Environmental Health Perspectives
Vol. 55, pp. 37-46, 1984

Paraquat: Model for Oxidant-Initiated Toxicity

by James S. Bus* and James E. Gibson*

Paraquat, a quaternary ammonium bipyridyl herbicide, produces degenerative lesions in the lung after systemic administration to man and animals. The pulmonary toxicity of paraquat resembles in several ways the toxicity of several other lung toxins, including oxygen, nitrofurantoin and bleomycin. Although a definitive mechanism of toxicity of paraquat has not been delineated, a cyclic single electron reduction/oxidation of the parent molecule is a critical mechanistic event. The redox cycling of paraquat has two potentially important consequences relevant to the development of toxicity: generation of "activated oxygen" (e.g., superoxide anion, hydrogen peroxide, hydroxyl radical) which is highly reactive to cellular macromolecules; and/or oxidation of reducing equivalents (e.g., NADPH, reduced glutathione) necessary for normal cell function. Paraquat-induced pulmonary toxicity, therefore, is a potentially useful model for evaluation of oxidant mechanisms of toxicity. Furthermore, characterization of the consequences of intracellular redox cycling of xenobiotics will no doubt provide basic information regarding the role of this phenomena in the development of chemical toxicity.

Introduction

Paraquat is a quaternary ammonium bipyridyl compound (1, 1′-dimethyl-4, 4′-bipyridylium dichloride) that is widely used as a broad spectrum herbicide. The mammalian toxicity of paraquat has been the focus of considerable research in recent years since systemic administration of this agent initiates a progression of degenerative and potentially lethal lesions in the lung. The pulmonary toxicity of paraquat is of particular interest because it resembles the toxicity produced by other agents which enter the lung via airborne or systemic circulation vectors. Although a definitive mechanism of toxicity of paraquat has not yet been delineated, it is apparent that a cyclic single electron reduction/oxidation of the parent molecule is a critical mechanistic event. The redox cycling of paraquat in biological systems has two potentially important consequences relevant to the development of toxicity: generation of "activated oxygen" (e.g., superoxide anion, hydrogen peroxide, hydroxyl radical) that is highly reactive to tissue components; and/or depletion of cellular reducing equivalents (e.g., NADPH) necessary for normal function.

The purpose of this review is twofold: first, to describe the pathological changes in lung of animals after paraquat administration, emphasizing the commonality to other pulmonary toxins; and second, to describe studies conducted to elucidate the mechanisms of paraquat toxicity which suggest it to be a model for oxidant-initiated injury to the lung.

*Chemical Industry Institute of Toxicology, P. O. Box 12137, Research Triangle Park, NC 27709.

Pathological Changes in the Lung

The response of the lung to paraquat cannot be viewed as specific for this agent, but rather is illustrative of a nonspecific response of the lung common to a variety of airborne or systemic toxins. The pathogenesis of the paraquat lung lesion has been well characterized and has been excellently summarized in two reviews by Smith and Heath (*1–2*). One useful means of comparing the histopathological aspects of paraquat lung toxicity with those of other lung toxins is to examine the response of three cell types frequently effected by such agents, the Type I and II alveolar pneumocytes and capillary endothelial cells.

The acute pulmonary toxicity of paraquat in animals has been described as occurring in two phases (*1–2*). In the initial "destructive" phase, alveolar epithelial cells were extensively damaged, and their subsequent disintegration often resulted in a completely denuded alveolar basement membrane. Pulmonary edema was also a characteristic of the destructive phase, and frequently was of sufficient severity to result in death of the animals. Animals surviving the initial destructive phase, which occurred in the first 1 to 4 days after acute paraquat treatment, progressed to what has been termed the "proliferative" phase. In this phase the alveoli were infiltrated with profibroblastic cells that rapidly differentiated into fibroblasts, resulting in the development of intra-alveolar fibrosis. The histopathologic outcome of the second phase may be influenced by the treatment regimen, however. Administration of repeated low doses of paraquat, which less severely damaged the alveolar epithelial cells, also induced hyperplasia of the Type II cells. This response may represent an attempt by the lung to repair the damaged epithelium.

Following a single dose of paraquat to animals, the earliest ultrastructural changes have been observed in the Type I alveolar epithelial cells approximately 4 to 6 hr after treatment (*3–5*) and were usually characterized by cellular and mitochondrial swelling, increased numbers of mitochondria, and the appearance of dark granules in the cytoplasm. Similar changes were also noted in mice exposed to an aerosol of paraquat for 15 min (*6*). If a high dose of paraquat was given (approximately LD_{50} or greater), the lesions in the Type I cells often progressed to the point of complete cellular disintegration, leaving areas of exposed basement membrane (*3–5, 7–10*). Injury to the Type I pneumocytes in rats fed 500 ppm paraquat in the diet was similar to that after an acute dose of paraquat, although the first ultrastructural lesions were not observed until 5 weeks of exposure (*11*).

The Type I cell has been observed as a common target for a wide spectrum of pulmonary toxins including the oxidant gases oxygen (*12,13*), ozone (*14*) and nitrogen dioxide (*15*) and the systemic agent bleomycin (*16*). The damage induced by these agents in this lung cell was basically similar to that observed with paraquat. In contrast to paraquat toxicity, however, both oxygen and bleomycin initially caused extensive damage in the capillary endothelial cells (*12,13,16*), which were remarkedly resistant to the toxic effects of paraquat (*4–6,10*).

Ultrastructural lesions in the alveolar Type II pneumocytes have also been observed shortly after acute paraquat exposure, although these lesions generally were not apparent until after the first lesions were seen in the Type I cells (*3–6*). Swollen mitochondria and damage to the lamellar bodies usually occurred between 8 to 24 hr after a high dose of paraquat (*4,5,17*). Progressive deterioration of the Type II cells continued, resulting in completely denuded alveolar basement membranes and debris filled alveolar spaces (*4,10*). The alveoli in these animals were infiltrated with profibroblastic cells which later differentiated into mature fibroblasts (*4*). The resulting intra-alveolar fibrosis obliterated the alveolar structure, such that the tissue appeared as a consolidated mass.

The apparent time lag in the response of the Type II cells compared to the Type I cell following acute paraquat exposure suggests that this cell population may be somewhat resistant to paraquat toxicity, although the mechanism for this resistance remains unclear. This apparent resistance was further supported in animal studies in which the dose of paraquat was decreased compared to the high dose studies described above. Vijeyaratnam and Corrin (*10*) observed that less severely affected parts of the lung appeared to undergo epithelial regeneration 7 to 14 days after a single dose of paraquat. Electron microscopic examination revealed the alveoli to be lined with cuboidal epithelial cells which closely resembled Type II pneumocytes except for a general lack of lamellar bodies. Similar phenomena have also been noted by other investigators who administered paraquat in the diet (*11*) or as repetitive intraperitoneal administrations (*8*). Thus, in animals where the dose of paraquat was sufficient to kill only the Type I pneumocytes, the surviving Type II cells repaired the damaged epithelium by proliferating and subsequently differentiating into Type I epithelial cells. This regenerative response of the lung to paraquat closely paralleled the response observed with a number of pulmonary toxins such as oxygen (*13*), ozone (*14*), nitrogen dioxide (*15*), butylated hydroxytoluene (*18*) and bleomycin (*16*).

It should be apparent from the preceding review that the pathogenesis of paraquat lung toxicity is by no means unique. Nonetheless, paraquat is a valuable research tool to explore mechanisms of chemically induced toxicity, since the lesions are easily and rapidly produced, and most importantly, a considerable body of information is available regarding the potential for paraquat to disturb cellular biochemistry via its ability to undergo cyclic reduction–oxidation reactions.

Mechanisms of Paraquat Toxicity

Redox Cycling of Paraquat

As early as 1933 it was shown that paraquat, which was used as an oxidation–reduction indicator (methyl viologen), could undergo a single electron reduction to a blue-colored free radical, in a reaction with a redox potential of -446 mV (*19,20*). Anaerobic conditions were necessary, however, to prevent immediate reoxidation of the radical to the parent compound. Later studies by Farrington et al. (*21*) demonstrated that paraquat radical, generated by pulse radiolysis of an aqueous aerobic solution of paraquat, reacted rapidly with oxygen, generating superoxide radical (O_2^-). This reaction is depicted schematically in Figure 1. Paraquat radical also readily reduced O_2^- to O_2^{-2}, which at physiologic pH in aqueous solution exists as hydrogen peroxide (H_2O_2). The reaction of paraquat with oxygen was subsequently confirmed in additional studies in which paraquat radical was produced by electrochemical (*22*) and pulse radiolysis or laser photolysis techniques (*23*).

Several studies have now demonstrated that biological systems are also capable of catalyzing the cyclic reduction–oxidation of paraquat. In 1968, Gage (*24*) reported that anaerobic incubation of liver microsomes with NADPH and paraquat produced the blue-colored radical. Under aerobic conditions this incubation system stimulated oxygen consumption which was not inhibited by carbon monoxide, suggesting that redox cycling was catalyzed via microsomal flavoproteins. The initial rate of oxygen consumption was approximately one-half the initial rate of NADPH oxidation, indicating probable formation of the two electron reduction product of oxygen, hydrogen peroxide.

A subsequent investigation by Ilett et al. (*25*)

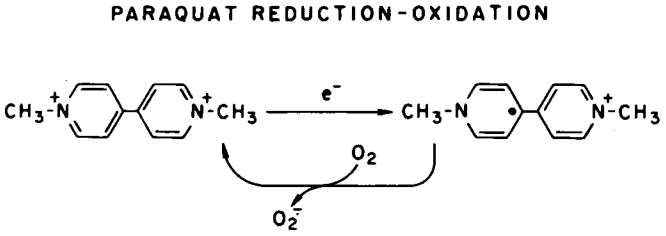

FIGURE 1. Paraquat reduction-oxidation.

confirmed the paraquat-induced stimulation of NADPH oxidation by rat liver microsomes and further found both rat lung and rabbit liver and lung microsomes to catalyze the reaction. Their observations that carbon monoxide did not inhibit NADPH oxidation, and that paraquat decreased the *in vitro* metabolism of bromobenzene and also did not produce a Type I or Type II microsomal spectral change, supported the conclusion of Gage (*24*) that paraquat uncoupled microsomal electron transport. It was further shown by these investigators and others that paraquat stimulated rat liver (*25–27*) and lung (*25,28,29*) microsomal hydrogen peroxide production, as measured by the conversion of methanol to formaldehyde. These observations again suggested the potential for a two-electron reduction of molecular oxygen catalyzed by paraquat.

In similar studies (*30*), rat lung, kidney and liver homogenates generated paraquat radical in the presence of NADPH. The reaction was not inhibited by carbon monoxide, cyanide or SKF-525A, which was consistent with the hypothesis that the microsomal-catalyzed reduction of paraquat occurred at some site prior to cytochrome P-450. Bus et al. subsequently (*31*) demonstrated that the anaerobic reduction of paraquat catalyzed by mouse lung microsomes and NADPH was inhibited by antibody to NADPH-cyctochrome P-450 reductase, implicating this flavoprotein in the microsomal reduction of paraquat.

Redox cycling of paraquat has also been implicated in studies evaluating the effects of paraquat on mammalian cell respiration. Gage (*24*) found that paraquat stimulated oxygen consumption *in vitro* when incubated with rat liver mitochondrial fragments inhibited with antimycin A and NADH or β-hydroxybutyrate. It was concluded that paraquat was most likely interacting with mitochondrial flavoprotein. The possibility that paraquat radical was a product of this interaction was supported by the observation that incubation of mitochondria with paraquat resulted in accumulation of the blue-colored paraquat radical in the media (*32*). More recently, Rossouw and Engelbrecht (*33,34*) reported a marked stimulation of cyanide insensitive (microsomal) respiration in lung homogenates and intact alveolar macrophages. In other studies (*35*), *in vitro* perfusion of rat lung with paraquat stimulated carbon-14-labeled glucose metabolism as reflected by a large increase (182%) in the activity of the pentose cycle and a smaller increase (30%) in mitochondrial metabolism. In that the paraquat treatment did not alter pyruvate production or the lactate-to-pyruvate ratio in the perfused lungs, it was suggested that paraquat increased the turnover of NADPH (as reflected by the increase in pentose cycle activity), which was viewed as consistent with an enzymatic reduction of paraquat.

As indicated in several studies described above (*24, 25*), the oxidation of reduced paraquat generated by mammalian tissues results in the apparent formation of hydrogen peroxide. Several studies have indicated that this reaction proceeds via formation of the single electron reduction product of molecular oxygen, O_2^-, as had been proposed from pulse radiolysis studies (*23, 36*). Davies and Davies (*37*) demonstrated that incubation of paraquat with rat liver microsomes and NADPH stimulated the autoxidation of epinephrine to adrenochrome, a reaction catalyzed by O_2^- (*38*). This observation was subsequently confirmed in studies utilizing rat liver (*28*) and lung (*28,29,39*). Addition of superoxide dismutase, which catalyzes (*40*) the reaction

$$O_2^- + O_2^- \xrightarrow{H^+} O_2 + H_2O_2$$

to the *in vitro* incubations inhibited paraquat-stimulated epinephrine autoxidation, implicating a O_2^- mediated reaction (*38*). It is interesting to note that rabbit lung microsomes did not stimulate O_2^- generation when incubated with paraquat (*29*), which correlates with a resistance of this species to paraquat lung toxicity (*41, 42*).

Redox cycling of paraquat has also been reported in nonmammalian systems such as plants and bacteria. Dodge (*43*) demonstrated the ability of isolated plant chloroplasts to form the paraquat radical under anaerobic conditions. In cultures of *Escherichia coli*, paraquat significantly stimulated cyanide-resistant respiration in a process that required both molecular oxygen and a source of electrons (*44,45*). It was concluded that paraquat undergoes a cyclic reduction–oxidation producing O_2^-, which was supported in a later study (*46*) in which paraquat radical accumulated in anaerobic cultures of *E. coli*. No blue-colored radical was noted in aerobic cultures. In addition, incubation of paraquat with a homogenate of *E. coli* and NADPH resulted in a superoxide dismutase-inhibitable reduction of cytochrome c. Since the amount of cytochrome c reduced per hour per milligram dry weight of cells in the homogenate correlated well with the cyanide-resistant oxygen consumption of whole cells expressed on a similar basis, it was also suggested that a soluble NADPH:paraquat reductase could generate sufficient O_2^- to account for most of the paraquat-dependent cyanide-resistant oxygen consumption by intact *E. coli*. A subsequent study (*47*) reported that the reduction of paraquat by *E. coli* soluble fraction and subsequent O_2^- formation was NADPH- and not NADH-dependent.

Role of Oxygen in Paraquat Toxicity

An increasing amount of evidence suggests that molecular oxygen is critical for paraquat toxicity in plants, mammals and bacteria. Early work by Mees (*48*) showed that oxygen was necessary for the herbicidal activity of the bipyridylium herbicides. These agents were not toxic to plant leaves under anaerobic conditions, despite the continuation of photosynthetic reactions capable of generating paraquat radicals. Exposure of the incubates to air resulted in the immediate onset of toxicity. Youngman and Dodge (*49*) recently reported that a copper chelate of D-penicillamine possessing significant superoxide dismutase activity (*50*) decreased the phytotoxic effects of paraquat in flax cotyledons. This observation supported the conclusion that $O_2^{\cdot-}$ mediated the phytotoxicity of paraquat.

In 1973, Fisher et al. (*21*) observed that paraquat pulmonary toxicity in rats was significantly enhanced by simultaneous exposure to one atmosphere of pure oxygen. This observation has subsequently been confirmed in other studies in which mice (*51*) and rats (*52–55*) were exposed to elevated concentrations of oxygen. Although the mechanism of oxygen-induced enhancement of paraquat toxicity is not precisely understood, it may well be related to the potential for both oxygen (*56,57*) and paraquat (as described above) to increase intracellular concentrations of $O_2^{\cdot-}$. The increased flux of $O_2^{\cdot-}$ generated by simultaneous paraquat and oxygen exposure may thus more readily overwhelm tissue oxidant defenses such as superoxide dismutase (*58*), resulting in a more rapid and severe onset of toxicity.

In contrast to elevated oxygen tensions, decreased oxygen tension protected against paraquat-induced lethality in mice (*59*). Mice dosed intraperitoneally with paraquat and then placed in a 14% oxygen environment that was subsequently decreased stepwise to 10% over the next 48 hr had a significantly greater survival rate than comparably dosed mice left in room air. Brief exposure of the mice contained in 10% oxygen to air resulted in a rapid increase in mortality. Compared to the results of the previous study (*59*), exposure of paraquat treated rats to 10% oxygen significantly enhanced lethality (*60*). However, the enhanced toxicity was associated with a significant increase in the concentration of paraquat in the lung, which the authors suggested may have been due to increased cardiac output caused by the hypoxic stress. This conclusion was supported by the observation that the rats were not acclimated to 10% oxygen prior to paraquat treatment.

Oxygen tension also affected the viability of cultured rat Type II pneumocytes incubated with paraquat (*61*). Under reduced oxygen tension the viability of paraquat-incubated cells was similar to control cells, while incubation under normobaric and hyperbaric (95%) oxygen progressively decreased viability compared to controls. Addition of superoxide dismutase did not ameliorate the toxicity of paraquat seen under normo- or hyperbaric conditions, although it was not determined if intracellular levels of superoxide dismutase were increased.

The requirement of oxygen for expression of paraquat toxicity was particularly apparent in several studies which evaluated paraquat toxicity in bacteria. Paraquat has been shown to be bacteriostatic under aerobic conditions for *E. coli*, a facultative anaerobe (*62*). Fisher and Williams (*63*) confirmed this observation and further demonstrated that paraquat did not inhibit growth of *E. coli* under strict anaerobic conditions, indicating a requirement for oxygen in toxicity.

In a subsequent series of studies (*44,45*) Hassan and Fridovich convincingly illustrated the critical role of oxygen in the toxicity of paraquat in *E. coli*. Aerobic incubation of *E. coli* with paraquat in a nutrient rich media markedly increased the intracellular activity of superoxide dismutase (*44*), an important defense enzyme modulating the toxicity of oxygen (*58*). Cells in which superoxide dismutase was induced by paraquat exposure were found to be resistant to oxygen toxicity (*44*). Exposure of *E. coli* to paraquat in media which prevented protein synthesis, and thereby an increase in superoxide dismutase activity, resulted in a significant increase in bacterial toxicity (*45*). In addition, *E. coli* containing high activities of superoxide dismutase were found to be much more resistant to paraquat toxicity than those with low enzyme activity.

In contrast to the observations of Hassan and Fridovich (*44,45*), Simons et al. (*60*) found the toxicity of paraquat to be similar in *E. coli* containing either low or high activities of superoxide dismutase. Their conclusion that paraquat bacterial toxicity was independent of oxygen radical formation was questioned by Hassan and Fridovich (*45*), however, who suggested that their results were most likely attributable to an extreme sensitivity of their bacterial cultures to oxygen and a lack of an abundant electron supply necessary for redox cycling of paraquat.

Potential Mechanisms of $O_2^{\cdot-}$-Mediated Toxicity

The ability of plant, bacterial and mammalian systems to readily catalyze $O_2^{\cdot-}$ formation via redox cycling of paraquat, as well as the sensitivity of paraquat treated organisms to oxygen, strongly suggests an important role for activated oxygen in the mechanism of paraquat toxicity. Superoxide anion has been shown to participate in a broad spectrum of potentially toxic reactions such as peroxidation of polyunsaturated lipid, depolymerization of hyaluronic acid, inactivation of proteins and damage to DNA (*58*). However, $O_2^{\cdot-}$ per se does not appear to be directly involved in many of these reactions. In 1934 Haber and Weiss (*64*) proposed the formation of hydroxyl radical ($OH^{\cdot}$), which is a much more potent oxidant than $O_2^{\cdot-}$, by a reaction of $O_2^{\cdot-}$ and H_2O_2 shown in Eq. (1).

$$O_2^{\cdot-} + H_2O_2 \rightarrow OH^- + OH^{\cdot} + O_2 \quad (1)$$

More recent studies (*65,66*) have suggested that this reaction is catalyzed in biological systems by complexed iron [Eqs (2–4)].

$$O_2^{\cdot-} + Fe^{+3} \rightarrow O_2 + Fe^{+2} \quad (2)$$

$$Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^{\cdot} + OH^- \quad (3)$$

$$O_2^{\cdot-} + H_2O_2 \rightarrow O_2 + OH^{\cdot} + OH^- \quad (4)$$

A generalized schematic describing the potential reactions of $O_2^{\cdot-}$ generated by paraquat redox cycling is depicted in Figure 2. Also illustrated in Figure 2 is the function of several antioxidant defense mechanisms which are important in modulating $O_2^{\cdot-}$-mediated toxicity. First, the enzyme superoxide dismutase rapidly scavenges $O_2^{\cdot-}$ by catalyzing its dismutation to O_2 and H_2O_2. Catalase then converts H_2O_2 to water and O_2. The net effect of both reactions is to reduce the intracellular concentration of reactants necessary for formation of $OH^{\cdot}$. Several mechanisms also function to prevent the membrane damaging process of lipid peroxidation initiated by activated oxygen. Lipid hydroperoxides, which spontaneously decompose to toxic lipid radicals in the presence of transition metal ions, can be enzymatically converted to nonradical forming lipid alcohols by glutathione (GSH) peroxidase, a selenium-containing enzyme. Reducing equivalents for this reaction are supplied via the concerted activities of GSH reductase and glucose-6-phosphate (G-6-P) dehydrogenase. In addition, the chain reaction process of lipid peroxidation can be terminated via the intervention of vitamin E, a naturally occurring lipid-soluble antioxidant. For more detailed information, Bus and Gibson (*67*) have reviewed the biological defenses against oxidant-induced tissue damage.

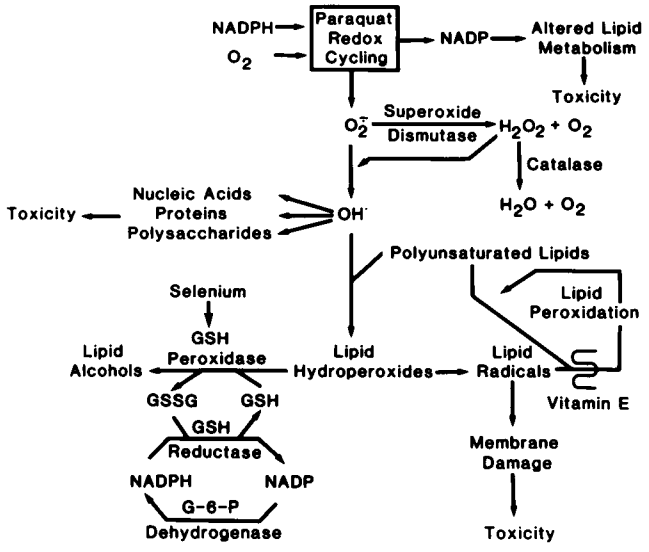

FIGURE 2. Potential mechanisms of paraquat toxicity.

Paraquat-Induced Lipid Peroxidation

The potential of paraquat to initiate the membrane damaging process of lipid peroxidation via $O_2^{\cdot-}$ generation has been suggested from a number of *in vitro* and *in vivo* studies. Studies by Pederson and Aust (*68*) investigating the mechanism of xanthine oxidase-promoted lipid peroxidation first suggested that $O_2^{\cdot-}$ could initiate lipid peroxidation, possibly via a singlet oxygen intermediate. These observations led to the proposal that paraquat could initiate peroxidation reactions via a similar mechanism (*31*), since this agent was known to generate $O_2^{\cdot-}$ in biological systems. Incubation of paraquat with NADPH, purified rat liver NADPH-cytochrome P-450 reductase (which catalyzed redox cycling of paraquat) and purified rat liver microsomal lipid stimulated peroxidation in a concentration-dependent manner (*31*). Addition of either superoxide dismutase or a singlet oxygen trapping agent inhibited peroxidation, which suggested that both forms of activated oxygen were involved in initiation of the reaction. More recent studies (*69,70*) have indicated, however, that singlet oxygen is most likely involved only in the propagation of lipid peroxidation reactions. The potential mechanisms by which activated oxygen is thought to initiate lipid peroxidation are described in a review by Bus and Gibson (*67*).

Investigations of paraquat-induced lipid peroxidation in more complex *in vitro* systems than those utilized by Bus et al. (*31*) have yielded conflicting results. Ilett et al. (*25*) reported that paraquat inhibited rat lung microsomal lipid peroxidation, even though paraquat stimulated both NADPH oxidation and hydrogen peroxide formation. Subsequent investigations have confirmed an *in vitro* paraquat inhibition of lipid peroxidation in microsomes isolated from rat lung and liver (*71,72*) and mouse liver (*26*).

In contrast, Talcott et al. (*27*) reported a stimulation of *in vitro* mouse lung microsomal peroxidation by paraquat. Since neither superoxide dismutase nor catalase altered the rate of paraquat-stimulated lipid peroxidation, it was proposed that paraquat catalyzed microsomal lipid peroxidation via a direct reduction of ferric ion by the paraquat radical. The resulting ferrous iron was suggested to directly initiate lipid peroxidation by reductive cleavage of pre-existing membrane lipid hydroperoxides to lipid radicals. Nonetheless, these investigators questioned whether lipid peroxidation was a primary mechanistic event in paraquat toxicity because an analogous agent which is not highly toxic to the lung, diquat, was even more potent than paraquat in stimulating lipid peroxidation. This conclusion is confounded, however, by the observation that paraquat, and not diquat, is accumulated in lung cells (most likely by Type I and/or Type II) by an apparent active transport mechanism (*73*). These investigators also reported that pretreatment of mice with an antioxidant, *N, N'*-diphenyl-*p*-phenylenediamine, or a high carbohydrate diet prevented *in vitro* paraquat stimulation of

lung microsomal lipid peroxidation (*74*). These pretreatments did not protect against paraquat-induced lethality, however, and no evidence for *in vivo* peroxidation as measured by conjugated diene formation was detected in lung microsomes isolated from paraquat-treated mice. Thus, it was concluded that although paraquat stimulated *in vitro* lipid peroxidation, this process did not correlate with *in vivo* toxicity.

Kornbrust and Mavis (*71*) found that paraquat inhibited *in vitro* lipid peroxidation in rat liver and lung microsomes but enhanced it in mouse lung and liver microsomes. In that the paraquat stimulated lipid peroxidation was NADPH-iron dependent, and was not inhibited by superoxide dismutase, it was suggested that paraquat catalyzed the reduction of ferric iron which initiated lipid peroxidation in a reaction similar to that proposed by Shu et al. (*74*). The role of lipid peroxidation as a mediator of *in vivo* toxicity was also questioned since both vitamin E and peroxidizable unsaturated fatty acid concentration was not altered in microsomes of rats administered a lethal dose of paraquat.

To date, studies attempting to demonstrate *in vitro* paraquat-stimulated lipid peroxidation have focused on the ability of this agent to peroxidize microsomal lipid, presumably because microsomes clearly catalyze paraquat redox cycling. Such studies may be complicated, however, by the fact that microsomes alone undergo NADPH- and oxygen-dependent lipid peroxidation, which is apparently catalyzed by NADPH-cytochrome P-450 reductase (*75*). Redox cycling of paraquat not only results in an uncoupling of microsomal electron transport (*76*) but also rapidly consumes NADPH and oxygen. All three of these effects would be expected to readily inhibit NADPH-dependent lipid peroxidation, which possibly accounts for the observations in many of the *in vitro* studies (*25,26,71,72*). The possibility that paraquat would stimulate microsomal lipid peroxidation if adequate precautions were taken to maintain both NADPH and oxygen concentrations in the incubate has in fact been demonstrated (*77,78*). In addition, it cannot be excluded that O_2^- generated by microsomal redox cycling of paraquat may initiate lipid peroxidation at other critical intracellular sites such as the mitochondrial membrane, which is particularly rich in unsaturated lipid. Lipid peroxidation of mitochondrial membranes has been correlated with swelling and lysis of the mitochondria (*79,80*), which is one of the earliest lesions detected by electron microscopy in paraquat poisoning (*1,2*). Hassan and Fridovich (*46*) have shown that O_2^- generated intracellularly in *E. coli* by paraquat redox cycling can diffuse extracellularly, despite the presence of intracellular superoxide dismutase. This observation suggests that intracellular O_2^- has a sufficient survival time to permit diffusion from its site of generation to other potential targets such as mitochondria within the cell.

The potential for paraquat to initiate lipid peroxidation at sites other than microsomes was suggested from a recent study utilizing isolated perfused rat livers (*81*). Phospholipid content was decreased and conjugated diene levels increased in microsomes and mitochondria following a 3-hr perfusion with 1mM paraquat. These effects were prevented by coperfusion with a copper–penicillamine complex possessing superoxide dismutase activity, and thus were consistent with the hypothesis that paraquat stimulated O_2^- mediated lipid peroxidation. However, it was also suggested that paraquat may induce effects other than peroxidation in liver since copper–penicillamine did not prevent a paraquat stimulated release of oxidized glutathione from the liver or a redistribution of fatty acid composition.

Much of the evidence that paraquat initiates lipid peroxidation *in vivo* has been obtained from studies in which the occurrence of this process is indirectly inferred. The mechanistic scheme depicted in Figure 2 predicts that alterations in defense mechanisms responsible for controlling lipid peroxidation should cause correlative changes in paraquat toxicity. This is in fact the case. Paraquat toxicity was significantly enhanced in mice fed selenium- or vitamin E-deficient diets (*82*). In addition, mice depleted of GSH, the source of reducing equivalents for GSH peroxidase, were also sensitized to paraquat toxicity (*82*), although this effect may in part be due to a loss of the intrinsic antioxidant properties of GSH. The enhancement of toxicity by selenium deficiency in rats, however, has been dissociated from the activity of GSH peroxidase, suggesting that selenium alone may be a direct factor in controlling lipid peroxidation (*83*). It is also interesting to note that selenium deficiency significantly elevated the hepatotoxicity of paraquat in mice, which indicated that diminution of a defense mechanism may shift the organ-specific organ toxicity of paraquat (*84*).

DiLuzio (*85*) observed that lipid peroxidation decreased tissue antioxidant concentrations, presumably because these agents are consumed in terminating lipid free-radical chain reactions. Paraquat, administered at an LD_{50} dose to mice, significantly decreased GSH in liver but not in lung (*86*). The decrease in GSH was speculated to result from increased utilization via the activity of GSH peroxidase, or possibly from a direct oxidation of GSH by paraquat-derived radicals. Paraquat also decreased the concentration of lipid-soluble antioxidants, which consist primarily of vitamin E, in a dose-dependent manner in lung but not in liver. This observation conflicted with a subsequent report (*77*) that vitamin E content was not affected in cell-free homogenates or microsomes isolated from paraquat-treated rats. It is unclear whether these differences may be due to the different species utilized.

Investigations of the lung toxicity of the oxidant gas ozone have led to the proposal that the GSH peroxidase system enzymes (GSH peroxidase, GSH reductase, G-6-P dehydrogenase) were induced in rats in response to oxidant stress (*87*). Consistent with this hypothesis,

exposure of rats to 100 ppm paraquat in the drinking water for 3 weeks significantly increased the activity of pulmonary GSH reductase and G-6-P dehydrogenase, but not GSH peroxidase (*86*). However, both GSH peroxidase and GSH reductase activities were increased in rats 48 hr after a single dose of paraquat (*88*). In this study the pulmonary activity of superoxide dismutase was also increased, indicating a possible response of the lung to paraquat-mediated O_2^- generation. The increase in enzymatic oxidant defenses as an indicator of a paraquat oxidant mechanism must be regarded with caution, however, because the increased pulmonary enzyme activity may represent a nonspecific response attributable to the marked shift in lung cell population which occurs with exposure to many pulmonary toxins (*89*).

The role of oxidant enzymatic defenses in modulating paraquat toxicity has been further supported by the observation of Bus et al. (*86*) that oxygen-tolerant rats (obtained by 85% oxygen pretreatment for 7 days) were more resistant to a lethal dose of paraquat. It was suggested that the cross tolerance of paraquat with oxygen may in part be due to the elevated lung activities of superoxide dismutase (*90*) and G-6-P-dehydrogenase (*21*) in oxygen-tolerant rats. This conclusion is tempered by a report (*91*) that oxygen treatment compromised uptake mechanisms of amine compounds into the lung. Thus, the cross tolerance phenomena may be a result of decreased uptake of paraquat into the lung.

Attempts to directly quantitate *in vivo* lipid peroxidation damage have met with mixed results. Expiration of ethane, a breakdown product of lipid peroxidation reactions, has recently been suggested as a valuable technique for *in vivo* assessment of lipid peroxidation (*67*). Reddy and co-workers (*88*) have shown ethane expiration was increased approximately twofold 2 hr after paraquat treatment. A subsequent study (*83*) found an approximate fourfold increase in ethane expiration in rats during the 6 hr immediately after an LD_{50} dose of paraquat. Steffen et al. (*92*), however, observed only a 26% increase in ethane evolution 4 hr after treatment of rats with a lethal dose of paraquat. This study was not comparable to the previous ones, however, in that the animals were placed in 100% oxygen after paraquat treatment. In studies using other indicators of lipid peroxidation damage, conjugated dienes were not elevated in the lungs of mice receiving a dose of paraquat equal to twice the LD_{50} (*74*); malondialdehyde formation was increased by paraquat in lungs of rats fed selenium-deficient diets, but not those fed selenium-adequate diets (*93*). Thus, it remains to be conclusively demonstrated that paraquat initiates lipid peroxidation as a primary toxic event *in vivo*. Future investigations of this potential mechanism will require improved techniques for quantitation of lipid peroxidation; existing methods may lack either sufficient sensitivity (since initial damage may be restricted to only a limited cell population in the lung) and/or selectivity.

Other Potential Superoxide-Mediated Reactions

It must be considered that O_2^- and $OH^\cdot$ generated by paraquat redox cycling may also react with critical macromolecules other than lipid, resulting in toxic effects to the cell. The hydroxyl radical, being an extremely potent oxidant, may readily alter nucleic acids, enzymes, and polysaccharides. The recent report of Ross et al. (*94*), who found that paraquat stimulated DNA strand breaks in cultured mouse lymphocytes, supports this possibility. Paraquat has also been observed to activate rat liver guanylate cyclase *in vitro* (*10*). Since the paraquat-induced activation was inhibited by superoxide dismutase, O_2^- was proposed as the agent responsible for activation. Giri and Krishna (*95*) subsequently demonstrated that paraquat stimulated guinea pig lung guanylate cyclase *in vivo*. These investigators postulated that increased cyclic GMP, which has been proposed as an intracellular signal for initiation of cell proliferation (*96*), may be the stimulus for the fibroproliferative changes characteristic of paraquat toxicity. Paraquat has been further implicated as a stimulus for collagen synthesis in rat lung (*97–100*), which *in vitro* experiments have suggested to be mediated via O_2^- (*99*).

Mechanisms Not Involving O_2^-

Paraquat redox cycling leads not only to the generation of O_2^- but also to a potential depletion of intracellular NADPH. Thus, it has been suggested that paraquat-stimulated NADPH depletion may be a primary toxic event, in that this effect would be expected to perturb several important cell processess (*49,71,101–103*).

Fisher et al. (*101*) found that paraquat increased the metabolism of [1-^{14}C]-glucose fourfold but did not alter the metabolism of [6-^{14}C]-glucose in isolated rat lung slices. In addition, paraquat also inhibited the incorporation of ^{14}C-acetate into lung lipids. From these observations it was suggested that paraquat redox cycling may have decreased available intracellular NADPH, causing the decrease in lipid biosynthesis, and that the activity of the pentose phosphate pathway increased in an attempt to maintain NADPH levels. A similar paraquat effect on radiolabeled glucose metabolism in rat lung slices was later reported by Rose et al. (*102*). These investigators also found that the activity of the pentose–phosphate pathway was increased in lungs of rats after intravenous administration of paraquat or diquat (a closely related bipyridylium herbicide capable of redox cycling but not accumulated into lung tissue). However, since only paraquat was toxic to the lung, it was concluded that the generation of free radicals, which was assumed from the stimulation of the pentose–phosphate pathway as a response to redox cycling, was not sufficient to cause cell toxicity. Nonetheless, the possibility was not excluded that radical generation in lung cells specifically accumulating paraquat could lead to toxicity.

To further characterize the potential role of NADPH depletion as a mechanism of paraquat toxicity, Witschi et al. (*55*) attempted to measure directly the concentrations of NADPH and NADP in lungs of rats treated with paraquat or diquat. Treatment with either bipyridylium compound produced similar decreases in the lung NADPH/NADP ratio, even though only paraquat persisted in lung tissue. Contrary to expectations, electron microscopic examination of the lung revealed that diquat was in fact toxic to the lung, although the lesion was much less pronounced that induced by paraquat and restricted to the Type I alveolar pneumocyte. Thus, it was concluded that there was no simple cause-effect relationship between the oxidation of NADPH and degree of lung damage. This conclusion was supported by the additional observation that simultaneous treatment with 100% oxygen enhanced both paraquat and diquat lethality, but did not alter the pattern of NADPH depletion.

Recently, several investigators have demonstrated that paraquat increased mixed disulfide concentrations in lung after *in vivo* treatment (*104*) and also in liver following *in vitro* perfusion (*105*). The mechanism of the increased mixed disulfide formation has been attributed to increased oxidized glutathione (GSSG) formation resulting from detoxification of lipid hydroperoxides (*104*) or from a decrease in NADPH which is necessary for maintenance of GSH in the reduced state (*105*). The formation of mixed disulfides has been suggested to result in alteration of the activity of several important cellular enzymes containing functional sulfhydryl groups, leading to potentially cytotoxic effects (*104,105*).

Summary

The pulmonary toxicity of paraquat is similar in many ways to the toxicity of several pulmonary toxins such as oxygen, nitrofurantoin, and bleomycin. The molecular properties of paraquat have made it a valuable tool for studying mechanisms of pulmonary injury that may be common to a number of agents. Although the mechanism of paraquat toxicity is not precisely understood, it remains clear that redox cycling is a critical event leading to cell injury. Further studies to characterize the consequences of intracellular redox cycling of xenobiotics will no doubt provide basic information regarding the role of this phenomena in development of toxicity.

REFERENCES

1. Heath, D., and Smith, P. The pathology of the lung in paraquat poisoning. In: Biochemical Mechanisms of Paraquat Toxicity (A. P. Autor, Ed.), Academic Press, New York, 1977, pp. 39–51.
2. Smith, P., and Heath, D. Paraquat. CRC Crit. Rev. Toxicol. 4: 411–445 (1976).
3. Kimbrough, R. D., and Gaines, T. B. Toxicity of paraquat to rats and its effect on rat lung. Toxicol. Appl. Pharmacol. 17: 679–690 (1970).
4. Smith, P., and Heath, D. The ultrastructure and time sequence of the early stages of paraquat lung in rats. J. Pathol. 114: 177–184 (1974).
5. Sykes, B. I., Purchase, I. F. H., and Smith, L. L. Pulmonary ultrastructure after oral and intravenous dosage of paraquat to rats. J. Pathol. 121: 233–241 (1977).
6. Popenoe, D. Effects of paraquat aerosol on mouse lung. Arch. Pathol. Lab. Med. 103: 331–334 (1979).
7. Kimbrough, R. D. Toxic effects of the herbicide paraquat. Chest 65: 655–675 (1974).
8. Smith, P., Heath, D., and Kay, J. M. The pathogenesis and structure of paraquat-induced pulmonary fibrosis in rats. J. Pathol. 114: 57–67 (1974).
9. Thurlbeck, W. M., and Thurlbeck, S. M. Pulmonary effects of paraquat poisoning. Chest 69: 2765–2805 (1976).
10. Vijeyaratnum, G. S., and Corrin, B. Experimental paraquat poisoning: a histological and electron-optical study of the changes in the lung. J. Pathol. 103: 123–129 (1971).
11. Kimbrough, R. D., and Linder, R. E. The ultrastructure of the paraquat lung lesion in the rat. Environ. Res. 6: 265–273 (1973).
12. Adamson, I. Y. R., Bowden, D. H., and Wyatt, J. P. Oxygen poisoning in mice. Ultrastructural and surfactant studies during exposure and recovery. Arch. Pathol. 90: 463–472 (1970).
13. Kapanci, Y., Weibel, E. R., Kaplan, H. P., and Robinson, F. R. Pathogenesis and reversibility of the pulmonary lesions of oxygen toxicity in monkeys. II. Ultrastructural and morphometric studies. Lab. Invest. 20: 101–118 (1969).
14. Stephens, R. J., Sloan, M. F., Evans, M. J., and Freeman, G. Alveolar type I cell response to exposure to 0.5 ppm O^3 for short periods. Exptl. Molec. Pathol. 20: 11–23 (1974).
15. Stephens, R. J., Freeman, G., and Evans, M. J. Early response of lung to low levels of nitrogen dioxide. Light and electron microscopy. Arch. Environ. Health 24: 160–179 (1972).
16. Adamson, I. Y. R., and Bowden, D. H. The pathogenesis of bleomycin-induced pulmonary fibrosis in mice. Am. J. Pathol. 77: 185–190 (1974).
17. Robertson, B., Grossman, G., and Ivemark, B. The alveolar lining layer in experimental paraquat poisoning. Acta Pathol. Microbiol. Scand. Sect. A. 84: 40–46 (1976).
18. Adamson, I. Y. R., Bowden, D. H., Cote, M. G, and Witschi, H. Lung injury induced by butylated hydroxytoluene. Lab Invest. 36: 26–32 (1977).
19. Michaelis, L., and Hill, E. S. The viologen indicators. J. Gen. Physiol. 16: 859–873 (1933).
20. Michaelis, L., and Hill, E. S. Potentiometric studies on semiquinones. J. Am. Chem. Soc. 55: 1481–1494 (1933).
21. Fisher, H. K., Clements, J. A., and Wright, R. R. Enhancement of oxygen toxicity by the herbicide paraquat. Am. Rev. Resp. Dis. 107: 246–252 (1973).
22. Thorneley, R. N. F. A convenient electrochemical preparation of reduced methyl viologen and a kinetic study of the reaction with oxygen using an anaerobic stopped-flow apparatus. Biochem. Biophys. Acta 333: 487–496 (1974).
23. Patterson, L. K., Small, R. D., Jr., and Scaiano, J. C. Reaction of paraquat radical cations with oxygen. A pulse radiolysis and laser photolysis study. Radiat. Res. 72: 218–225 (1977).
24. Gage, J. C. The action of paraquat and diquat on the respiration of liver cell fractions. Biochem. J. 109: 757–761 (1968).
25. Ilett, K. F., Stripp, B., Menard, R. H., Reid, W. D., and Gillette, J. R. Studies on the mechanism of the lung toxicity of paraquat: Comparison of tissue distribution and some biochemical parameters in rats and rabbits. Toxicol. Appl. Pharmacol. 28: 216–226 (1974).
26. Steffen, C., and Netter, K. J. On the mechanism of paraquat action on microsomal oxygen reduction and its relation to lipid peroxidation. Toxicol. Appl. Pharmacol. 47: 593–602 (1979).
27. Talcott, R. E., Shu, H., and Wei, E. T. Dissociation of microsomal oxygen reduction and lipid peroxidation with the electron acceptors, paraquat and menadione. Biochem. Pharmacol. 28: 665–671 (1979).
28. Montgomery, M. R. Interaction of paraquat with the pulmonary microsomal fatty acid desaturase system. Toxicol. Appl. Pharmacol. 36: 543–554 (1976).

29. Montgomery, M. R. Paraquat toxicity and pulmonary superoxide dismutase: an enzymic deficiency of lung microsomes. Res. Commun. Chem. Path. Pharmacol. 16: 155–158 (1977).
30. Baldwin, R. C., Pasi, A., MacGregor, J. T., and Hine, C. T. The rates of radical formation from the dipyridylium herbicides paraquat, diquat, and morfamquat in homogenates of rat lung, kidney and liver: an inhibitory effect of carbon monoxide. Toxicol. Appl. Pharmacol. 32: 298–304 (1975).
31. Bus, J. S., Aust, S. D., and Gibson, J. E. Superoxide- and singlet oxygen-catalyzed lipid peroxidation as a possible mechanism for paraquat (methyl viologen) toxicity. Biochem. Biophys. Res. Commun. 58: 749–755 (1974).
32. Kopaczyk-Locke, K. *In vitro* and *in vivo* effects of paraquat on rat liver mitochondria. In: Biochemical Mechanisms of Paraquat Toxicity (A. P. Autor, Ed.), Academic Press, New York, 1977, pp. 93–114.
33. Roussouw, D. J., and Engelbrecht, F. M. The effect of paraquat on the respiration of lung cell fractions. S. Afr. Med. J. 54: 1101–1104 (1978).
34. Roussouw, D. J., and Engelbrecht, F. M. The effect of paraquat on the aerobic metabolism of rabbit alveolar macrophages and lung fibroblasts. S. Afr. Med. J. 55: 20–23 (1979).
35. Bassett, D. J. P., and Fisher, A. B. Alterations of glucose metabolism during perfusion of rat lung with paraquat. Am. J. Physiol. 234: E653–E659 (1978).
36. Farrington, J. A., Ebert, M., Land, E. J., and Fletcher, K. Bipyridylium quaternary salts and related compounds. V. Pulse radiolysis studies of the reaction of paraquat radical with oxygen. Implications for the mode of action of bipyridyl herbicides. Biochim. Biophys Acta 314: 372–381 (1973).
37. Davies, D. A., and Davies, D. L. Effect of *d*-propranolol and superoxide dismutase on paraquat reduction and adrenochrome formation by rat liver microsomes. Fed. Proc. 33: 228 (1974).
38. Misra, H. P., and Fridovich, I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247: 3170–3174 (1972).
39. Sasame, N. A., and Boyd, M. R. Superoxide and hydrogen peroxide production and NADPH oxidation stimulated by nitrofurantoin in lung microsomes—possible implications for toxicity. Life Sci. 24: 1091–1096 (1979).
40. McCord, J. M., and Fridovich, I. Superoxide dismutase—an enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. 244: 6049–6055 (1969).
41. Butler, C., and Kleinerman, J. Paraquat in the rabbit. Brit. J. Ind. Med. 28: 67–71 (1971).
42. Clark, D. G., McElligott, T. F., and Hurst, E. W. The toxicity of paraquat. Brit. J. Ind. Med. 23: 126–132 (1966).
43. Dodge, A. D. The mode of action of the bipyridylium herbicides, paraquat and diquat. Endeavour 30: 130–135 (1971).
44. Hassan, H. M., and Fridovich, I. Regulation of synthesis of superoxide dismutase in *Escherichia coli*. J. Biol. Chem. 252: 7667–7672 (1977).
45. Hassan, H. M., and Fridovich, I. Superoxide radical and the enhancement of oxygen toxicity of paraquat in *Escherichia coli*. J. Biol. Chem. 253: 8143–8148 (1978).
46. Hassan, H. M., and Fridovich, I. Paraquat and *Escherichia coli*. Mechanism of production of extracellular superoxide radical. J. Biol. Chem. 254: 10846–10852 (1979).
47. Hassan, H. M., and Fridovich, I. Intracellular production of superoxide radical and of hydrogen peroxide by redox active compounds. Arch. Biochem. Biophys. 196: 385–395 (1979).
48. Mees, G. C. Experiments on the herbicidal action of 1,1′-ethylene-2,2′-dipyridylium dibromide. Ann. Appl. Biol. 48: 601–612 (1960).
49. Youngman, R. J., and Dodge, A. D. Mechanism of paraquat action: inhibition of the herbicidal effect by a copper chelate with superoxide-dismutating activity. Z. Naturforsch. 34: 1032–1035 (1979).
50. Lengfelder, E., and Elstner, E. F. Determination of the superoxide dismutating activity of *D*-penicillamine copper. Hoppe-Seyler's Z. Physiol Chem. 359: 751–757 (1978).
51. Bus, J. S., and Gibson, J. E. Postnatal toxicity of chronically administered paraquat in mice and interactions with oxygen and bromobenzene. Toxicol. Appl. Pharmacol. 33: 461–470 (1975).
52. Autor, A. P. Reduction of paraquat toxicity by superoxide dismutase. Life Sci. 14: 1309–1319 (1974).
53. Douze, J. M. C., and Van Heijst, A. N. P. The paraquat intoxication: oxygen a real poison. Acta Pharmacol. Toxicol. 41: 241–245 (1977).
54. Kehrer, J. P., Haschek, W. M., and Witschi, H. The influence of hyperoxia on the acute toxicity of paraquat and diquat. Drug Chem. Toxicol. 2: 397–408 (1979).
55. Witschi, H., Kacew, A., Hirai, K.-I., and Cote, M. G. *In vivo* oxidation of reduced nicotinamide-adenine dinucleotide phosphate by paraquat and diquat in rat lung. Chem.-Biol. Interact. 19: 143–160 (1977).
56. Deneke, S. M., and Fanburg, B. L. Normobaric oxygen toxicity of the lung. N. Engl J. Med. 303: 76–86 (1970).
57. Frank, L., and Massaro, D. Oxygen toxicity. Am. J. Med. 69: 117–126 (1980).
58. Hassan, H. M., and Fridovich, I. Superoxide dismutases: detoxication of a free radical. In: Enzymatic Basis of Detoxication, Vol. 1 (W. B. Jakoby, Ed.), Academic Press, New York, 1980, pp. 311–332.
59. Rhodes, M. L., Zavalla, D. C., and Brown, D. Hypoxic protection in paraquat poisoning. Lab. Invest. 35: 496–500 (1976).
60. Simons, R. S., Jackett, P. S., Carroll, M. E. W., and Lowrie, D. B. Superoxide independence of paraquat toxicity in *Escherichia coli*. Toxicol. Appl. Pharmacol. 37: 271–280 (1976).
61. Raffin, T. A., Simon, L. M., Douglas, W. H. J., Theodore, J., and Robin, E. D. The effects of variable O_2 tension and of exogenous superoxide dismutase on type II pneumocytes exposed to paraquat. Lab. Invest. 42: 205–208 (1980).
62. Davison, C. L., and Papirmeister, B. Bacteriostasis of *Escherichia coli* by the herbicide paraquat. Proc. Soc. Exptl. Biol. Med. 136: 359–364 (1971).
63. Fisher, H. K., and Williams, G. A. Paraquat is not bacteriostatic under anaerobic conditions. Life Sci. 19: 421–424 (1976).
64. Haber, F., and Weiss, J. The catalytic decomposition of hydrogen peroxide by iron salts. Proc. Roy. Soc. (London) A 147: 332–351 (1934).
65. McCord, J. M., and Day, E. D., Jr. Superoxide dependent production of hydroxyl radical catalyzed by iron-EDTA complex. FEBS Letters 86: 139–142 (1978).
66. Rigo, A., Stevenato, R., Finazzi-Agro, A., and Rotilio, G. An attempt to evaluate the rate of the Haber-Weiss reaction by using $OH^{\cdot}$ radical scavengers. FEBS Letters 80: 130–132 (1977).
67. Bus, J. S., and Gibson, J. E. Lipid peroxidation and its role in toxicology. Rev. Biochem. Toxicol. 1: 125–149 (1979).
68. Pederson, T. C., and Aust, S. D. The role of superoxide and singlet oxygen in lipid peroxidation promoted by xanthine oxidase. Biochem. Biophys. Res. Commun. 52: 1071–1078 (1973).
69. King, M. M., Lai, E. K., and McCay, P. B. Singlet oxygen production associated with enzyme-catalyzed lipid peroxidation in liver microsomes. J. Biol. Chem. 250: 6496–6502 (1975).
70. Svingen, B. A., O'Neal, F. O., and Aust, S. D. The role of superoxide and singlet oxygen in lipid peroxidation. Photochem. Photobiol. 28: 803–809 (1978).
71. Kornbrust, D. J., and Mavis, R. D. The effect of paraquat on microsomal lipid peroxidation *in vitro* and *in vivo*. Toxicol. Appl. Pharmacol. 53: 323–332 (1980).
72. Montgomery, M. R., and Niewoehner, D. E. Oxidant-induced alterations in pulmonary mixed-function oxidation: acute effects of paraquat and ozone. J. Environ. Sci. Health C13: 205–219 (1979).
73. Rose, M. S., Smith, L. L., and Wyatt, I. Evidence for energy-dependent accumulation of paraquat into rat lung. Nature 252: 314–315 (1974).
74. Shu, H., Talcott, R. E., Rice, S. A., and Wei, E. T. Lipid peroxidation and paraquat toxicity. Biochem. Pharmacol. 28: 327–331 (1979).
75. Pederson, T. C., Buege, J. A., and Aust, S. D. Microsomal

electron transport. The role of reduced nicotinamide adenine dinucleotide phosphate-cytochrome c reductase in microsomal lipid peroxidation. J. Biol. Chem. 248: 7134–7141 (1973).
76. Krieger, R. I., Lee, P. W., Black, A., and Fukuto, T. R. Inhibition of microsomal aldrin epoxidation by diquat and several related bipyridylium compounds. Bull. Contam. Toxicol. 9: 1–3 (1973).
77. Trush, M. A., Mimnaugh, E. G., Ginsburg, E., and Gram, T. E. *In vitro* stimulation by paraquat of reactive oxygen-mediated lipid peroxidation in rat lung microsomes. Toxicol. Appl. Pharmacol. 60: 279–286 (1981).
78. Trush, M. A., Mimnaugh, E. G., Ginsburg, E., and Gram, T. E. Studies on the *in vitro* interaction of mitomycin c, nitrofurantoin and paraquat with pulmonary microsomes; stimulation of reactive oxygen-mediated lipid peroxidation. Biochem. Pharmacol. 31: 805–814 (1982).
79. Hofsten, P. E., Hunter, F. E., Jr., Gibicki, J. M., and Weinstein, J. Formation of "lipid peroxide" under conditions which lead to swelling and lysis of rat liver mitochondria. Biochem. Biophys. Res. Commun. 7: 276–281 (1962).
80. Hunter, F. E., Gibicki, J. M., Hofsten, P. E., Weinstein, J., and Scott, A. Swelling and lysis of rat liver mitochondria induced by ferrous ions. J. Biol. Chem. 238: 828–835 (1963).
81. Brigelius, R., and Lengfelder, E. Influence of Cu-penicillamine on the paraquat toxicity: investigation of lipid metabolism in the isolated perfused rat liver. In: Metalloproteins: Structure, Function and Clinical Aspects (U. Weser, Ed.), Georg-Thieme Verlag, Stuttgart, 1979, pp. 150–156.
82. Bus, J. S., Aust, S. D., and Gibson, J. E. Lipid peroxidation: A possible mechanism for paraquat toxicity. Res. Commun. Chem. Path. Pharmacol. 11: 31–38 (1975).
83. Burk, R. F., Lawrence, R. A., and Lane, J. M. Liver necrosis and lipid peroxidation in the rat as the result of paraquat and diquat administration. J. Clin. Invest. 65: 1024–1031 (1980).
84. Cagen, S. Z., and Gibson, J. E. Liver damage following paraquat in selenium-deficient and diethyl maleate-pretreated mice. Toxicol. Appl. Pharmacol. 40: 193–200 (1977).
85. DiLuzio, N. R. Antioxidants, lipid peroxidation, and chemical induced liver injury. Fed. Proc. 32: 1875–1881 (1973).
86. Bus, J. S., Cagen, S. Z., Olgaard, M., and Gibson, J. E. A mechanism for paraquat toxicity in mice and rats. Toxicol. Appl. Pharmacol. 35: 501–513 (1976).
87. Chow, C. K., and Tappel, A. L. An enzymatic protective mechanism against lipid peroxidation damage to lungs of ozone exposed rats. Lipids 7: 518–524 (1972).
88. Reddy, K. A., Litov, R. E., and Omaye, S. T. Effect of pretreatment with antiinflammatory agents on paraquat toxicity in the rat. Res. Commun. Chem. Pathol. Pharmacol. 17: 87–100 (1977).
89. Witschi, H., and Côte, M. G. Primary pulmonary responses to toxic agents. CRC Crit. Rev. Toxicol. 5: 23–66 (1977).
90. Crapo, J. D., and Tierney, D. F. Superoxide dismutase and pulmonary oxygen toxicity. Am. J. Physiol. 226: 1401–1407 (1974).
91. Block, E. R., and Fisher, A. B. Hyperoxia and lung serotonin clearance: a new observation. Chest (Suppl.) 77: 289–291 (1977).
92. Steffen, C., Muliawan, H., and Kappus, H. Lack of *in vivo* lipid peroxidation in experimental paraquat poisoning. Naunyn-Schmiederberg's Arch. Pharmacol. 310: 241–243 (1980).
93. Omaye, S. T., Reddy, K. A., and Cross, C. E. Enhanced lung toxicity of paraquat in selenium-deficient rats. Toxicol. Appl. Pharmacol. 43: 237–247 (1978).
94. Ross, W. E., Block, E. R., and Chang, R.-Y. Paraquat induced DNA damage in mammalian cells. Biochem. Biophys. Res. Commun. 91: 1302–1308 (1979).
95. Giri, S. N., and Krishna, G. A. The effect of paraquat on guanylate cyclase activity in relation to morphological changes of guinea pig lungs. Lung 157: 127–134 (1980).
96. Goldberg, N. D., Haddox, M. K., Estensen, R., White, J. G., Lopez, C., and Hadden, J. W. Evidence of a dualism between cyclic GMP and cyclic AMP in the regulation of cell proliferation and other cell properties. In: Cyclic AMP in Immune Response and Tumor Growth (L. M. Lichtenstein and C. W. Parker, Eds.), Springer, Berlin, 1973, pp. 247–262.
97. Greenberg, D. B., Lyons, A., and Last, J. A. Paraquat-induced changes in the rate of collagen biosynthesis by rat lung explants. J. Lab. Clin. Med. 92: 1033–1042 (1978).
98. Hollinger, M. A., and Chvapil, M. Effect of paraquat on rat lung prolyl hydroxylase. Res. Commun. Chem. Pathol. Pharmacol. 16: 159–162 (1977).
99. Hussain, M. Z., and Bhatnagar, R. S. Involvement of superoxide in the paraquat-induced enhancement of lung collagen synthesis in organ culture. Biochem. Biophys. Res. Commun. 89: 71–76 (1979).
100. Thompson, W. D., and Patrick, R. A. Collagen prolyl hydroxylase levels in experimental paraquat poisoning. Brit. J. Exptl. Pathol. 9: 288–291 (1978).
101. Fisher, H. K., Clements, J. A., Tierney, D. F., and Wright, R. R. Pulmonary effects of paraquat in the first day after injection. Am. J. Physiol. 228: 1217–1223 (1975).
102. Rose, M. S., Smith, L. L., and Wyatt, I. The relevance of pentose phosphate pathway stimulation in rat lung to the mechanism of paraquat toxicity. Biochem. Pharmacol. 25: 1763–1767 (1976).
103. Smith, L. L., and Rose, M. S. Biochemical changes in lungs exposed to paraquat. In: Biochemical Mechanisms of Paraquat Toxicity (A. P. Autor, Ed.), Academic Press, New York, 1977, pp. 187–197.
104. Keeling, P. L., Smith, L. L., and Aldridge, W. N. The formation of mixed disulphides in rat lung following paraquat administration. Correlation with changes in intermediary metabolism. Biochim. Biophys. Acta 716: 249–257 (1982).
105. Brigelius, R., Lenzen, R., and Sies, H. Increase in hepatic mixed disulphide and glutathione disulfide levels elicited by paraquat. Biochem. Pharmacol. 31: 1637–1641 (1982).