oxygenation injury in isolated perfused rat heart. *Free Radicals Res. Commun.* 3:69–78
102. Peterson, D. A., Asinger, R. W., Elsperger, K. J., Homans, D. C., Eaton, J. W. 1985. Reactive oxygen species may cause myocardial perfusion injury. *Biochem. Biophys. Res. Commun.* 127: 87–93

103. Cuerello, S., Ceconi, C., Bigoli, C., Ferrari, R., Albertini, A., et al. 1985. Changes in cardiac glutathione status after ischaemia-reperfusion. *Experientia* 41:42–43
104. Das, D. K., Engelman, R. M., Otani, H., Rousou, J. A., Breyer, R. H., Lemeshow, S. 1986. Effects of superoxide-dismutase and catalase on myocardial energy metabolism during ischemia and reperfusion. *Clin. Physiol. Biochem.* 4:187–98
105. de Leiris, J., Richard, V. Pestre, S. 1984. Calcium antagonists and experimental myocardial ischaemia and infarction. In *Calcium Antagonists and Cardiovascular Disease*, ed. L. H. Opie, pp. 105–15. New York: Raven
106. Gallagher, K. P., Bude, A. T., Pace, D., Geren, R. A., Schlafer, M. 1986. Failure of superoxide dismutase and catalase to alter size of infarction in the concious dog after 3 hours of occlusion followed by reperfusion. *Circulation* 73:1065–1076
107. Koyama, I., Buckley, G. B., Williams, G. M., Im, M. T. 1986. The role of oxygen free radicals in mediating the reperfusion injury of cold-preserved ischaemic kidneys. *Transplantation* 40:590–95
108. Attala, S. L., Toledo-Pareyra, L. H., Mackenzie, G. H., Cederna, J. P. 1985. Influence of oxygen-derived free radical scavengers on ischaemic livers. *Transplantation* 40:584–89
109. Marubayashi, S., Dohi, K., Ochi, K., Kawasaki, T. C. 1986. Role of free radicals in ischaemic rat liver cell injury: Prevention of damage by α-tochopherol administration. *Surgery* 99:184–93
110. Wolfe, W. G., DeVries, W. C. 1975. Oxygen toxicity. *Ann. Rev. Med.* 26:203–17
111. Yam, J., Frank, L., Roberts, R. J. 1978. Oxygen toxicity. Comparison of lung biochemical responses in neonatal and adult rats. *Pediat. Res.* 12:115–19
112. Frank, L. 1982. Protection from O_2 toxicity by preexposure to hyperoxia: Lung antioxidant enzyme role. *J. Appl. Physiol.* 53:475–82
113. Van der Valk, P., Gille, J. J. P., Oostra, A. B., Roubos, E. W., Sminia, T., Joenje, H. 1985. Characterisation of an oxygen tolerant cell line derived from chinese hamster ovary. *Cell Tissue Res.* 239:61–68
114. Deneke, S. M., Lynch, B. A., Fanburg, B. L. 1985. Transient depletion of lung glutathione by diethylmaleate enhances oxygen toxicity. *J. Appl. Physiol.* 58:571–74
115. Iwata, M., Takagi, K., Sataké, T., Sugiyama, S., Ozawa, T. 1986. Mechanisms of oxygen toxicity in rat lungs. *Lung* 164:93–106
116. Patterson, C. E., Butler, J. A., Bryne, F. D., Rhodes, M. L. 1985. Oxidant lung injury: Intervention with sulfhydryl reagents. *Lung* 163:23–32
117. Ehrenkranz, R. A., Ablow, R. C., Warshaw, J. B. 1982. Effect of vitamin E on the development of oxygen-induced lung injury in neonates. *Ann. NY Acad. Sci.* 393:452–66
118. Troll, W., Wiesner, R. 1985. The role of oxygen radicals as a possible mechanism of tumor promotion. *Ann. Rev. Pharmacol. Toxicol.* 25:509–28
119. Perchellet, J. P., Perchellet, E. M., Orten, D. K., Schneider, B. A. 1986. Decreased ratio of reduced/oxidised glutathione in mouse epidermal cells treated with tumor promotors. *Carcinogenesis* 7:503–6
120. Perchellet, J. P., Abney, N. L., Thomas, R. M., Guislain, Y. L., Perchellet, E. M. 1987. Effects of combined treatments with selenium, glutathione and vitamin E on glutathione peroxidase activity, ornithine decarboxylase induction and complete and multistage carcinogenesis in mouse skin. *Cancer Res.* 47:477–85
121. Corrocher, R., Caseril, M., Bellisola, G., Gabrielli, G. B., Nicoli, N., et al. 1986. Severe impairment of antioxidant systems in human hepatoma. *Cancer* 58:1658–62
122. Bannister, W. H., Federici, G., Heath, J. K., Bannister, J. V. 1986. Antioxidant systems in tumor cells. The levels of antioxidant enzymes, ferritin and total iron in a human hepatoma cell line. *Free Radicals Res. Commun.* 1:361–67
123. Diilio, C., Sacchetta, P., Del Boccio, G., La Rovere, G., Federici, G. 1985. Glutathione peroxidase, glutathione-S-transferase and glutathione reductase activities in normal and neoplastic human breast tissue. *Cancer Lett.* 29:37–42
124. Shinkai, J. K., Mukai, M., Akedo, M. 1986. Superoxide radical potentiates invasive capacity of rat ascites hepatoma cells in vitro. *Cancer Lett.* 32:7–13
125. Henson, P. M., Johnston, R. B. 1986. Tissue injury in inflammation. Oxidants, proteinases and cationic proteins. *J. Clin. Invest.* 79:669–74
126. Jadot, G., Michelson, A. M., Puget, K. 1986. Anti-inflammatory activity of superoxide dismutases studied in adjuvant-induced polyarthritis in rats. *Free Radicals Res. Commun.* 2:27–42

127. Adachi, T., Fukuto, M., Ito, Y., Hirano, Y., Sugiura, M., et al. 1986. Effect of superoxide dismutase on glomerular nephritis. *Biochem. Pharmacol.* 35: 341–45
128. Guice, K. S., Miller, D. E., Oldham, K. T., Townsend, C. M., Thompson, J. C. 1986. Superoxide dismutase and catalase: A possible role in established pancreatitis. *Am. J. Surg.* 151:161–68
129. Hirschelmann, R., Bekemeier, H. 1986. Influences of the iron chelating agent desferrioxamine on two rat inflammatory models. *Free Radicals Res. Commun.* 2:125–27
130. Shingu, M., Yoshioka, K., Nobunaga, M., Yoshida, A. K. 1985. Human vascular smooth muscle and endothelial cells lack catalase activity and are susceptible to hydrogen peroxide. *Inflammation* 9:309–20
131. Niwa, Y., Somiya, K., Michelson, A. M., Puget, K. 1985. Effect of liposomal-encapsulated superoxide dismutase on active oxygen-related diseases. A preliminary study. *Free Radicals Res. Commun.* 1:137–53
132. Somiya, K., Niwa, Y., Michelson, A. M. 1986. Effects of liposome superoxide dismutase on human neutrophil activity. *Free Radicals Res. Commun.* 1:329–37
133. Beutler, E., Pagelow, C. 1986. Erythrocyte glutathione synthetase deficiency leads not only to glutathione but also to glutathione-S-transferase deficiency. *J. Clin. Invest.* 77:38–41
134. Calabrese, E. J., Geiger, C. P. 1986. Low erythrocyte glucose-6-phosphate dehydrogenase (G-6-Pd) activity and suceptibility to carbaryl-induced methemoglobin formation and glutathione depletion. *Bull. Environ. Contam. Toxicol.* 36:506–9
135. El Hazmi, M. A. F., Warry, A. S. 1985. Glutathione reductase deficiency in association with sickel cell and thalassemia genes in Saudi populations. *Hum. Hered.* 35:326–32
136. Matsunaga, T., Seger, R., Höger, P., Tiefenaur, L., Hitzig, W. H. 1985. Congenital acatalasemia: A study of neutrophil function after provocation with hydrogen peroxide. *Pediat. Res.* 19:1187–1190
137. Greenwald, R. A. 1985. Therapeutic benefits of oxygen radical scavenger treatments remain unproven. *J. Free Radicals Biol. Med.* 1:173–77