Phospholipid synthesis in isolated alveolar type II cells exposed *in vitro* to paraquat and hyperoxia

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Isolated alveolar epithelial type II cells were exposed to paraquat and to hyperoxia by gas diffusion through the thin Teflon bottom of culture dishes. After exposure, type II cells were further incubated in the presence of labelled substrates to assess their capacity to synthesize lipids. Hyperoxia alone (90% O₂; 5 h) had minor effects on lipid metabolism in the type II cells. At low paraquat concentrations (5 and 10 µM), hyperoxia enhanced the paraquat-induced decrease of [*Me*-¹⁴C]choline incorporation into phosphatidylcholines. The incorporation rates of [*Me*-¹⁴C]choline, [¹⁻¹⁴C]palmitate, [¹⁻¹⁴C]glucose and [^{1,3-3}H]glycerol into various phospholipid classes and neutral lipids were decreased by paraquat, depending on the concentration and duration of the exposure. The incorporation of [¹⁻¹⁴C]acetate into phosphatidylcholines, phosphatidylglycerols and neutral lipids appeared to be very sensitive to inactivation by paraquat. At 5 µM-paraquat the rate of [¹⁻¹⁴C]acetate incorporation was decreased to 50% of the control values. The rate of [¹⁻¹⁴C]palmitate incorporation into lipids was much less sensitive; it even increased at low paraquat concentrations. At 10 µM-paraquat both NADPH and ATP were significantly decreased. It is concluded that lipid synthesis in isolated alveolar type II cells is extremely sensitive to paraquat. At low concentrations of this herbicide, lipid synthesis, and particularly fatty acid synthesis, is decreased. The effects on lipid metabolism may be partly related to altered NADPH and ATP concentrations.

INTRODUCTION

Paraquat dichloride (1,1'-dimethyl-4,4'-bipyridilium dichloride; methyl viologen) is an effective and commercially important herbicide. It is very toxic to humans and animals, and the lung is the primary target organ (Rose *et al.*, 1974). The toxic effects of paraquat on the lung result in pulmonary oedema, fibrosis and haemorrhage. These events are secondary to damage of the alveolar epithelium (Smith & Heath, 1974; Keeling *et al.*, 1981). Morphological studies have indicated that alveolar epithelial type II cells are the cells that are affected first and most extensively by paraquat (Waddell & Marlowe, 1980; Keeling *et al.*, 1981; Hirai *et al.*, 1985). These cells accumulate paraquat by an energy-dependent process (Forman *et al.*, 1982; Horton *et al.*, 1986). It has been shown that paraquat is directly toxic to rat type II cells *in vitro* (Skillrud & Martin, 1984).

The epithelial alveolar type II cell is the main, if not the exclusive, producer of surfactant. This highly surface-active material lines the alveoli and is essential for normal lung function (Clements, 1977; Kikkawa & Smith, 1983). Impairment of the surfactant system produces respiratory distress comparable with that observed in the early stage of paraquat poisoning. This has led several investigators to postulate that the surfactant system is a target in paraquat toxicity. Because phospholipids, and particularly phosphatidylcholines, are quantitatively the major components of lung surfactant, most studies were devoted to investigate the effects of paraquat on the metabolism of phospholipids (for review, see Haagsman & Van Golde, 1985).

The results of these studies showed that paraquat alters phospholipid metabolism in whole lung (Fisher *et al.*, 1975; Malmquist, 1980; Engelbrecht *et al.*, 1981; Keeling *et al.*, 1982). So far no studies have been reported that dealt with the effects of paraquat on lipid metabolism in isolated alveolar epithelial type II cells. As type II cells are the primary target cells in paraquat poisoning and the producers of surfactant lipids, it was decided to study the effects of paraquat on phospholipid synthesis in isolated alveolar type II cells.

Exposure to paraquat has been advocated as a model for oxidant-initiated toxicity (Bus & Gibson, 1984). An impairment of surfactant production has been implicated as one of the initial effects leading to injury in response to hyperoxia (cf. Haagsman & Van Golde, 1985). Therefore, characterization of paraquat-induced alterations of type II cell metabolism may, in addition, provide useful information regarding the effects of hyperoxia on surfactant production by alveolar type II cells.

METHODS AND MATERIALS

Animals

Male Wistar rats (180–200 g; Central Institute for the Breeding of Laboratory Animals, Zeist, The Netherlands) were used in all experiments. They were fed *ad libitum* on a standard laboratory diet (RMH-B; Hope Farms, Woerden, The Netherlands) which contained 1670 mg of choline/kg dry wt.

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Isolation of type II cells

Alveolar type II cells were isolated by trypsin treatment, density-gradient centrifugation and differential adherence in primary monolayer culture (Mason, 1982). The cells were cultured for 18 h in plastic film dishes (Petriperm; Heraeus, Hanau, Germany) with a 25 μ m-thick Teflon bottom as described by Haagsman *et al.* (1985). The culture medium consisted of Dulbecco's modified Eagle medium supplemented with 10% (v/v) fetal bovine serum and 50 μ g of gentamycin/ml.

Exposure of type II cells to oxygen and paraquat

Before exposure, fresh medium without serum was added to the cells. Subsequently, the dishes were closed in such a way that no gas entered the dishes except by diffusion through the Teflon bottom. Culture dishes were placed in a 36-litre Perspex fumigation box at 37 °C and exposed to O₂/CO₂ (9:1). Control cells were exposed to N₂/O₂/CO₂ (36:9:5). Exposures were carried out at different concentrations of paraquat. Standard exposure time was 5 h. After the exposure, cells were stained and counted as described by Haagsman *et al.* (1985). Within each cell preparation different combinations of parameters were tested.

Incubation of type II cells with radioactive substrates

After exposure to a high O₂ concentration and/or paraquat, the type II cells, while still attached to the culture dishes, were rinsed three times with a standard medium consisting of 125 mM-NaCl, 5 mM-KCl, 2.5 mM-Na₂HPO₄, 2.5 mM-CaCl₂, 1.2 mM-MgSO₄, 17 mM-Hepes (pH 7.4) and 5.6 mM-glucose. Subsequently, cells were incubated for 2 h with radioactive substrates at 37 °C in 4 ml of standard medium supplemented with 0.1 mM-choline and 0.2 mM-palmitate (complexed to bovine serum albumin in the molar ratio 5.3:1) as described previously (Haagsman *et al.*, 1985). However, the incubations designed to study the metabolism of [*Me*-¹⁴C]choline were terminated differently. After those incubations, the cells were washed and scraped from the dishes in 2 × 2 ml of ice-cold methanol/water (5:4, v/v).

Analysis of phospholipids, intermediates and cofactors

After addition of lung lipid extract (0.2 μ mol of P), lipids were extracted as described by Sundler *et al.* (1974). The phospholipids were separated by two-dimensional t.l.c. on silica gel G plates impregnated with boric acid (Poorthuis *et al.*, 1976). Radiolabel in choline metabolites was determined as described previously (Post *et al.*, 1982), except that 3 μ mol of CDP-choline was added as carrier instead of betaine.

ATP was assayed after extraction of type II cells with 2 × 2 ml of 0.4 M-HClO₄/2 mM-EDTA (in freshly double-distilled water) by the luciferin/luciferase method (ATP bioluminescence CLS; Boehringer, Mannheim, Germany).

NADPH and NADP⁺ were assayed as described by Jørgensen & Rasmussen (1979). Type II cells were washed with standard medium and extracted with 1.2 ml of 0.1 M-KOH (for NADPH determinations) or 1.2 ml of 0.1 M-HCl (for NADP⁺ determinations). Internal standards were used to calculate recoveries. The extracts were transferred to Eppendorf vials, heated for 7 min at 60 °C, cooled on ice, neutralized, and, after an additional

10 min at 0 °C, centrifuged for 15 min at 12000 *g*. A 0.5 ml portion of the supernatant was taken for the assay.

Assay of cholinephosphate cytidyltransferase

Cholinephosphate cytidyltransferase (EC 2.7.7.15) was assayed as follows. Cells were scraped from the dishes (with 2 × 2 ml of standard medium) and centrifuged for 6 min at 145 *g*. Cells were homogenized in 0.22 ml of a solution containing 145 mM-NaCl, 10 mM-Tris/HCl (pH 7.4), 1 mM-EDTA and 10 mM-NaF with the aid of a 7 ml Dounce homogenizer (10 strokes with a loose pestle and 20 strokes with a tight pestle). The resulting homogenates were transferred to Eppendorf vials and centrifuged for 3 min at 12000 *g*. The supernatants were centrifuged for 5 min at 132000 *g* in a Beckman Airfuge to obtain a cytosolic and a microsomal fraction. Cytosolic or microsomal fractions (10–60 μ g of protein) were incubated at 37 °C in 100 μ l of medium of the following composition: 50 mM-Tris/succinate (pH 6.5), 10 mM-magnesium acetate, 5 mM-CTP (adjusted to pH 6.5 with Na₂CO₃), 1 mM-[*Me*-¹⁴C]choline phosphate (1 μ Ci/assay) and, with cytosolic fractions, 1 mM-phosphatidylglycerol (made from egg phosphatidylcholine). Assays were terminated after 20 min (cytosolic fractions) or 10 min (microsomal fractions) by boiling the incubation mixture for 2 min. After the addition of 2 μ mol of CDP-choline and centrifugation for 10 min at 2000 *g*, the supernatants were analysed by t.l.c. on silica gel G plates, with 0.5% NaCl/methanol/13.4 M-NH₃ (10:10:1, by vol.) as developing solvent. The formation of CDP-choline was linear with time and protein within the indicated range. Protein was determined as described by Lowry *et al.* (1951), with bovine serum albumin as standard.

Materials

[1-¹⁴C]Acetate, [1-¹⁴C]palmitate, D-[U-¹⁴C]glucose and [1,3-³H]glycerol were purchased from The Radiochemical Centre (Amersham, Bucks., U.K.). [*Me*-¹⁴C]Choline chloride was obtained from NEN Chemicals (Dreieichenhain, Germany). Silica gel was bought from Merck Chemicals (Darmstadt, Germany). Glutathione reductase, NADPH and NADP⁺ were purchased from Boehringer (Mannheim, Germany). Glucose-6-phosphate dehydrogenase and paraquat chloride were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other chemicals were supplied by J. T. Baker Chemical Co. (Deventer, The Netherlands).

Data presentation

Unless otherwise indicated, data given in each Table or Figure are from at least three different type II cell preparations. Values are expressed as means $\pm$ s.d., and are compared by ANOVA with Scheffe's procedure. A significance level of 95% was chosen.

RESULTS

Effects of hyperoxia and paraquat on choline metabolism

Type II cells were cultured in plastic film dishes with a 25 μ m Teflon bottom. In this way cells can be directly exposed to gases by diffusion. Exposure of type II cells for 5 h to 90% O₂ alone had no significant effect on

Table 1. Effect of paraquat on choline metabolism in alveolar type II cells

Type II cells in Dulbecco's medium were exposed to different concentrations of paraquat. The gas phase was either N₂/O₂/CO₂ (36:9:5) (control cells) or O₂/CO₂ (9:1) (treated cells). After the exposure choline metabolism was studied. Cells were incubated for 2 h in standard medium supplemented with 0.1 mM-[Me-¹⁴C]choline (18000 d.p.m./nmol) and 0.2 mM-palmitate (complexed to bovine serum albumin in the molar ratio 5.3:1). After the incubation the amount of label incorporated into choline phosphate, CDP-choline and phosphatidylcholine was determined: * indicates significant difference from control ($P < 0.05$); † indicates significant difference from paraquat (5 μ M) ($P < 0.05$).

Paraquat added	[Me- ¹⁴ C]Choline incorporation (nmol/h per 10 ⁶ cells)		
	Choline phosphate	CDP-choline	Phosphatidylcholine
None (control)	1.83 ± 0.24	0.064 ± 0.007	1.28 ± 0.10
5 μ M	2.56 ± 0.76	0.055 ± 0.004	1.11 ± 0.17
10 μ M	2.01 ± 0.18	0.055 ± 0.004	1.04 ± 0.23
20 μ M	1.57 ± 0.22†	0.058 ± 0.010	0.81 ± 0.26*†
50 μ M	1.09 ± 0.32†	0.053 ± 0.010	0.37 ± 0.17*†

phosphatidylcholine synthesis from labelled choline. In the presence of low concentrations (5 or 10 μ M) of paraquat, but not at 50 μ M-paraquat, hyperoxia (90% O₂; 5 h) enhanced the inhibitory effect of paraquat on the incorporation of [Me-¹⁴C]choline into phosphatidylcholines by 20–30% (results not shown). In the experiments described hereafter, the exposures of type II cells to paraquat were routinely performed at the high O₂ concentration to ensure maximal redox cycling of paraquat.

The incorporation of [Me-¹⁴C]choline into phosphatidylcholines was decreased by paraquat in a dose-dependent manner (Table 1). In contrast, in each experiment the amount of label recovered in choline phosphate after preincubation of cells with 5 μ M- and 10 μ M-paraquat was higher than in the controls. At higher paraquat concentrations, the amount of labelled choline phosphate was significantly lower (Table 1). The label in CDP-choline did not change significantly as a function of the paraquat concentration. These results may suggest that at low paraquat concentrations choline phosphate accumulates due to a decreased functional activity of cholinephosphate cytidylyltransferase. In two cell preparations the activity of this enzyme was assayed after paraquat exposure. In both experiments the cytosolic activity of the enzyme, assayed in the presence of 1 mM-phosphatidylglycerol, was higher (31% and 56% respectively) after exposure to 10 μ M-paraquat, whereas the activity was somewhat lower compared with the controls (12% and 22% respectively) after exposure to 50 μ M-paraquat. However, the increase in the cytosolic activity could not be matched with a decrease of microsomal activity, which has been suggested to be the functional activity of cholinephosphate cytidylyltransferase (Pelech & Vance, 1984).

Effect of hyperoxia and paraquat on the incorporation of other radiolabelled precursors into lipids

The incorporation of [1-¹⁴C]palmitate into phosphatidylcholine first increased as a function of the paraquat concentration (Fig. 1). The highest incorporation rate was consistently observed at 10 μ M-paraquat; at higher concentrations the rate decreased. These effects were not only observed when phosphatidylcholine synthesis was studied. Similar incorporation patterns were observed if the incorporation of [1-¹⁴C]palmitate into phosphatidyl-

Fig. 1. Effects of hyperoxia and paraquat on the incorporation of [1-¹⁴C]palmitate into lipids by type II cells

Cells were exposed for 5 h to 90% O₂ and various concentrations of paraquat. There were two controls: one exposed to 90% O₂ (indicated with 0 μ M-paraquat) and one with 18% O₂ (also without paraquat; indicated as C). After the exposure, cells were incubated for 2 h in standard medium in the presence of 0.2 mM-[1-¹⁴C]-palmitate (9000 d.p.m./nmol) and 0.1 mM-choline. The incorporation of label into phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylglycerols (PG) and neutral lipids (NL) was determined. The experiment was repeated twice with similar results.

ethanolamines, phosphatidylglycerols or neutral lipids was studied (Fig. 1).

Fig. 2. Effects of hyperoxia and paraquat on the incorporation of $[1-^{14}\text{C}]$ acetate into lipids by type II cells

Cells were exposed for 5 h to 90% O_2 and various concentrations of paraquat. The controls were as in Fig. 1. After the exposure, cells were incubated for 2 h in standard medium in the presence of 1 mM- $[1-^{14}\text{C}]$ acetate (4500 d.p.m./nmol), 0.1 mM-choline and 0.2 mM-palmitate. The incorporation of label into phosphatidylcholines (PC), phosphatidylglycerols (PG) and neutral lipids (NL) was determined. The experiment was repeated twice with similar results.

The incorporation of $[1-^{14}\text{C}]$ acetate (1 mM) into phosphatidylcholines, phosphatidylglycerols and neutral lipids was much more sensitive to hyperoxia and paraquat exposure. Hyperoxia (90%) for 5 h resulted in significantly lower incorporation rates of $[1-^{14}\text{C}]$ acetate into phosphatidylcholines, phosphatidylglycerols and neutral lipids. Inclusion of as little as 5 μM -paraquat in the exposure medium decreased the incorporation rate of $[1-^{14}\text{C}]$ acetate into phosphatidylcholines, phosphatidylglycerols and neutral lipids to about 50% of the control values (Fig. 2). Thus the rate of fatty acid synthesis is extremely sensitive to inactivation by hyperoxia and paraquat.

The incorporation of D- $[U-^{14}\text{C}]$ glucose into lipids was also studied as a function of the concentration of paraquat. Phosphatidylcholines and neutral lipids were separated and hydrolysed to determine the incorporation of label into the fatty-acyl moiety and glycerol backbone respectively. About 10% of the label incorporated into phosphatidylcholines and neutral lipids was recovered in the fatty-acyl moiety of the lipids (Fig. 3a). Although the incorporation into the glycerol backbone and the fatty-acyl moiety were both decreased by paraquat, the incorporation of D- $[U-^{14}\text{C}]$ glucose into the fatty-acyl

Fig. 3. Effect of paraquat on the incorporation of D- $[U-^{14}\text{C}]$ glucose and $[1,3-^3\text{H}]$ glycerol into lipids by type II cells

Cells were exposed for 5 h to 90% O_2 and various concentrations of paraquat. After the exposure, cells were incubated for 2 h in standard medium in either the presence of 5.6 mM-D- $[U-^{14}\text{C}]$ glucose (2000 d.p.m./nmol; a) or 0.1 mM- $[1,3-^3\text{H}]$ glycerol (120000 d.p.m./nmol; b) and 0.1 mM-choline and 0.2 mM-palmitate. The incorporation of label into phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylglycerols (PG) and neutral lipids (NL) was determined. The experiment was repeated twice with similar results.

moiety was more sensitive to paraquat (Fig. 3). This suggests that fatty acid synthesis is more sensitive to inactivation by hyperoxia and paraquat than glycolysis is. The incorporation of D- $[U-^{14}\text{C}]$ glucose into phosphatidylethanolamines and phosphatidylglycerols also decreased as a function of paraquat concentration.

The incorporation of $[1,3-^3\text{H}]$ glycerol into lipids was less susceptible to paraquat (Fig. 3b). Except for the incorporation into the fatty-acyl moiety (which was very low), the incorporation rates into the various lipids did not change, or were even slightly increased, after exposure to low concentrations of paraquat (Fig. 3b). However, at 20 μM -paraquat the incorporation rates of $[1,3-^3\text{H}]$ glycerol were lower, although the decrease was smaller than that measured for the incorporation rate of D- $[U-^{14}\text{C}]$ glucose.

Effects of hyperoxia and paraquat on NADPH, NADP^+ and ATP concentrations

Because paraquat is known to deplete NADPH by its cyclical reduction and re-oxidation (Bus & Gibson, 1984), the observed sensitivity of fatty acid synthesis may be a consequence of low NADPH concentrations. Fig. 4 shows that NADPH concentrations were indeed significantly lower after incubation of type II cells for 5 h in the presence of 10 μM -paraquat. Despite a possibly higher activity of the pentose phosphate pathway (Fisher

Fig. 4. Effect of paraquat on NADPH and NADP⁺ concentrations in type II cells

Cells were exposed for 5 h to 90% O₂ and various concentrations of paraquat. After the exposure NADPH and NADP⁺ concentrations were determined. Numbers of cell preparations are given in parentheses: * indicates significant difference from control ($P < 0.05$).

Table 2. Effect of paraquat on ATP concentrations in alveolar type II cells

Type II cells were incubated for 5 h in Dulbecco's medium with different concentrations of paraquat. The gas phase was either N₂/O₂/CO₂ (36:9:5) (control cells) or O₂/CO₂ (9:1) (treated cells). After the incubation, cells were scraped from the dishes in 2 × 2 ml of 0.4 M-HClO₄/2 mM-EDTA, and ATP was assayed. * indicates significant difference from control ($P < 0.05$); † indicates significant difference from O₂ ($P < 0.05$).

Treatment	ATP concentration (nmol/10 ⁶ cells)
Control	2.8 ± 0.8
O ₂	2.3 ± 0.9
O ₂ + paraquat (5 μM)	2.4 ± 0.8
O ₂ + paraquat (10 μM)	1.8 ± 0.7*†
O ₂ + paraquat (20 μM)	1.2 ± 0.6*†

& Reichert, 1984), type II cells were not able to maintain normal NADPH concentrations. At 50 μM-paraquat NADPH concentrations were very low. However, the concentration of NADPH + NADP⁺ was also decreased significantly compared with the controls, which indicates that at this concentration of paraquat breakdown or leakage of nicotinamide nucleotides may occur.

After exposure of type II cells to 10 μM-paraquat, ATP concentrations were significantly decreased (Table 2). At 20 μM-paraquat ATP concentrations were less than 50% of the control value. Thus exposure to low concentrations of paraquat results in an altered energy state of the type II cells, which in turn may seriously influence type II cell function.

Effects of exposure time to hyperoxia and paraquat on the incorporation of radiolabelled precursors into lipids

The uptake of paraquat by type II cells is proportional to the exposure time (Forman *et al.*, 1982; Horton *et al.*, 1986). Therefore, it was decided to investigate the effect of the duration of the exposure on the rate of lipid synthesis. Type II cells were exposed for various times to 20 μM-paraquat. After the exposure, the incorporation rate of radiolabelled precursors into lipids was determined. Fig. 5 shows that the inhibition of the rate of phosphatidylcholine synthesis from labelled choline depended on the duration of the preincubation with 20 μM-paraquat. The incorporation of [1-¹⁴C]palmitate into phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols and neutral lipids was also dependent on the exposure time to 20 μM-paraquat (Fig. 6). These results suggest that the amount of paraquat accumulated in type II cells may determine the effect on lipid metabolism. This effect may be exerted long after type II cells have taken up paraquat. Again, lipid synthesis from [1-¹⁴C]acetate was the most sensitive process to inactivation by paraquat. A 1 h preincubation with 20 μM-paraquat resulted in a decrease in the rate

Fig. 5. Incorporation of [Me-¹⁴C]choline into phosphatidylcholines as a function of the preincubation time in the presence of paraquat

Type II cells were exposed for various times to 90% O₂ and 20 μM-paraquat. After the exposure, cells were incubated for 2 h in standard medium in the presence of 0.1 mM-[Me-¹⁴C]choline (10000 d.p.m./nmol) and 0.2 mM-palmitate, and the incorporation of label into phosphatidylcholines was determined. The experiment was repeated twice, with similar results: ○, control; ●, paraquat-treated.

Fig. 6. Incorporation of $[1-^{14}\text{C}]$ palmitate into lipids as a function of the preincubation time in the presence of paraquat

Type II cells were exposed for various times to 90% O_2 and 20 μM -paraquat. After the exposure, cells were incubated for 2 h in standard medium in the presence of 0.2 mM- $[1-^{14}\text{C}]$ palmitate (9000 d.p.m./nmol) and 0.1 mM-choline and the incorporation of label into phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylglycerols (PG) and neutral lipids (NL) was determined. The experiment was repeated twice, with similar results: $\circ$, control; $\bullet$, paraquat-treated.

of synthesis of phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols and neutral lipids, to about 50% of the control values (Fig. 7).

DISCUSSION

The widely used herbicide paraquat causes severe lung damage, and its lethality is markedly enhanced by exposure to high concentrations of O_2 (Fisher *et al.*, 1973; Douze & Van Heijst, 1977; Keeling *et al.*, 1981; Bus & Gibson, 1984). Studies *in vivo* suggest that surfactant lipid synthesis is decreased by paraquat (Fisher *et al.*, 1975; Malmquist, 1980; Engelbrecht *et al.*, 1981; Keeling *et al.*, 1982), and that the alveolar epithelial type II cell is the specific target in paraquat toxicity (Waddell & Marlowe, 1980; Keeling *et al.*, 1981; Hirai *et al.*, 1985). Paraquat may be used as a model for oxidant-initiated toxicity (Bus & Gibson, 1984). In some studies, isolated alveolar type II cells were used to investigate the uptake of paraquat (Forman *et al.*, 1982; Horton *et al.*, 1986) and paraquat-induced injury (Skillrud & Martin, 1984). One of the important functions of type II cells is the production of surfactant

Fig. 7. Incorporation of $[1-^{14}\text{C}]$ acetate into lipids as a function of the preincubation time in the presence of paraquat

Type II cells were exposed for various times to 90% O_2 and 20 μM -paraquat. After the exposure, cells were incubated for 2 h in standard medium in the presence of 1 mM- $[1-^{14}\text{C}]$ acetate (4500 d.p.m./nmol), 0.1 mM-choline and 0.2 mM-palmitate, and the incorporation of label into phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylglycerols (PG) and neutral lipids (NL) was determined. The experiment was repeated twice, with similar results: $\circ$, control; $\bullet$, paraquat-treated.

lipids. In one study the A-549 cell line was employed to investigate the effects of paraquat on lipid metabolism *in vitro*. However, rather high concentrations of paraquat (1 mM) and long exposure times (36 h) were used (Saito *et al.*, 1982). So far, no studies had been carried out in which isolated alveolar type II cells were used to investigate the effects of paraquat on lipid metabolism. In the present paper we describe effects of low concentrations of paraquat on lipid metabolism in isolated type II cells. In order to achieve exposure of the cells in close proximity to the gas phase, type II cells were cultured in plastic-film dishes with a 25 μm Teflon bottom. This model was used previously to expose type II cells to ozone (Haagsman *et al.*, 1985; Haagsman & Van Golde, 1985).

The metabolism of $[Me-^{14}\text{C}]$ choline is altered by paraquat in a dose-dependent manner (Table 1). At low concentrations of paraquat (5 and 10 μM) the amount of label recovered in choline phosphate was higher than in the controls, whereas the label in phosphatidylcholines was decreased (Table 1). This may be explained by decreased functional activity of cholinephosphate cytidyltransferase. The increased cytosolic activity observed in two experiments could be in line with the incorporation data, because the functional activity has been suggested to reside in the microsomal fraction (Pelech & Vance, 1984). However, no significant

decrease was observed in the microsomal activity. More work needs to be done to investigate whether the functional activity of cholinephosphate cytidyltransferase is indeed altered by exposure to low paraquat concentrations. At higher paraquat concentrations the incorporation of [*Me*-¹⁴C]choline into phosphatidylcholine decreased further, but also the recovery of label in choline phosphate decreased. These effects may be explained by lower ATP concentrations observed at high paraquat (20 μ M) concentrations; these may be too low to ensure a maximal rate of the choline kinase-catalysed reaction. By using a type II cell volume of 440 μ m³ (Young *et al.*, 1981) and neglecting compartmentation, the calculated cellular ATP concentration of control cells is 6.4 mM. However, after treatment with 20 μ M-paraquat the concentration falls to 2.7 mM, which is below the K_m of choline kinase for ATP (3 mM) observed for the enzyme from whole lung (Ulane *et al.*, 1978). The lower rate of phosphatidylcholine synthesis may also be a consequence of a decreased flux through the diacylglycerol path. In ozone toxicity the rate of lipid synthesis is severely depressed, owing to a decreased activity of glycerolphosphate acyltransferase (Haagsman *et al.*, 1985). This enzyme, which catalyses the first acylation of glycerol 3-phosphate, is localized for the most part in the endoplasmic reticulum in type II cells and is very susceptible to inactivation by oxidation (Batenburg *et al.*, 1986). Unlike in ozone toxicity, the activity of glycerolphosphate acyltransferase is not altered by exposure to paraquat, not even at high concentrations (results not shown). These results are in line with the hypothesis that the ozone-induced decrease in glycerolphosphate acyltransferase activity is due to a direct oxidation of this enzyme by ozone (Haagsman *et al.*, 1985).

Exposure of type II cells to low concentrations of paraquat results in an increased incorporation rate of [¹⁴C]palmitate into phosphatidylcholines (Fig. 1). The increased incorporation rate may be due to a decreased supply of fatty acids synthesized *de novo*. The rate of fatty acid synthesis, as monitored by the incorporation of [¹⁴C]acetate, is very sensitive to inactivation by paraquat. A concentration of 5 μ M-paraquat decreases the rate of fatty acid synthesis, and the incorporation of these newly synthesized fatty acids into lipids, to about 50% of the control values (Fig. 2). This suggests that acetyl-CoA formation from acetate, or fatty acid synthesis itself, is affected by paraquat, because the use of [¹⁴C]acetate eliminates the effect of changes in pyruvate formation. The incorporation rate of D-[U-¹⁴C]glucose into the fatty-acyl moiety of phosphatidylcholines and neutral lipids is more sensitive to inactivation by paraquat than the incorporation into the glycerol moiety of these lipids (Fig. 3). These results indicate that fatty acid synthesis is the most sensitive process in lipid synthesis to inactivation by paraquat. Because NADPH is essential for fatty acid synthesis, the observed effect of paraquat on fatty acid synthesis may be the result of the observed decrease in the NADPH/NADP⁺ ratio after exposure of type II cells to paraquat (Fig. 4). Measurements of enzyme activities of fatty acid-synthesizing enzymes after paraquat exposure are required to assess whether fatty acid synthesis itself is directly affected by paraquat exposure. The decreased rate of fatty acid synthesis in rat lung slices after treatment of the animals with paraquat has been

attributed to the formation of mixed disulphides between protein thiol groups and glutathione (Keeling *et al.*, 1982).

The incorporation of [1,3-³H]glycerol into the glycerol backbone of the glycerolipids is less affected by paraquat than the incorporation of D-[U-¹⁴C]glucose into the glycerol backbone. This suggests that label entering glycerolipids after phosphorylation of [1,3-³H]glycerol is less diluted by triose phosphate derived from glycolysis. Fisher & Reicherter (1984) showed that the glycolytic rate in type II cells does not change significantly after exposure to 3 mM-paraquat for 1.5 h. Thus a lesser degree of dilution is probably not a consequence of a change in the flux through the triose phosphate pool, but rather is due to a lower rate of conversion because of the more oxidized state of the cell.

The effect of paraquat on lipid metabolism is dependent on the duration of the exposure of type II cells to this herbicide (Figs. 5, 6 and 7). This indicates that the intracellular concentration of paraquat determines the observed effects on type II cell metabolism, because the uptake of paraquat is time-dependent (Forman *et al.*, 1982; Horton *et al.*, 1986). Time-dependent effects on type II cells have also been observed when cellular injury by 100 μ M-paraquat was studied after 4, 8 and 16 h exposure (Skilrud & Martin, 1984). The incorporation of [1-¹⁴C]acetate into lipids was again the most sensitive process (Fig. 7). After 1 h incubation in the presence of 20 μ M-paraquat, the rate of fatty acid synthesis and incorporation of fatty acids synthesized *de novo* into lipids decreased to 50% of the control values.

At very low concentrations the herbicide paraquat is able to interfere with type II cell metabolism. One of the important functions of the type II cell is to synthesize surfactant lipids. Lipid metabolism is affected by paraquat at low concentrations. Therefore it is conceivable that impairment of the surfactant system is, at least partially, responsible for the observed pulmonary effects after paraquat intoxication. The results of the present study with isolated type II cells strongly suggest that paraquat is not a satisfactory model of oxidant stress in general. Although paraquat may exert its cytotoxicity by virtue of its capacity to generate superoxide (Bagley *et al.*, 1986), or by the formation of mixed disulphides (Keeling *et al.*, 1982), type II cell metabolism may be affected primarily by the oxidation of reducing equivalents (e.g. NADPH). Studies using type II cells loaded with superoxide or catalase are needed to delineate the mechanisms via which paraquat affects lipid metabolism in type II cells.

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