Biochemical Pharmacology, Vol. 40, No. 7, pp. 1533–1539, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

AN EVALUATION OF THE REDOX CYCLING POTENCIES OF PARAQUAT AND NITROFURANTOIN IN MICROSOMAL AND LUNG SLICE SYSTEMS

AISHAH ADAM, LEWIS L. SMITH* and GERALD M. COHEN

Toxicology Unit, Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, U.K.; and *Biochemical Toxicology Section, ICI plc, Central Toxicology Laboratories, Alderley Park, Macclesfield, Cheshire SK10 4TJ, U.K.

(*Received* 20 *March* 1990; *accepted* 14 *May* 1990)

Abstract—The redox cycling abilities of the pulmonary toxins paraquat and nitrofurantoin have been compared with those of the potent redox cyclers, diquat and menadione in lung and liver microsomes by using the oxidation of NADPH and consumption of oxygen. The relative potencies of these compounds to undergo redox cycling were in the order: diquat ≃ menadione ≫ paraquat ≃ nitrofurantoin. This was partly attributed to the much lower affinity (K_m) of lung and liver microsomes for paraquat and nitrofurantoin than for diquat and menadione. The potential to redox cycle was assessed in an intact cellular system by determining the oxygen consumption of rat lung slices in the presence (10^{-6}, 10^{-5} and 10^{-4} M) or absence of each of the four substrates. At concentrations of paraquat (10^{-5} M) known to be accumulated by lung slices, a small but significant stimulation of lung slice oxygen uptake was observed. Nitrofurantoin (10^{-4}–10^{-6} M) did not affect lung slice oxygen uptake in lung slices, an observation consistent with its being a poor redox cycling compound, which is not actively accumulated into lung cells. This data has important implications in assessing the risk of exposure to paraquat. Low levels of paraquat would not be expected to cause lung damage because insufficient compound is present in the lung to exert its toxicity by redox cycling (due to the high K_m observed).

Cellular damage resulting from the injurious effects of free radicals may lead to a number of conditions including cancer, ageing and drug-induced toxicity [1–3]. It has been proposed that the pulmonary toxicities of the herbicide, paraquat [2, 4] and the antibiotic, nitrofurantoin [2, 5] are related to their ability to form free radicals which can react with molecular O_2 to form the superoxide anion radical ($O_2^{\cdot-}$). Superoxide anion radicals or other activated oxygen radical species formed from them, such as hydroxyl radicals ($OH^{\cdot}$ [6, 7] and singlet O_2 ($^1O_2$) [8] may damage cells by a number of mechanisms including peroxidation of polyunsaturated fatty acids [9, 10] or depletion of cellular reducing equivalents such as NADPH [11]. Furthermore, there may be a disturbance of cellular thiol status, either by depletion of intracellular reduced glutathione [12] or formation of protein-mixed disulphides. Protection against "oxidative stress" is normally afforded by cellular antioxidant defences such as superoxide dismutase, catalase, the glutathione peroxidase–glutathione reductase enzyme systems [4, 13], vitamin E [14] and vitamin C [15]. Cellular injury results when pro-oxidant effects exceed the antioxidant defence capability.

Paraquat (1,1′-dimethyl-4,4′-bipyridinium) has gained notoriety largely as a consequence of self-inflicted poisoning [16–18]. Lung damage is a prominent pathological feature and is often the primary cause of death of the patient [19]. Much work has been carried out to elucidate the mechanism of toxicity of paraquat in order to develop a specific antidote. However, the precise mode of toxic action is still contentious. Some details of the mechanism of toxicity have been clearly established. Paraquat is selectively accumulated into the lung compared with other tissues by an energy-dependent transport system which is probably present for the accumulation of endogenous polyamines [20, 21] and/or cystamine [22]. This accumulation occurs primarily in alveolar type I and II cells and in Clara cells [23] and these cell types are selectively damaged by paraquat in comparison with other lung cell types [23]. It is generally accepted that the critical biochemical event in paraquat toxicity is the cyclical one electron reduction and reoxidation of paraquat utilizing cellular reducing equivalents and producing superoxide anion radical [2, 4].

Patients treated for prolonged periods with nitrofurantoin (*N*-(5-nitro-2-furfurylidene)-1-aminohydantoin) have been reported to develop fatal lung fibrosis similar to that induced by paraquat [24, 25], which has led to the suggestion that both compounds produce lung damage through a similar mechanism [26]. Nitrofurantoin undergoes redox cycling under aerobic conditions with appropriate electron donors [27] but, unlike paraquat, it is not selectively accumulated into the lungs and it binds covalently to cellular macromolecules [28]. Although both compounds redox cycle in *in vitro* systems [26, 29], their redox cycling potencies have not been quantified. We have therefore determined the abilities of paraquat and nitrofurantoin to redox cycle in comparison to two other potent redox cycling compounds, diquat and menadione, in an attempt to relate redox cycling ability to toxicity.

Diquat (*N*,*N*′-ethylene-2,2′-bipyridinium), a herbicide structurally related to paraquat, produces minimal lung damage *in vivo* [30], probably due to its inability to be taken up by the pulmonary

polyamine uptake system and so accumulate in the lung [31]. Menadione [2-methyl-1,4-naphthoquinone] is a potent redox cycler with liver microsomes [32] and has been extensively utilized as an investigative tool in biological studies involving oxidative stress.

The role of redox cycling in mediating the toxicity of paraquat and nitrofurantoin has been widely investigated [4, 5]. Under appropriate experimental conditions, the free radical forms of both these compounds and activated O_2 species produced following their autoxidation have been demonstrated [27]. Rat liver and lung microsomes have been shown to mediate the one-electron reduction of paraquat, nitrofurantoin, diquat and menadione probably via NADPH-cytochrome P-450 reductase [10, 26, 33], and were used to study the redox cycling potencies of these compounds. However the ease by which they undergo redox cycling is unclear. We have determined the relative potencies of paraquat and nitrofurantoin to redox cycle in microsomal systems by measuring their utilization of O_2 and NADPH in comparison to two potent redox cycling compounds, diquat and menadione. Using liver or lung microsomes, the relative potencies of the compounds to undergo redox cycling were: diquat $\simeq$ menadione $\gg$ paraquat $\simeq$ nitrofurantoin. With lung slices only small increases in O_2 consumption were observed in the presence of any of these compounds indicating that such O_2 utilization comprised only a small percentage of total lung O_2 consumption.

MATERIALS AND METHODS

Nitrofurantoin was purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Menadione was obtained from Aldrich Chemical Co. (Gillingham, Dorset, U.K.). Paraquat and diquat were kindly provided by I.C.I. Plant Protection Division (Surrey, U.K.). Other chemicals were obtained either from the Sigma Chemical Co. or B.D.H. (Poole, Dorset, U.K.).

Preparation of microsomes. Washed hepatic and pulmonary microsomes were prepared from fed, male Wistar-derived Alderley Park rats, essentially by the method of Ernster *et al.* [34]. The organs were perfused with ice-cold 1.15% KCl, minced and homogenized with a Polytron homogeniser at full speed for 30 and 45 sec for liver and lung, respectively. The washed microsomal pellet was resuspended in Tris–EGTA buffer (100 mM Tris, 5 mM EGTA; pH 7.4). Microsomal protein was determined by the method of Lowry *et al.* [35].

NADPH oxidation. The oxidation of NADPH at 37° was followed at 340 nm on a Shimadzu MPS 2000 spectrophotometer as previously described [12]. Liver and lung microsomes were used at concentrations of 0.2 and 0.4 mg protein/mL, respectively. Paraquat and diquat were dissolved in buffer (100 mM Tris, 5 mM EGTA; pH 7.4), whilst nitrofurantoin and menadione were dissolved in dimethylsulphoxide (1%) (DMSO).

The activity of microsomal NADPH-cytochrome *c* reductase was determined spectrophotometrically by following the reduction of cytochrome *c* (Sigma type III) by NADPH at 550 nm on a Shimadzu MPS 200 spectrophotometer. The specific activity of the enzyme was calculated using an extinction coefficient of 19.6/mM/cm [36].

O_2 consumption. The microsomal consumption of O_2 was measured on a Yellow Spring Clark oxygen electrode maintained at 37°, as previously described [37]. Microsomes were used at a concentration of 0.2 mg protein/mL.

Rat lung slices and respirometry studies. Rat lung slices were prepared from fed, 180–200 g male, Wistar-derived, Alderley Park rats [38]. O_2 consumption of rat lung slices (35–45 mg wet wt) at 37° was measured using a Gilson respirometer [39]. Paraquat and diquat were dissolved in Krebs–Ringer phosphate buffer, pH 7.4, whilst nitrofurantoin and menadione were dissolved in 0.1% DMSO.

RESULTS

NADPH oxidation and O_2 consumption

The initial rates of rat liver and lung microsomal NADPH oxidation were stimulated by all four compounds. However, the stimulation produced by diquat and menadione was greater than that produced by paraquat and nitrofurantoin (Fig. 1a and b). Similarly, all four compounds stimulated the initial rates of rat liver microsomal O_2 consumption, with diquat and menadione producing the greatest stimulation (Fig. 2).

To determine the stoichiometry of these reactions, the amounts of NADPH oxidized and O_2 utilized by each of the four substrates were calculated after 1 and 5 min incubation. After 1 min, neither paraquat nor nitrofurantoin caused a greater than stoichiometric oxidation of NADPH (Fig. 1) and consumption of O_2 (Fig. 2). In contrast, menadione and diquat (5–10 μM), oxidized NADPH and utilized O_2 in amounts that were approximately 10-fold their concentration in the systems (Figs 1 and 2). However, after 5 min of incubation, both paraquat and nitrofurantoin (5–10 μM) utilized O_2 and oxidized NADPH in amounts approximately 4–5-fold greater than the concentration of substrate present in the incubation (Table 1). Diquat and menadione again caused similar disproportionate oxidation of NADPH and consumption of O_2 (Table 1).

Kinetic parameters

Lung and liver microsomal NADPH oxidation were studied in the presence of various concentrations of paraquat (20 μM–5 mM), diquat (10 μM–1 mM), nitrofurantoin (20 μM–100 μM) and menadione (2 μM–60 μM). K_m and V_{max} values were calculated from Lineweaver–Burk, Eadie–Hofstee, Hanes–Woolf and Direct Linear plots using a computer programme (ENZPACK) (Table 2). Values of K_m for paraquat and nitrofurantoin were of a similar order of magnitude and were about 20-fold greater than those for diquat and menadione. V_{max} values were either expressed per mg of microsomal protein or were standardized to a unit of specific activity of microsomal NADPH–cytochrome *c* reductase. V_{max} for nitrofurantoin in liver microsomes was lower than for the other compounds (Table 2).

O_2 consumption of lung slices

In order to obtain some indication of their ability

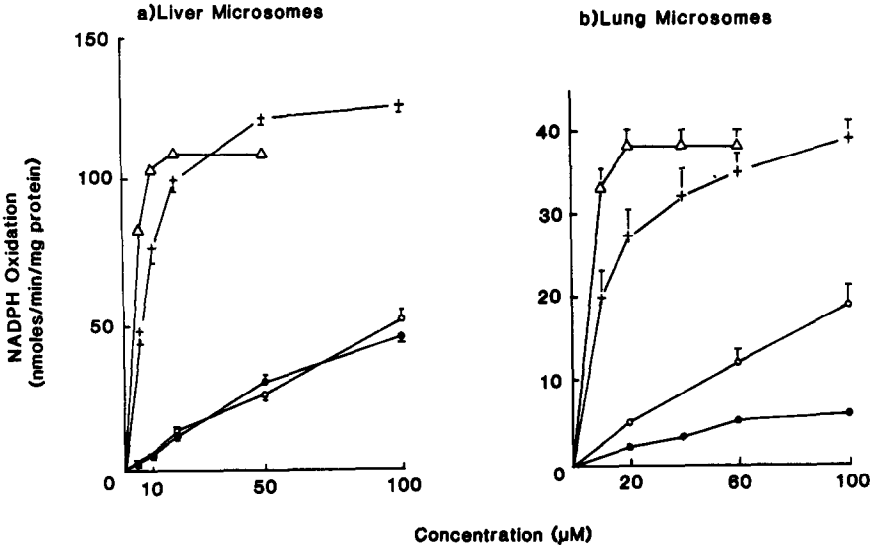

Fig. 1. Effect of substrates on initial rates of NADPH oxidation. Oxidation of NADPH by liver and lung microsomes incubated with paraquat (○——○), NF (●——●), DQ (+——+) and menadione (△——△) was followed at 340 nm at 37° for 1 min. Values shown have been corrected for basal rates of microsomal NADPH oxidation (12.17 ± 0.33 nmol NADPH/mg protein/min). Each point represents the mean ± SE of three experiments with three determinations per substrate concentration per experiment, except for menadione in liver microsomes and nitrofurantoin in lung microsomes.

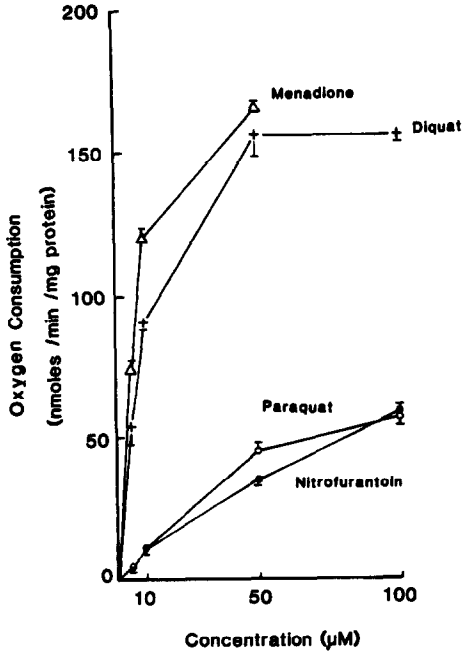

Fig. 2. Effect of substrates on initial rates of O_2 consumption. The initial rates of liver microsomal O_2 consumption incubated with paraquat (○——○), NF (●——●), DQ (+——+) and menadione (△——△) were determined at 37° for 1 min. Values shown have been corrected for basal rates of microsomal O_2 consumption (15.95 ± 0.53 nmol O_2/mg protein/min). Each point represents the mean ± SE of three separate experiments with two determinations per substrate concentration per experiment.

to redox cycle in a cellular system, the O_2 consumption of lung slices in the presence or absence of paraquat, diquat, nitrofurantoin or menadione was followed for 4 hr of incubation at 37°. In control lung slices, the rate of O_2 uptake was linear over this period (90–120 μL O_2 100/mg wet wt/hr).

A significant increase ($P < 0.01$) in the O_2 uptake of lung slices was observed with 10 μM paraquat (Fig. 3a). Although 100 μM paraquat also caused a stimulation in lung slice O_2 uptake, the increase observed was smaller (Fig. 3a). No significant increase in lung slice O_2 uptake was observed when slices were incubated with a lower concentration of paraquat (1 μM) (Fig. 3a). Nitrofurantoin (1–100 μM) did not produce any significant effects on lung slice O_2 consumption (Fig. 3b).

Low concentrations of menadione and diquat (1 μM and 10 μM) did not affect the lung slice O_2 uptake (Fig. 3c and d). However, 100 μM diquat produced a small, but significant increase ($P < 0.01$) in O_2 consumption after 1 hr (Fig. 3d). In contrast, 100 μM menadione produced a significant time-dependent depression ($P < 0.01$) in the O_2 uptake of lung slices when compared to controls (Fig. 3c).

DISCUSSION

Microsomal NADPH and O_2 utilization

Diquat and menadione elicited a nonstoichiometric increase in the utilization of NADPH and O_2 in liver and lung microsomal systems (Figs 1 and 2). In contrast, although both paraquat and nitrofurantoin stimulated microsomal NADPH oxidation and O_2 consumption, neither of the compounds caused a nonstoichiometric increase in the

Table 1. Amounts of NADPH and O_2 utilized after 5 min of substrate addition to liver microsomal systems

Substrate concentration (nmol)	nmoles NADPH oxidized/ mg protein/5 min				nmol O_2 consumed/ mg protein/5 min			
	PQ	NF	DQ	Md	PQ	NF	DQ	Md
5	22.6	20.1	187.7	246.2	27.3	27.7	200.8	204.8
10	42.4	38.6	244.6	259.6	44.2	16.9	302.3	341.9
20	76.9	65.8	273.9	244.2	ND	ND	ND	ND
50	128.3	129.2	280.8	236.1	134.0	104.5	443.4	485.5
100	198.4	193.5	273.3	ND	199.2	184.9	449.0	ND

ND: not determined.

Values shown have been corrected for the amount of NADPH oxidized in 5 min (53.9 ± 1.7 nmol NADPH/mg protein) and the amount of O_2 utilized in 5 min (81.6 ± 1.7 nmol O_2/mg protein) by liver microsomes in the absence of substrates. Each value represents the means of three different experiments.

Table 2. Kinetic parameters of microsomal NADPH oxidation with various substrates

Substrates	Rat liver microsomes			Rat lung microsomes		
	K_m (μM)	V_{max}*	V_{max}†	K_m (μM)	V_{max}*	V_{max}†
PQ	143 ± 13.7	148 ± 3	0.73 ± 0.02	198 ± 6.2	54 ± 0.9	0.79 ± 0.01
DQ	6.2 ± 0.7	124 ± 2.0	0.60 ± 0.02	17 ± 0.4	45 ± 0.3	0.88 ± 0.01
Menadione	5.1 ± 0.2	132 ± 1.5	1.27 ± 0.33	6.9 ± 0.4	45 ± 0.9	0.69 ± 0.01
NF‡	102.6 ± 19.4	53.2 ± 6.6	0.28 ± 0.03	ND	ND	ND

* V_{max}: nmol NADPH oxidized per mg microsomal protein per min.

† V_{max}: nmol NADPH oxidized per unit specific activity of NADPH–cytochrome *c* reductase.

‡ NF: values were obtained from extrapolated results. ND: not determined.

Results are means ± SE of four different methods of calculations (Lineweaver–Burk, Eadie–Hofstee, Hanes–Woolf and Direct Linear) on results from three different experiments per substrate used. The specific activity of NADPH–cytochrome *c* reductase for liver and lung microsomes was 198 ± 3.5 and 57 ± 5.8 nmol cytochrome *c* reduced/mg protein/min, respectively.

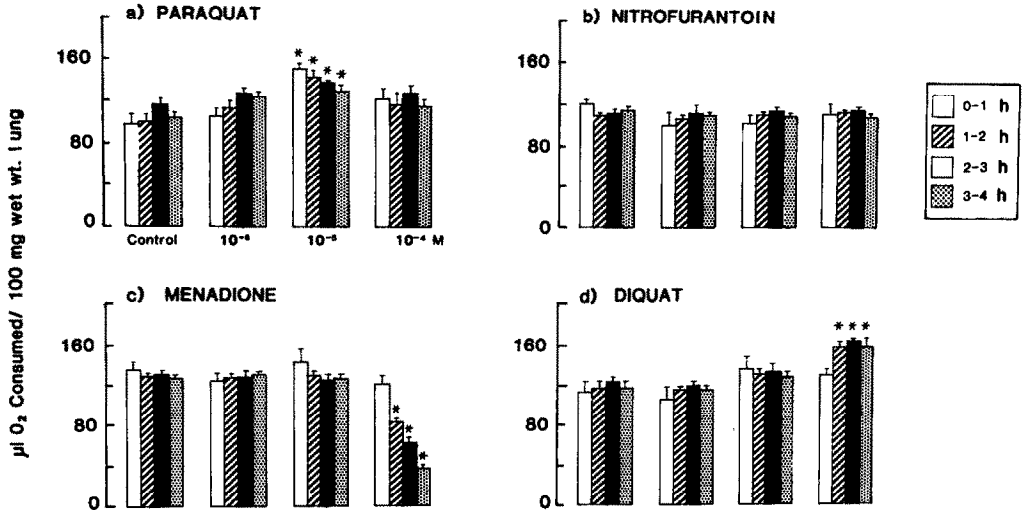

Fig. 3. O_2 consumption of rat lung slices. Lung slices were incubated with (a) paraquat, (b) nitrofurantoin, (c) menadione and (d) DQ at concentrations of 10^{-6}, 10^{-5} and 10^{-4} M at 37°. Control slices were incubated either in buffer or in 0.1% DMSO. Values indicate the hourly increment in O_2 consumption and represent the means of three experiments performed on six rats. *Significantly different from control ($P < 0.01$, *t*-test).

utilization of NADPH and O_2 after 1 min of incubation (Figs 1 and 2). However, after 5 min of incubation, paraquat and nitrofurantoin utilized a disproportionate amount of O_2 and NADPH when compared to the amounts of substrate present (Table 1). Thus it is apparent that in microsomal systems, the relative potency of the compounds to redox cycle was diquat ≃ menadione ≫ paraquat ≃ nitrofurantoin.

The poor redox cycling of paraquat and nitrofurantoin was accurately reflected in the kinetic parameters (Table 2). Values of K_m for diquat and menadione were comparable and much lower than for paraquat (in lung and liver microsomes) and nitrofurantoin (in liver microsomes) (Table 2). The K_m value, which reflects the affinity of NADPH–cytochrome *c* reductase for the substrate is a composite of two reactions; one-electron reduction of the substrate, utilizing NADPH, and probably catalysed by NADPH-cytochrome P-450 reductase and autoxidation of the reduced substrate to yield the parent compound with concomitant production of activated O_2 species. Since interaction of one-electron reduced forms of substrates with molecular O_2 is diffusion limited [2, 26, 40, 41], we can assume that the K_m values reflect the one-electron reduction reactions only. Thus, NADPH–cytochrome *c* reductase appears to have much lower affinities for paraquat and nitrofurantoin than for diquat and menadione. V_{max} values for the different substrates were comparable in both lung and liver microsomes, except for nitrofurantoin, which was lower (Table 2). Values of V_{max} for paraquat, diquat and menadione were similar in both lung and liver microsomes when expressed on the basis of NADPH–cytochrome *c* reductase activity (Table 2) because the activity of the pulmonary NADPH-cytochrome P-450 reductase is lower than that of the liver [42].

Lung slice O_2 consumption

In order to assess the potential contribution of the redox cycling to the toxicity of these compounds *in vivo*, the effects of the compounds on O_2 utilization was studied in lung slices, a model system which retained intact cellular structure. A significant increase in lung slice O_2 uptake was observed with 10^{-5} M paraquat but not with 10^{-6} M paraquat (Fig. 3a). As paraquat has been shown in the present study to be a poor redox cycler, high intracellular concentrations are necessary to produce a significant increase in lung slice O_2 uptake as a result of redox cycling. These would be achieved by active accumulation into susceptible cell types. In fact, with 10^{-6} M paraquat, rat lung slices have been shown to accumulate ten times the amount of paraquat intracellularly after 2 hr of incubation at 37° [43]. Thus, the stimulation in O_2 uptake observed with 10^{-5} M paraquat could be due to its redox cycling in susceptible cells. At higher concentrations, redox cycling may lead to toxicity, causing a decrease in O_2 consumption of lung slices incubated with 10^{-4} M compared to those incubated with 10^{-5} M paraquat.

Lung slices incubated with 10^{-6} M and 10^{-5} M menadione and diquat did not significantly affect lung slice O_2 uptake (Fig. 3c and d). However, lung slices incubated with 10^{-4} M diquat utilized significantly more O_2 than the controls. At this concentration, enough redox active diquat molecules may have passively diffused into susceptible cells to redox cycle without exceeding cellular defences (Fig. 3d). In contrast, menadione at the same concentration caused significant inhibition of lung slice O_2 uptake (Fig. 3c), probably due to toxicity to susceptible cells, either by redox cycling, and/or covalent binding to cellular macromolecules [12]. The inability of lower concentrations of menadione (10^{-6} and 10^{-5} M) to stimulate lung slice O_2 uptake may be due to toxicity of the quinone to lung cells, which may primarily involve a covalent binding, rather than a redox cycling mechanism.

Nitrofurantoin did not affect the lung slice O_2 uptake (Fig. 3b), an observation consistent with its being a poor redox cycler as well as it not being actively taken up into the lungs. However, other factors such as mitochondrial respiration, besides redox cycling may effect lung slice O_2 consumption. Nitrofurantoin has been shown to inhibit liver mitochondrial respiration [44], as well as to bind covalently to cellular macromolecules and these observations may also partially explain its apparent lack of effect on lung slice O_2 uptake.

Implications for risk assessment

The kinetic parameters that were established for the redox cycling of paraquat in the lung may have implications in defining the toxicity of paraquat and assessing the hazard that results when animals or man are exposed to small quantities of the herbicide. The data suggests that the intrinsic toxicity of paraquat is relatively low. This is particularly true when compared with the related dipyridyl, diquat, which, although not nearly as toxic to the lung, is quantitatively a much more effective redox cycling agent (compare K_ms for microsomal NADPH utilization between paraquat and diquat, Table 2). However, in lung slices paraquat causes a greater increase in O_2 consumption than diquat indicating that with intact lung slices it is more able to redox cycle. This is consistent with previous findings and illustrates the absolute importance of the selective uptake of paraquat into specific lung cells. Without this process, the lung would not be able to accumulate toxic levels of paraquat. The absence of a similar accumulation process for diquat is the most probable explanation as to why this herbicide, which is the more potent redox cycler, does not cause significant lung damage *in vivo*.

The K_m for NADPH oxidation by paraquat (198 μM) may also provide an insight for the reason why paraquat does not provoke insidious pulmonary toxicity in individuals exposed to low levels of the herbicide. Until the intracellular concentration of paraquat reaches concentrations that significantly reduce NADPH levels (and concomitantly generate O_2^-) no toxicity will be observed. The relatively high K_m found with paraquat for this process means that significant toxicity will only occur when the lung is able to accumulate these levels of paraquat from the plasma. This has been observed by others [45] since it can be calculated that unless a critical amount of paraquat is present in the plasma no toxicity will be produced. In other words, unless a threshold

concentration is reached in the lung, no damage results. This data provides some quantifiable evidence that this is the case since the damaging consequence of redox cycling will not be manifest until the intracellular concentration of paraquat is well into the micromolar range.

Acknowledgements—We should like to thank Mrs M. Fagg for the typing of the manuscript and Mr D. King for preparation of the figures.

REFERENCES

1. Mason RP, Harrelson WG, Kalyanaraman B, Mottley C, Peterson FJ and Holtzman JL, Free radical metabolites of chemical carcinogens. In: *Free Radicals, Lipid Peroxidation and Cancer* (Eds. McBrien DCH, Slater TF), pp. 377–400. Academic Press, London, 1982.
2. Kappus H and Sies H, Toxic drug effects associated with oxygen metabolism: redox cycling and lipid peroxidation. *Experientia* **37**: 1233–1358, 1981.
3. Trush MA, Mimnaugh EG and Gram TE, Commentary: Activation of pharmacologic agents to radical intermediates. Implications for the role of free radicals in drug action and toxicity. *Biochem Pharmacol* **31**: 3335–3346, 1982.
4. Smith LL, Rose MS and Wyatt I, The pathology and biochemistry of paraquat. Oxygen free radicals and tissue damage. *Ciba Found. Series* **65**- 321–314, 1979.
5. Boyd MR, Catignani GL, Sasame HA, Mitchell JR and Stiko AW, Acute pulmonary injury in rats by nitrofurantoin and modification by vitamin E, dietary fat, and oxygen. *Am Rev Respir Dis* **120**: 93–99, 1979.
6. McCord JM and Day Jr, ED, Superoxide-dependent production of hydroxyl radical catalyzed by iron–EDTA complex. *FEBS Lett* **86**: 139–142, 1978.
7. Halliwell B, Superoxide-dependent formation of hydroxyl radicals in the presence of iron chelates. *FEBS Lett* **92**: 321–326, 1978.
8. Khan AU, Singlet molecular oxygen from superoxide anion and sensitized fluorescence of organic molecules. *Science* **168**: 476–477, 1970.
9. Smith MT, Thor H and Orrenius S, The role of lipid peroxidation in the toxicity of foreign compounds to liver cells. *Biochem Pharmacol* **32**: 763–764, 1983.
10. Bus JS and Gibson JE, Lipid peroxidation and its role in toxicology. *Rev Biochem Toxicol* **1**: 125–149, 1979.
11. Keeling PL and Smith LL, Relevance of NADPH depletion and mixed disulphide formation in rat lung to the mechanism of cell damage following paraquat administration. *Biochem Pharmacol* **31**: 3243–3249, 1982.
12. Gant TW, Rao DNR, Mason RP and Cohen GM, Redox cycling and sulphydryl arylation: their relative importance in the mechanism of quinone cytotoxicity to isolated hepatocytes. *Chem Biol Interact* **65**: 157–173, 1988.
13. Frank L, Prolonged survival after paraquat. Role of the lung antioxidant enzyme systems. *Biochem Pharmacol* **30**: 2319–2324, 1981.
14. Tappel AL, Vitamin E and free radical peroxidation of lipids. *Ann NY Acad Sci* **203**: 12, 1972.
15. Matkovics B, Barabas K, Szabo L and Berencsi G, *In vivo* study of the mechanism of protective effects of ascorbic acid and reduced glutathione in paraquat poisoning. *Gen Pharmacol* **11**: 455–461, 1980.
16. Bullivant C, Accidental poisoning by paraquat: report of two cases in man. *Br Med J* **1**: 1272–1273, 1966.
17. Matthew H, Logan A, Woodruff MFA and Heard B, Paraquat poisoning—lung transplantation. *Br Med J* **3**: 759–763, 1968.
18. Burman D, Hodson AK and Mott MG, Paraquat poisoning. *Lancet* **1** (7743): 201, 1971.
19. Sykes BI, Purchase IFH and Smith LL, Pulmonary ultrastructure after oral and intravenous dosage of paraquat to rats. *J Path* **121**: 233–241, 1977.
20. Smith LL, The identification of an accumulation system for diamines and polyamines into the lung and its relevance to paraquat toxicity. *Arch Toxicol* **5**: 1–14, 1982.
21. Horton JK, Brigelius R, Mason RP and Bend JR, Paraquat uptake into freshly isolated rabbit lung epithelial cells and its reduction to the paraquat radical under anaerobic conditions. *Molec Pharmacol* **29**: 484–488, 1986.
22. Lewis CPL, Haschek WM, Wyatt I, Cohen GM and Smith LL, The accumulation of cystamine and its metabolism to taurine in rat lung slices. *Biochem Pharmacol* **38**: 481–488, 1989.
23. Wyatt I, Soames AR, Clay MF and Smith LL, The accumulation and localisation of putrescine, spermidine, spermine and paraquat in the rat lung. *Biochem Pharmacol* **37**: 1909–1918, 1988.
24. Israel KS, Brashear RE, Sharma HM, Yum MN and Glover JL, Pulmonary fibrosis and nitrofurantoin. *Am Rev Resp Dis* **108**: 353–357, 1973.
25. Kursh ED, Mostyn EM and Persky L, Nitrofurantoin pulmonary complications. *J Urol* **113**: 392–395, 1975.
26. Mason RP and Holtzman JL, The role of catalytic superoxide formation in the O_2 inhibition of nitroreductase. *Biochem Biophys Res Commun* **67**: 1267–1274, 1975.
27. Sasame HA and Boyd MR, Superoxide and hydrogen peroxide production and NADPH oxidation stimulated by nitrofurantoin in lung microsomes: possible implications for toxicity. *Life Sci* **24**: 1091–1096, 1979.
28. Boyd MR, Stiko AW and Sasame HA, Metabolic activation of nitrofurantoin—possible implications for carcinogenesis. *Biochem Pharmacol* **28**: 601–606, 1979.
29. Montgomery MR, Interaction of paraquat with the pulmonary microsomal fatty acid desaturase system. *Toxicol Appl Pharmacol* **36**: 543–554, 1976.
30. Conning DM, Fletcher K and Swan AAB, Paraquat and related bipyridyls. *Br Med Bull* **25**: 245–249, 1969.
31. Rose MS, Smith LL and Wyatt I, Evidence for energy-dependent accumulation of paraquat into rat lung. *Nature* **252**: 314–315, 1974.
32. Miller MG, Rodgers A and Cohen GM, Mechanisms of toxicity of naphthoquinones to isolated hepatocytes. *Biochem Pharmacol* **35**: 1177–1184, 1986.
33. Eklow-Lastbom L, Rossi L, Thor H and Orrenius S, Effects of oxidative stress caused by hyperoxia and diquat: a study in isolated hepatocytes. *Free Radic Res Commun* **2**: 601–606, 1986.
34. Ernster L, Siekevitz P and Palade GE, Enzyme structure relationships in the endoplasmic reticulum of rat liver: morphological and biochemical study. *J Cell Biol* **15**: 541–562, 1962.
35. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
36. Yonetani T, Studies on cytochrome *c* peroxidase. II. Stoichiometry between enzyme, H202 and ferrocytochrome *c* and enzymic determination of extinction coefficients of cytochrome *c*. *J Biol Chem* **240**: 4509–4514, 1965.
37. Thornalley PJ, d'Arcy Doherty M, Smith MT, Bannister JV and Cohen GM, The formation of active oxygen species following activation by 1-naphthol, 1,2- and 1,4-naphthoquinone by rat liver microsomes. *Chem Biol Interact* **38**: 195–206, 1984.
38. Rose MS, Smith LL 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.
39. Smith LL, Wyatt I and Rose MS, Factors affecting the efflux of paraquat from rat lung slices. *Toxicology* **19**: 197–207, 1981.
40. Farrington JA, Ebert M, Land EJ and Fletcher K, Bipyridylium quarternary 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.
41. Farrington JA, Ebert M and Land EY, Bipyridylium quarternary salts and related compounds. Pulse radiolysis studies of the reaction of paraquat radical analogues with oxygen. *J Chem Soc Faraday Trans 1* **74**: 665–675, 1978.
42. Matsubara T and Tochino Y, Electron transport systems of lung microsomes and their physiological functions. Intracellular distribution of oxidative enzymes in lung cells. *J Biochem* **70**: 981–991, 1971.
43. Rose MR, Lock EA, Smith LL and Wyatt I, Paraquat accumulation: tissue and species specificity. *Biochem Pharmacol* **25**: 419–423, 1976.
44. Lim LO, Bortell R and Neims AH, Nitrofurantoin inhibition of mouse liver mitochondrial respiration involving NAD-linked substrates. *Toxicol Appl Pharmacol* **84**: 493–499, 1986.
45. Hart TB, Paraquat—a review for safety in agricultural and horticultural use. *Human Toxicol* **6**: 13–18, 1987.