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DISSOCIATION OF MICROSOMAL OXYGEN REDUCTION AND LIPID PEROXIDATION WITH THE ELECTRON ACCEPTORS, PARAQUAT AND MENADIONE*

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Abstract—Paraquat, diquat and menadione, electron acceptors which interact with the microsomal electron transport chain, were used to investigate the relationship between microsomal lipid peroxidation and microsomal oxygen reduction. All three compounds stimulated hydrogen peroxide production and the rate of superoxide production by mouse liver microsomes. However, while paraquat and diquat stimulated microsomal lipid peroxidation (2-fold in liver microsomes and 6- to 10-fold in lung microsomes), menadione was a potent inhibitor. Superoxide dismutase and catalase had no effect on paraquat-stimulated lipid peroxidation. Diquat, at concentrations sufficient to stimulate superoxide production, was unable to stimulate lipid peroxidation. Based on the above observations, a mechanism of paraquat- and diquat-initiated lipid peroxidation independent of superoxide and peroxide generation is proposed. The stimulatory effects of paraquat and diquat on lung microsomal lipid peroxidation are also discussed in relation to the lipid peroxidation hypothesis of paraquat lung toxicity.

The role of oxygen reduction in microsomal lipid peroxidation is incompletely understood. While neither superoxide nor hydrogen peroxide seems to be the proximal initiator of lipid peroxidation, both of these oxygen species may be obligatory intermediates, i.e. precursors of the initiating species [1–3]. To investigate further the role of oxygen reduction in microsomal lipid peroxidation, we studied superoxide, hydrogen peroxide and lipid peroxide production in microsomal assay mixtures supplemented with paraquat, diquat and menadione. The latter are electron acceptors which are reduced by the microsomal flavoprotein, NADPH-cytochrome reductase [4, 5], and are reoxidized by oxygen [6–8]. Contrary to the coupling between oxygen reduction and lipid peroxidation implicit in the above view [1–3], the results described in this report indicate that in the presence of paraquat and diquat, microsomal lipid peroxidation may not depend on concurrent oxygen reduction. Instead, we propose a mechanism based on the interaction of the paraquat free radical, iron, and lipid hydroperoxides. The importance of paraquat- and diquat-stimulated microsomal lipid peroxidation in liver and lung to the lipid peroxidation hypothesis of paraquat lung toxicity is also discussed.

MATERIALS AND METHODS

Adult male Swiss–Webster albino mice were used as the source of liver and lung microsomes for these studies. Diquat was a generous gift of Imperial Chemical Industries Ltd., Alderley Park, England. Other reagents were obtained from commercial sources.

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Preparation of microsomes

Mice were killed by cervical dislocation, and the livers or lungs were rapidly excised, perfused with cold 0.9% saline, and homogenized in 5 vol. of 1.15% KCl with a Polytron homogenizer. The microsomes were then isolated from the homogenates by differential centrifugation [9]. Liver microsomes were prepared by pooling tissues from four mice; six to twelve mice were used for preparing lung microsomes. The isolated liver and lung microsomes were resuspended in 1.15% KCl and the protein content was measured by a biuret procedure [10].

In vitro *assays*

Microsomal hydrogen peroxide production was assayed by following the oxidation of methanol to formaldehyde in the presence of NADPH and catalase [4, 5]. The incubation mixture for these assays consisted of 50 mM Tris–HCl buffer, pH 7.4, 0.2 M methanol, 1800 units catalase (Sigma Chemical Co., St. Louis, MO)/ml, 1 mM NADPH, 1 mg of microsomal protein/ml and various concentrations of electron acceptors. Since methanol can be oxidized directly by the microsomal ethanol-oxidizing system (MEOS), it was necessary to estimate the contribution of catalase-independent methanol oxidation. The catalase-independent rate was estimated by including a catalase inhibitor, 1 mM sodium azide, in the incubation mixture, and measuring residual formaldehyde production by the Nash procedure [11]. The difference between this rate and the rate obtained in the absence of azide was considered to be the endogenous rate of catalase-coupled methanol oxidation, which in turn was equivalent to the endogenous rate of microsomal hydrogen peroxide production. Stimulated rates of hydrogen peroxide production were obtained by subtracting the endogenous rate from the rates obtained in the presence of paraquat, diquat or menadione.

NADPH-dependent microsomal lipid peroxidation was assayed as previously described [9], by measuring the malondialdehyde formed as a result of the breakdown of peroxidized lipids [12]. In the malondialdehyde assay, liver or lung microsomes (0.5 or 0.75 mg protein/ml respectively) were incubated in a solution containing 50 mM Tris–HCl buffer, pH 7.4, 0.5 mM NADPH, 50μM $FePP_i$ and, in some cases, 200μM paraquat. Aliquots were withdrawn at various times and assayed for malondialdehyde content with the thiobarbituric acid procedure [12]. In other experiments, lipid peroxidation was assessed by spectral measurement of conjugated dienes in extracts of peroxidized microsomes. Incubation conditions for these assays were as described for the malondialdehyde assay. Lipids were extracted from aliquots of the incubation mixture (5–10 ml) pipetted into flasks containing 20 ml $CHCl_3$–methanol (2:1) at 0 and 20 min after NADPH addition. The extracted lipids were weighed and spectra were recorded with a Unicam 1750 spectrophotometer according to the method described by Recknagel and Ghoshal [13]. The ultraviolet absorption at 235 nm, characteristic of conjugated diene absorption [13], was considered to be proportional to the extent of lipid peroxidation.

The oxidation of epinephrine to adrenochrome, an index of microsomal superoxide generation [14], was followed with an Aminco DW-2 spectrophotometer in the dual wavelength mode. The incubation mixtures consisted of Tris–HCl buffer, pH 7.4, liver microsomes (1 mg protein/ml), 0.5 mM NADPH, 200μM epinephrine and various concentrations of paraquat, diquat or menadione. The change in absorbance with time at 480 minus 650 nm was recorded after the addition of NADPH at time zero for at least 10 min. In some experiments with diquat, superoxide production was monitored by following the production of the lactoperoxidase–superoxide complex III [15]. The incubation mixture for these spectophotometric assays consisted of liver microsomes (1 mg protein/ml) suspended in 50 mM Tris–HCl buffer, pH 7.4, 0.5 mM NADPH, 5μM lactoperoxidase (Sigma Chemical Co.), and in some cases, 50μM $FePP_i$ and various concentrations of diquat.

All assays performed in these studies were done at 37°.

RESULTS

Hydrogen peroxide, superoxide, and lipid peroxide production by liver microsomes incubated with electron acceptors

Table 1 summarizes the results of concentration dependence studies performed with three electron acceptors, paraquat, diquat and menadione, on three microsomal electron transport processes.

Hydrogen peroxide production. Saturating concentrations of each electron acceptor stimulated methanol oxidation 10-fold over the endogenous rate. Sodium azide completely abolished the stimulatory effects (not shown); thus, it may be concluded that the 10-fold increases in methanol oxidation rates were entirely due to increases in microsomal hydrogen peroxide production. The half-saturating stimulatory concentrations of paraquat, diquat and menadione, estimated by double reciprocal plots, were 50, 13 and 7μM respectively.

Superoxide production. A saturating concentration of each electron acceptor increases the steady state levels of superoxide 1.5- to 2-fold, as estimated from the linear, steady state phase of the adrenochrome production curves. The half-saturating stimulatory concentrations of paraquat, diquat and menadione were estimated to be approximately 6, 0.6 and 3μM respectively. In control experiments, superoxide dismutase was found to abolish the stimulatory effects.

Lipid peroxidation. Saturating concentrations of paraquat and diquat stimulated 2-fold the production of malondialdehyde, a breakdown product of lipid

Table 1. Effects of electron acceptors on microsomal oxygen reduction and lipid peroxidation in rat liver microsomes*

	Electron transfer reaction					
	Lipid peroxidation		Hydrogen peroxide production		Superoxide accumulation†	
Electron acceptor	Max. effect	Concn for $\frac{1}{2}$ max. (μM)	Max. effect	Concn for $\frac{1}{2}$ max. (μM)	Max. effect	Concn for $\frac{1}{2}$ max. (μM)
Paraquat	2 × Stim.	25	10 × Stim.	50	1.5 to 2 × Stim.	6
Diquat	2 × Stim.	14	10 × Stim.	13	1.5 to 2 × Stim.	0.6
Menadione	100% Inhib.	0.4	10 × Stim.	7	1.5 to 2 × Stim.	3

* Graded concentrations of paraquat, diquat or menadione were included in buffered incubation mixtures prepared for the assay of lipid peroxidation [9], hydrogen peroxide production [4], or superoxide production [15]. Lipid peroxidation was estimated by measuring the accumulation of malondialdehyde in aliquots of the incubation mixture, hydrogen peroxide was assayed by determining the rate of catalase-dependent methanol oxidation to formaldehyde, and superoxide accumulation was measured indirectly by the adrenochrome production assay [9, 4, 15]. Double reciprocal plots were used to estimate the half-maximal concentrations of paraquat, diquat amd menadione for the stimulation of hydrogen peroxide production, and, in the cases of paraquat and diquat, for the stimulation of lipid peroxidation. The half-maximal concentration of menadione required for inhibition of lipid peroxidation was estimated by interpolation of a per cent inhibition vs. menadione concentration curve. Half-maximal stimulatory concentrations of electron acceptor for superoxide production were estimated by interpolation: a family of adrenochrome kinetic progress curves was generated with each electron acceptor, using graded concentrations.

† Steady state levels, as estimated from the linear portion of adrenochrome kinetic progress curves.

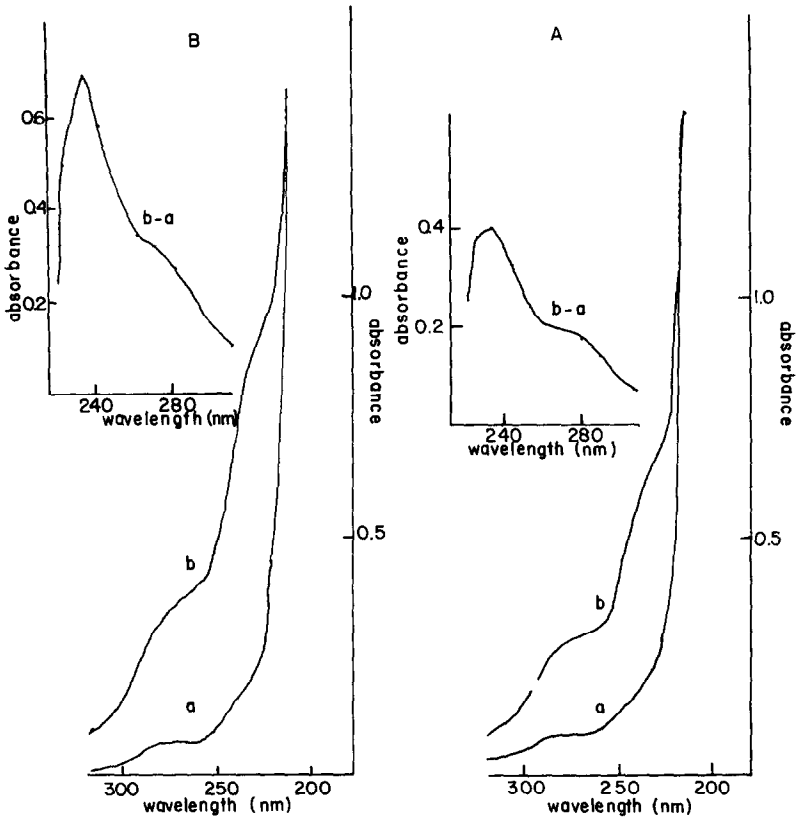

Fig. 1. Effect of paraquat on conjugated diene accumulation in liver microsomes. Liver microsomes, 0.5 mg protein/ml, were incubated at 37° in 50 mM Tris–HCl, pH 7.4, containing 0.5 mM NADPH, 50μ M $FePP_i$ with the addition (Fig. 1B) or without the addition (Fig. 1A) of 200μ M paraquat. Aliquots (5 ml) were withdrawn for lipid extraction at 0 and 20 min after NADPH addition. The lipids were extracted with 20 ml chloroform–methanol (2:1) as described by Recknagel and Ghoshal [13]. The ultraviolet absorption spectra of the extracted lipids (0.1 mg/ml in methanol) were obtained with a Unicam 1750 spectrophotometer. Curves a of Fig. 1A and 1B are the spectra of the lipids extracted from zero time aliquots; curves b are from the 20-min samples. The b minus a curves are the difference spectra resulting from 20 min of peroxidation in the absence (Fig. 1A) and presence (Fig. 1B) of 200 μM paraquat. The absorption maximum of these difference spectra, 235 nm, is characteristic of conjugated diene absorption, an index of the extent of lipid peroxidation [13].

Table 2. Effects of ferric pyrophosphate, catalase, superoxide dismutase, and sodium azide on paraquat-stimulated, NADPH-dependent lipid peroxidation *

Additions to complete system	Omissions from complete system	Malondialdehyde (nmoles/10 min/mg protein)
None	None	28.1 ± 2.58
None	$FePP_i$	0.4 ± 0.72†
None	Paraquat	15.7 ± 1.31‡
Catalase (1800 units/ml)	None	27.8 ± 1.80
Sodium azide (1 mM)	None	27.2 ± 2.17
Superoxide dismutase (10 μg/ml)	None	24.5 ± 3.28

* The complete lipid-peroxidizing system consisted of liver microsomes (0.5 mg protein/ml) suspended in 50 mM Tris–HCl buffer, pH 7.4, containing 0.5 mM NADPH, 50μ M $FePP_i$, and 200μ M paraquat. Incubations, carried out at 37°, were initiated by the addition of NADPH. Aliquots were withdrawn at 0 and 10 min for the determination of malondialdehyde [13]. Additions to, or omissions from, the complete system were as indicated in the table. Results are expressed as the mean ± S.E. for five preparations of microsomes.

† $P < 0.001$.

‡ $P < 0.05$.

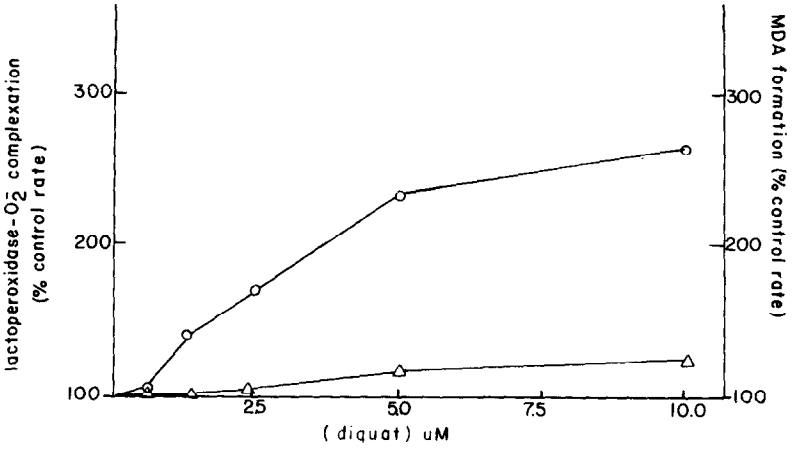

Fig. 2. Concentration dependence of diquat-stimulated lactoperoxidase–superoxide complexation and of diquat-stimulated malondialdehyde formation. The incubation mixture for these assays consisted of liver microsomes, 1 mg protein/ml, suspended in 50 mM Tris–HCl buffer, pH 7.4, containing 5μM lactoperoxidase, 0.5 mM NADPH (added at zero time), 50μM $FePP_i$, and various concentrations of diquat. To monitor lactoperoxidase–superoxide complexation, the change in absorbance at 589 minus 650 nm was recorded for 270 sec after the addition of NADPH. Malondialdehyde assays were done in Erlenmeyer flasks, shaken for 10 min in a 37° water bath. Circles: lactoperoxidase-superoxide complexation; and triangles: MDA formation.

peroxides. Menadione, on the other hand, completely inhibited the paraquat-stimulated malondialdehyde production. The half-maximal stimulatory concentrations of paraquat and diquat, and the half-maximal inhibitory concentration of menadione were estimated to be 25, 14 and 0.4μM respectively.

The paraquat-stimulated lipid peroxidation in liver microsomes assayed by malondialdehyde determination was confirmed by measurements of conjugated diene accumulation [13]. As shown in Fig. 1, the inclusion of paraquat in the assay mixture increased the accumulation of conjugated dienes 2-fold, as expected from the previous results with the malondialdehyde assays (Table 1).

Relationship between oxygen reduction and lipid peroxidation in liver microsomes

Inspection of the data in Table 1 indicates that submicromolar concentrations of diquat are sufficient to stimulate superoxide production, but not lipid peroxidation, suggesting that these two processes may not be tightly coupled in liver microsomes. Consistent with this suggestion is the observation that menadione was both a potent stimulator of superoxide production and a potent inhibitor of lipid peroxidation.

The provocative diquat results prompted further experiments to delineate more precisely the diquat concentration ranges effective for stimulation of superoxide production and lipid peroxidation. In these experiments, superoxide production was monitored by continuous recording of the absorption due to the superoxide–lactoperoxidase complex III [15]. In parallel incubations, diquat-stimulated lipid peroxidation was assayed in the presence of lactoperoxidase. The results of these experiments, depicted in Fig. 2, confirm the conclusion drawn from the initial experiments (Table 1): low concentrations of diquat, insufficient to stimulate microsomal lipid peroxidation, effectively stimulated superoxide formation.

In the case of paraquat, no clear separation of stimulatory thresholds was obtained in the initial experiments (Table 1). However, in further experiments, we obtained evidence dissociating oxygen reduction from the stimulation of lipid peroxidation by supplementing the paraquat-stimulated lipid-peroxidizing system with catalase, sodium azide, or superoxide dismutase. None of these additions affected the lipid peroxidation rate (Table 2), suggesting that neither superoxide nor hydrogen peroxide is obligatory in the paraquat-stimulated system. The one component which appears to be critical to paraquat-stimulated, NADPH-driven lipid peroxidation in liver microsomes is ferric pyrophosphate (Table 2). The omission of ferric pyrophosphate from the incubation mixture diminished malondialdehyde formation by 95 per cent, confirming for the paraquat-stimulated system the previously observed importance of iron in microsomal lipid peroxidation [16–18]. The role of iron in facilitating paraquat- or diquat-stimulated microsomal lipid peroxidation will be further considered in Discussion.

Table 3. Lipid peroxidation in lung microsomes incubated with paraquat or diquat*

Electron acceptor	Maximal effect	Concn for $\frac{1}{2}$ max. effect (μM)
Paraquat	6–10 × Stimulation	80
Diquat	6–10 × Stimulation	6

* Lung microsomes, 0.75 mg protein/ml, were incubated at 37° in the presence and absence of various concentrations of paraquat or diquat. The incubation mixture contained 50 mM Tris–HCl buffer, pH 7.4, 0.5 mM NADPH, and 50μM $FePP_i$. Aliquots of the incubation mixtures were withdrawn at various times and assayed for malondialdehyde content [12]. Half-maximal stimulatory concentrations were estimated by double reciprocal plots.

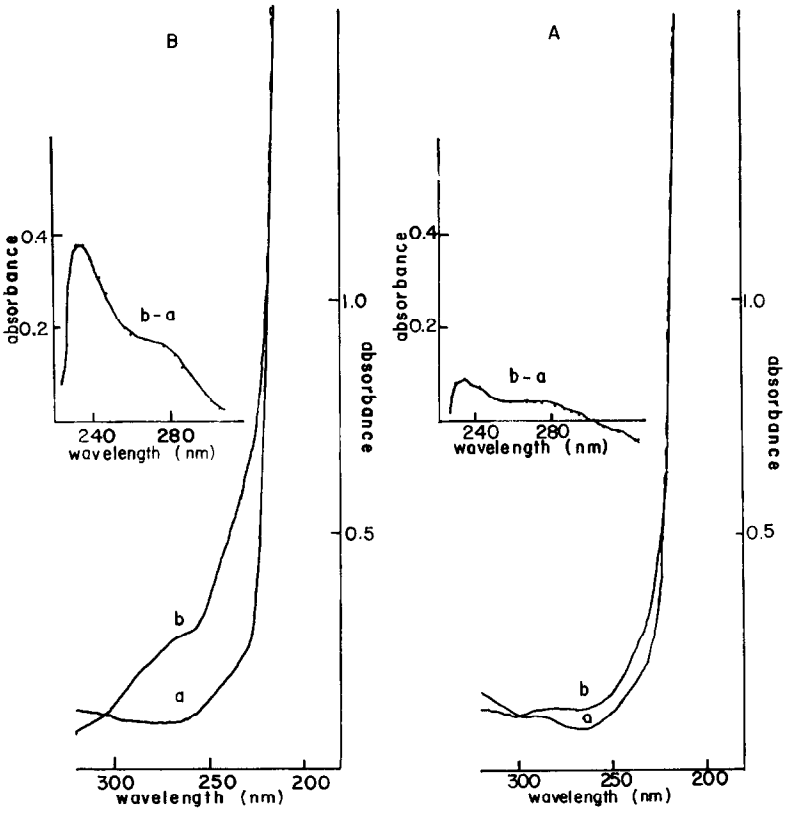

Fig. 3. Effect of paraquat on conjugated diene accumulation in lung microsomes. Lung microsomes (0.75 mg protein/ml) were incubated at 37° in the buffered mixture described in the legend to Fig. 1, with the addition (Fig. 3B) or without the addition (Fig. 3A) of 200μ M paraquat. Conjugated diene absorption spectra were obtained from extracted lipids as described in the legend of Fig. 1. The b minus a curves are the spectra corresponding to 20 min of lipid peroxidation, and were obtained with methanol solutions (0.25 mg/ml) of the extracted microsomal lipids.

Lipid peroxide production by mouse lung microsomes incubated with paraquat or diquat

In rodents, the target organ for paraquat toxicity, but not diquat toxicity, is considered to be the lung [19–21]. Therefore, it was of interest to determine the abilities of these two electron acceptors to stimulate lipid peroxidation in lung microsomes. Lipid peroxidation rates, as assessed by either the malondialdehyde assay or the measurement of conjugated dienes, were stimulated 6- to 10-fold when saturating concentrations of either paraquat or diquat were included in the assay mixture (Table 3 and Fig. 3). The half-maximal stimulatory concentrations were estimated to be 80μ M for paraquat and 6μ M for diquat (Table 3).

DISCUSSION

Studies with liver microsomes: possible mechanism of paraquat- and diquat-stimulated lipid peroxidation

While the requirement for iron in lipid peroxidation is well-established [16–18] and has been confirmed for the paraquat-supplemented system, the role of reduced oxygen is still open to question, especially in light of the data presented in Results. Fong *et al.* [2] have proposed a mechanism for the initiation of lipid peroxidation based on the interaction of ferric iron, superoxide, ferrous iron, and hydrogen peroxide. In this mechanism, superoxide reduces ferric iron; the resulting ferrous iron reductively cleaves hydrogen peroxide:

$$Fe^{3+} + O_2^{\cdot-} \rightarrow Fe^{2+} + O_2 \quad (1)$$

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

The hydroxyl radical generated in reaction 2 is hypothesized to initiate the lipid peroxidative chain reaction. If superoxide-dependent iron reduction is a prerequisite for the production of the initiating species, then superoxide dismutase would be expected to inhibit the initiation of lipid peroxidation. However, neither Fong *et al.* [2] Pederson and Aust [22], nor we (see Table 2) have been able to observe superoxide dismutase inhibition of microsomal lipid peroxidation. The suggestion that the superoxide is inaccessible to superoxide dismutase because of its inability to diffuse to the soluble enzyme seems unlikely in view of the observed reaction between superoxide and lactoperoxidase, also a soluble enzyme, and unlikely in view of the water solubility of the cofactors that stimulate lipid peroxidation (*vide infra*). Similarly, our data (Table 2) argue against the participation of hydrogen peroxide as a substrate for generating the ultimate reactive species involved in initiating lipid peroxidation, because the addition of excess catalase, or the azide inhibition of

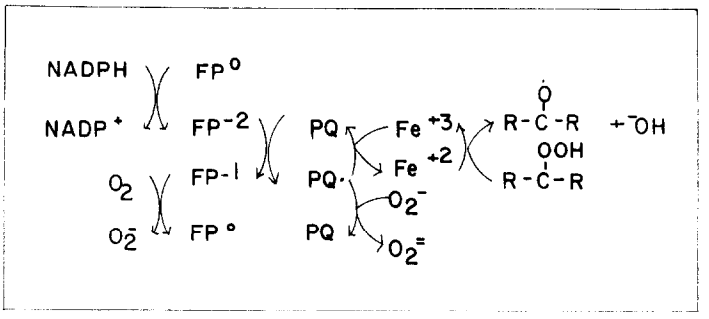

Fig. 4. Possible mechanism for paraquat- and diquat-initiated lipid peroxidation. FP is the flavoprotein, NADPH cytochrome *c* (P-450) reductase. FP° is the oxidized form of this enzyme, FP^{-2} is the fully reduced form, and FP^{-1}, the half-reduced semiquinone. The origin of the lipid hydroperoxides has not been established.

endogenous catalase, did not affect the rate of paraquat-stimulated lipid peroxidation.

However, the initiation sites of lipid peroxidation, unsaturated fatty acid side chains of phospholipids, may be sequestered within the hydrophobic matrix of the membrane, and the extent of their communication with the aqueous mixture is not known. It is possible that superoxide anion is generated in the hydrophobic region and is inaccessible to superoxide dismutase for a finite period of time. Microsomally generated H_2O_2 could be similarly sequestered in a catalase-insensitive hydrophobic pool and, if ferric pyrophosphate could enter this pool, lipid peroxidation might be initiated through a catalase-insensitive Haber–Weiss reaction [2]. However, in our hands, ferric pyrophosphate was freely soluble in the aqueous incubation mix, but insoluble in chloroform. Moreover, lipid-soluble ferric chloride did not initiate lipid peroxidation [17]. Together, these observations suggest a requirement for a water-soluble iron cofactor, and may indicate that peroxidation is initiated at the membrane–buffer interface. This observation is consistent with the observations that NADPH-cytochrome *c* reductase, a membrane-bound enzyme involved in lipid peroxidation [1], readily transfers electrons to the water-soluble pyridinium salts paraquat and diquat, which then stimulate lipid peroxidation if ferric pyrophosphate is present (Tables 1 and 2).

These observations are not consistent with an initiation mechanism strictly involving lipid-soluble components. On balance, it seems more likely that paraquat (or diquat) and ferric pyrophosphate react with the target fatty acids at the membrane–buffer interface, and, if superoxide anion and hydrogen peroxide participate in reactions with these water-soluble components, their participation should be eliminated by catalase and superoxide dismutase.

Our data are consistent with the hypothesis that paraquat or diquat stimulates lipid peroxidation through the interaction of iron, free radicals, and preformed lipid hydroperoxides independently of oxygen reduction:

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

$$Fe^{2+} + R{-}COOH \rightarrow R{-}CO\cdot + {}^{-}OH \quad (4)$$

Reaction 3, analogous to the iron reduction reaction proposed by Fong *et al.* [2] (reaction 1), substitutes the paraquat free radical for superoxide as the source of the electron for ferric iron. The feasibility of such a reaction was demonstrated with the dithionite-reduced paraquat free radical: Addition of ferric pyrophosphate to oxygen-free solutions of paraquat free radicals bleached the blue solution colorless*, indicating that the ferric pyrophosphate can oxidize the paraquat free radicals (unpublished observations). In reaction 4 we propose that ferrous iron formed in reaction 3 reductively cleaves endogenous lipid hydroperoxide to generate alkoxy radicals, the initiating species. Reaction 4 is analogous to the initiation event proposed by Fong *et al.* [2] where lipid hydroperoxide rather than hydrogen peroxide serves as the precursor of the initiating species. A mechanism involving ferrous iron and hydroperoxides, similar to reaction 4 above, has also been proposed by Noguchi and Nakano [18] as the initiation event for xanthine-induced lipoxygenation. Direct evidence for the involvement of endogenous hydroperoxides is currently lacking but the observation that addition of hydroperoxide to liver microsomes leads to rapid and substantial lipid peroxidation [23, 24] whereas hydrogen peroxide addition does not (unpublished observations), lends support to this possibility.

A diagram of the interaction of paraquat (and diquat) with the microsomal electron transport chain is presented in Fig. 4. In this scheme, a paraquat free radical, formed by electron transfer from NADPH cytochrome P-450 reductase (FP), has alternative fates: It may be oxidized either by superoxide, forming hydrogen peroxide [25], or it may be oxidized by ferric iron (reaction 3). The ferrous iron, derived from the interaction of the paraquat free radical and ferric iron, may then initiate lipid peroxidation by reductive cleavage of lipid hydroperoxides to alkoxy radicals (reaction 4). The latter event remains to be demonstrated directly, but the proposed mechanism (Fig. 4) seems highly plausible in view of the dependency of paraquat-stimulated lipid peroxidation on iron and the apparent absence of dependency on superoxide and hydrogen peroxide (Table 2).

NADPH may be required to maintain the iron cofac-

* In this set of experiments, solutions of dithionite-reduced paraquat (100 μM) were pipetted into tubes containing equal volumes of ferric pyrophosphate, 50–500 μM. All solutions were buffered at pH 7.5 with 0.1 M Tris–HCl. Bleaching of paraquat occurred instantaneously in the tubes containing ferric pyrophosphate greater than or equal to 100 μM.

tor in the ferrous oxidation state to ensure continual input of electrons into the peroxidizing system. The lipid hydroperoxides may be present endogenously as has been suggested [22] or they may be generated *in vitro* by an unspecified reaction with oxygen. If the latter is the case, NADPH may supply electrons for oxygen activation. The origin of lipid hydroperoxides remains to be investigated; the mechanism shown in Fig. 4 concerns iron dependent, paraquat- or diquat-facilitated, reactions that may occur after lipid hydroperoxides are formed.

Lipid peroxidation as a mechanism of paraquat lung toxicity

Paraquat, *in vivo*, causes severe pulmonary edema and hemorrhage with degeneration of the type I (alveolar epithelial) cells [19]. While lipid peroxidation has been proposed as a mechanism underlying the effects of paraquat on lung [20], the ability of paraquat to stimulate microsomal lipid peroxidation has been questioned [5]. In our preparations, paraquat proved to be an effective stimulator of microsomal lipid peroxidation in the presence of NADPH and ferric pyrophosphate (Table 2). Consistent with the organ specificity of paraquat, the stimulation was most pronounced in lung microsomes (Table 3).

Although the *in vitro* observations seem to support the lipid peroxidation hypothesis of paraquat lung toxicity, it must be noted that diquat, which is not highly toxic to lung [21], was also a potent stimulator of lung microsomal lipid peroxidation (Table 3). In fact, when considered on a molar basis, diquat was an order-of-magnitude more potent than paraquat in stimulating lipid peroxidation. This observation is difficult to reconcile with the lipid peroxidation hypothesis of paraquat lung toxicity even when one takes into account the greater ability of lung tissue to concentrate paraquat over diquat [26]. These results prompted a reinvestigation of the lipid peroxidation hypothesis of paraquat lung toxicity, to be described in a separate report [27].

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