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THE ENHANCEMENT OF PARAQUAT TOXICITY IN RATS BY 85% OXYGEN: LETHALITY AND CELL-SPECIFIC LUNG DAMAGE

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Summary.—When rats were dosed s.c. with paraquat or diquat and then exposed to air or 85% oxygen, the lethality of paraquat was enhanced approximately 10-fold by 85% oxygen exposure, whereas the lethality of diquat was enhanced only 2-fold. This increase in toxicity was not caused by an increase in the lung concentration of either bipyridyl.

The lungs of rats which had been dosed with paraquat (2·5 and 20 mg/kg) or diquat (10 and 20 mg/kg) and exposed to air or 85% oxygen were examined morphologically at various times up to 24 h after dosing. By 24 h after dosing, the extent of damage appeared to be generally similar for those doses of paraquat that killed the same proportion of animals when combined with air or 85% oxygen. The combination of 20 mg paraquat/kg and air exposure caused alveolar epithelial Type I and Type II cell damage. Following 2·5 mg paraquat/kg and 85% oxygen exposure or 20 mg diquat/kg and 85% oxygen exposure the Type II alveolar epithelial cells were more severely damaged than the Type I epithelial and endothelial cells. In contrast, there was no cell damage after 1 or 2 days of exposure to 85% oxygen alone, and when lung damage did develop after 4 days of exposure, it was the capillary endothelial cells which were primarily affected. Thus the toxic effects of paraquat to the Type II alveolar epithelial cells are enhanced by exposure to oxygen.

The ability of the lung to accumulate paraquat was measured in lung slices that had been taken from rats dosed with paraquat or diquat and exposed to air or 85% oxygen for 2, 8 or 24 h. Paraquat accumulation was inhibited at times after dosing when the alveolar epithelial cells appeared to be damaged. This is consistent with the hypothesis that paraquat is primarily accumulated by the alveolar epithelial cells.

We have concluded that (i) the toxic effects of paraquat to the alveolar epithelial cells of the lung are markedly enhanced when paraquat-treated rats are exposed to 85% oxygen, and (ii) the combination of low concentrations of paraquat (2·5 mg/kg) and 85% oxygen or high concentrations of diquat (20 mg/kg) and 85% oxygen damages the Type II alveolar epithelial cells.

THE BIPYRIDYL HERBICIDE PARAQUAT (1,1′-dimethyl-4,4′-bipyridylium) is toxic to both man and experimental animals (Clark, McElligott and Hurst, 1966). When given systemically to rats, several organs are damaged, the lung being the most severely affected, and death generally results from anoxia (Clark *et al.*, 1966; Clark and Hurst, 1970). One of the therapeutic measures for anoxia in human cases of paraquat poisoning is the administration of air supplemented with additional oxygen. However, it has been shown that the lethality of paraquat to rats is markedly enhanced by exposure to 40, 60, 80 and 100% oxygen (Fisher, Clements and Wright, 1973; Douze and Van Heijst, 1977; Kehrer, Haschek and Witschi, 1979).

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Morphological studies of the lungs of rats poisoned with paraquat or oxygen have shown that these agents primarily damage different cell types in the lung. Following paraquat poisoning the alveolar epithelial Type I and Type II cells are the first cells damaged (Vijeyaratnam and Corrin, 1971; Smith and Heath, 1974; Sykes, Purchase and Smith, 1977), whereas following oxygen poisoning the capillary endothelial cells are primarily damaged (Kistler, Caldwell and Weibel, 1967; Schaffner, Felig and Trachtenberg, 1967). Although other authors have shown that oxygen exposure accelerates the development of lung damage caused by paraquat (Fisher *et al.*, 1973; Kehrer *et al.*, 1979) the pathogenesis and cell types primarily affected by the interaction are not known. In the study presented here we have examined the possibility that the cause of enhanced paraquat toxicity was due to alveolar epithelial-cell damage by paraquat, and capillary endothelial-cell damage by oxygen. The development of lung cell damage was characterized at various times up to 24 h after dosing rats with paraquat or the related bipyridyl diquat combined with exposure to air or 85% oxygen.

Diquat was used in these studies because this chemically related herbicide has similar chemical and physical properties to paraquat, but produces only minimal lung damage during air exposure (Clark and Hurst, 1970; Witschi *et al.*, 1977), although its toxicity is enhanced by oxygen exposure (Kehrer *et al.*, 1979). Finally, we have also used these treatments to examine the hypothesis (Smith, Lock and Rose, 1976) that the active accumulation of paraquat by the lung (Rose, Smith and Wyatt, 1974) is reduced in lung slices from rats with damaged alveolar epithelial cells.

MATERIALS AND METHODS

Materials.—Oxygen (99·9% pure) was obtained from Air Products Limited, Walkden, Lancashire, U.K. Paraquat dichloride (1,1′-dimethyl-4,4′-bipyridylium) and diquat dichloride monohydrate (1,1′-ethylene-2,2′-bipyridylium) (both 99·9% pure) were obtained from I.C.I. Limited, Plant Protection Division, Jealott's Hill Research Station, Berkshire, U.K. Radiolabelled compounds [methyl-^{14}C] paraquat (30 mCi/mmol), [ethylene-^{14}C]-diquat (30 mCi/mmol) were purchased from the Radiochemical Centre, Amersham U.K. Halothane B.P. (Fluothane) was obtained from I.C.I. Limited, Macclesfield, Cheshire, U.K.

Krebs–Ringer phosphate (KRP) buffer (pH 7·4) contained NaCl (130 mM), KCl (5·2 mM), $CaCl_2$ (1·9 mM), $MgSO_4$ (1·29 mM), Na_2HPO_4 (10 mM) and glucose (11 mM). The chemicals were all obtained from B.D.H. Chemicals Limited, Poole, Dorset, U.K.

Treatment of animals.—Male, Wistar-derived, Alderley Park strain specific-pathogen-free rats (190–230 g body wt) were used for all experiments. They were maintained in an air-conditioned animal room with a 12 h light–dark cycle, and had access to food and water unless otherwise stated. The rats were dosed s.c. (2·0 ml/kg body wt) with saline or with the required amount of paraquat or diquat and immediately exposed to air or 85% oxygen (10 l/min flow rate, at 21–25°) for the required period of time in perspex exposure chambers (volume 60 l for all experiments except lethality studies—volume 330 l). In order to measure the concentration of bipyridyl in the lung, the rats were dosed (s.c.) with the required amount of paraquat or diquat containing ^{14}C-labelled paraquat or diquat (20 μCi/kg). Oxygen concentrations and temperature were recorded continuously using a Beckman Fieldlab or 0260 oxygen analyser (Beckman Instruments Inc., Fullerton, U.S.A.). The relative humidity was recorded continuously using a Shaw 4-way autoscan hygrometer (Shaw Instruments Ltd, Bradford, Yorkshire, U.K.).

Lethality studies.—After each treatment (1, 2·5, 5, 10, 15 or 20 mg paraquat ion/kg, or 5, 10 or 20 mg diquat ion/kg) the rats were exposed to air or 85% oxygen for 10 days. Deaths were recorded daily. LD_{50} values were determined by logit analysis (Finney, 1971).

Lung paraquat of diquat concentration.—After each treatment (saline, 1, 2·5, 5, 10, 15 or 20 mg paraquat ion/kg, or 10 or 20 mg diquat ion/kg) and exposure to air or 85% oxygen, the rats were killed with halothane at 2, 8 or 24 h after dosing. The lungs and heart were quickly removed and the lungs were perfused with 10 ml KRP buffer (at room temperature) *via* the pulmonary artery. The 3 smallest right lobes (anterior, middle and azygous) were removed and after drying at 100° were weighed and dissolved in 2 ml Soluene 350 (Packard Instrument Company, Caversham, U.K.) containing 0·5 ml water. The dissolved sample was mixed with 20 ml Dimilume scintillator (Packard

Instrument Company) and radioactivity was measured by liquid scintillation counting. Internal standards were used to correct for quenching. Since (i) paraquat and diquat are not metabolized by the rat (Daniel and Gage, 1966), and (ii) the amount of bipyridyl in tissues is similar when measured using analytical procedures or using ^{14}C-labelled paraquat or diquat, the lung bipyridyl concentrations can be calculated using the specific activity of the dosing solution.

Lung morphology studies.—After each treatment (saline, 2·5 or 20 mg paraquat ion/kg, or 10 or 20 mg diquat ion/kg) and exposure to air of 85% oxygen, the rats (5 animals per group) were killed with halothane at 1, 2, 4, 8, 12, 16 or 24 h after dosing. The thoracic cavity was opened and a cannula inserted into the trachea and tied in place with a surgical thread. Fixative (Modified Karnovskys—mixed formaldehyde/glutaraldehyde—McDowell and Trump, 1976) was instilled into the lungs until they just filled the thoracic cavity. A slice of tissue for electron microscopy was taken from the middle of the left lobe from all animals except the 2·5 mg/kg paraquat/85% oxygen groups at 16 and 24 h and the 20 mg/kg paraquat/85% oxygen animals at 12 h. These groups showed focal macroscopic lesions on most or all of the lung lobes and a sample for electron microscopy was taken to include one or more of these lesions. No macroscopic abnormalities were found in other groups. The tissue was diced into 1 mm cubes, post-fixed in buffered osmium tetroxide fixative (pH 7·3) and dehydrated using graded alcohols and epoxy propane before embedding in epoxy resin. Sections 1 μm thick, stained with toluidine blue, were examined under the light microscope, and suitable areas were selected for further study in the electron microscope. These areas (approximately 0·5 mm × 1 mm) generally included a terminal bronchiole with surrounding alveolar tissue. If the 1 μm sections showed evidence of alveolar-wall abnormalities under the light microscope, these areas were also studied by electron microscopy. Animals showing evidence of changes at the light-microscopy level were restricted to those exposed to 85% oxygen for 4 days, those receiving 2·5 mg/kg paraquat and exposed to oxygen for 16 or 24 h, 20 mg paraquat/kg in oxygen for 12 h, 20 mg paraquat/kg in air for 24 h and one animal receiving 20 mg diquat/kg and placed in oxygen for 16 h. For all rats studied the remainder of the lung was processed and embedded in paraffin wax, sectioned at 5–6 μm and stained with haematoxylin and eosin for further evaluation with the light microscope.

^{14}C paraquat accumulation by lung slices.—After each treatment (saline, 1, 2·5, 5, 10, 15 or 20 mg paraquat ion/kg, or 20 mg diquat ion/kg) and exposure to air or 85% oxygen, the rats were killed with halothane at 2, 8 or 24 h after dosing (see above). The lungs and heart were quickly removed, and the lungs were perfused with 10 ml KRP buffer (at room temperature) *via* the pulmonary artery. The left lobe was chopped into 0·6 mm thick slices using a McIlwain tissue chopper (Mickle Laboratory Engineering Company Limited, Guildford, Surrey, U.K.). Approximately 80 mg of these slices were placed in 3 ml KRP buffer in a 25 ml conical flask at 37°. After 30 min pre-incubation, shaking at 140 strokes per min, paraquat (final concentration 10^{-5}M containing 0·1 μCi of ^{14}C-paraquat) was added and the incubation was continued for a further 60 min. The slices were removed from the medium, blotted on filter paper to remove excess fluid, and weighed. The slices were then dissolved in 1 ml Soluene, and 10 ml Dimilume scintillator (Packard Instrument Company) was added. Radioactivity was measured by liquid scintillation counting. A 100 μl aliquot of the incubation medium was made up to 1·0 ml with water, 10 ml Instagel added and the radioactivity measured. Internal standards were used to correct for quenching. The results are expressed as a ratio of radioactivity in the slices compared with the medium (Smith *et al.*, 1976).

Statistics.—The control and treated means were compared using Student's *t* test. The level of significance was chosen as $P < 0{\cdot}05$.

RESULTS

Lethality studies

Doses of paraquat or diquat that were not lethal to the rats during air exposure became lethal when the animals were placed in 85% oxygen (Table I). Furthermore, the mean time to death was shorter in rats exposed to 85% oxygen instead of air (Table I). LD_{50} values were estimated to be 18 mg/kg for paraquat in combination with air, 1·8 mg/kg for paraquat in combination with 85% oxygen, 20 mg/kg for diquat in combination with air and 10 mg/kg for diquat in combination with 85% oxygen. 85% oxygen exposure for 10 days was itself not lethal to the rats.

Lung concentration of paraquat or diquat

Exposure to air or 85% oxygen for 2 or 8 h did not affect the amount of paraquat in the lung (Table II). However, by 24 h after dosing with 2·5 or 5 mg/kg there was less paraquat in the lung of rats that were exposed to 85% oxygen compared with

TABLE I.—*Percentage mortality and mean time to death*

Treatment[a]	Air-exposed		85% O_2-exposed	
	% Lethality	Mean time to death (h)	% Lethality	Mean time to death (h)
Paraquat				
20 mg/kg	90	57	100	18
15 mg/kg	25	63	100	12
10 mg/kg	0	0	100	12
5 mg/kg	NM	NM	85	28
2·5 mg/kg	NM	NM	75	45
1 mg/kg	NM	NM	0	0
Diquat				
20 mg/kg	50	48	100	17
10 mg/kg	0	0	50	41
5 mg/kg	NM	NM	0	0

[a] Rats were dosed s.c. with various concentrations of paraquat or diquat (20 rats per experiment) and then exposed to air or 85% oxygen. Deaths were recorded daily until 10 days after dosing. 85% oxygen was not itself a lethal treatment.
NM = not measured.

TABLE II.—*Concentration of paraquat or diquat in rat lung*

Treatment[a]	nmol bipyridyl/100 mg dry wt					
	Air-exposed			85% Oxygen-exposed		
	2 h	8 h	24 h	2 h	8 h	24 h
Paraquat						
1 mg/kg	NM	NM	NM	1·3 ± 0·1 (5)	1·0 ± 0·1 (6)	0·9 ± 0·1 (5)
2·5 mg/kg	3·5 ± 0·4 (5)[b]	2·6 ± 0·2 (5)	2·0 ± 0·2 (5)	3·6 ± 0·2 (10)	2·2 ± 0·1 (6)	1·2 ± 0·2 (5)[c]
5 mg/kg	5·7 ± 0·5 (5)	5·7 ± 0·6 (5)	3·4 ± 0·2 (5)	6·6 ± 0·8 (5)	4·7 ± 0·3 (6)	1·1 ± 0·1 (5)[c]
10 mg/kg	9·9 ± 0·5 (5)	9·8 ± 0·8 (5)	7·4 ± 0·5 (5)	14·1 ± 1·3 (5)	9·6 ± 0·7 (5)	NM[d]
15 mg/kg	17·2 ± 1·1 (5)	14·3 ± 1·2 (10)	11·2 ± 0·9 (5)	18·9 ± 0·7 (5)	12·3 ± 0·9 (5)	NM[d]
20 mg/kg	24·1 ± 0·8 (5)	19·1 ± 1·5 (9)	12·3 ± 0·2 (5)	25·1 ± 0·9 (5)	18·2 ± 1·3 (5)	NM[d]
Diquat						
10 mg/kg	2·6 ± 0·1 (5)	1·2 ± 0·1 (5)	0·4 ± 0·1 (5)	3·1 ± 0·1 (5)	1·3 ± 0·1 (5)	0·7 ± 0·1 (4)
20 mg/kg	5·7 ± 0·3 (5)	5·1 ± 0·4 (5)	0·9 ± 0·1 (5)	7·5 ± 0·4 (5)	5·0 ± 0·4 (5)	1·3 ± 0·1 (3)

[a] Rats were dosed (s.c.) with various concentrations of paraquat or diquat and exposed to air or 85% oxygen for 2, 8 or 24 h before killing in halothane. The lungs were perfused with buffer and the 3 smallest right lobes were used for measuring the lung bipyridyl concentration.
[b] Results are presented as mean ± s.e. (number of animals per determination in parentheses).
[c] Significantly different from air exposed, paraquat-dosed animals ($P < 0.05$).
[d] Animals do not survive to 24 h after dosing. NM = not measured.

similarly treated rats that were exposed to air (Table II). Exposure to air or 85% oxygen for 2, 8 or 24 h did not affect the amount of diquat in the lung (Table II).

Morphological studies

For comparison, the appearances of normal rat alveolar tissue are shown in Fig. 1. Exposure to 85% oxygen for 1 or 2 days did not cause any observable lung damage on both light and electron microscopy. However, after 4 days of exposure the lungs appeared mildly hypercellular under the light microscope, with some thickening of alveolar walls. Ultrastructurally, some swelling and disruption of capillary endothelial cells was found, occasionally resulting in a denuded basement membrane. Alveolar epithelial cells were normal (Fig. 2).

Using both light and electron microscopy, no morphological changes were seen in the lungs of rats dosed (s.c.) with 2·5 mg paraquat/kg and placed in air for 24 h. However, after 2·5 mg paraquat/kg

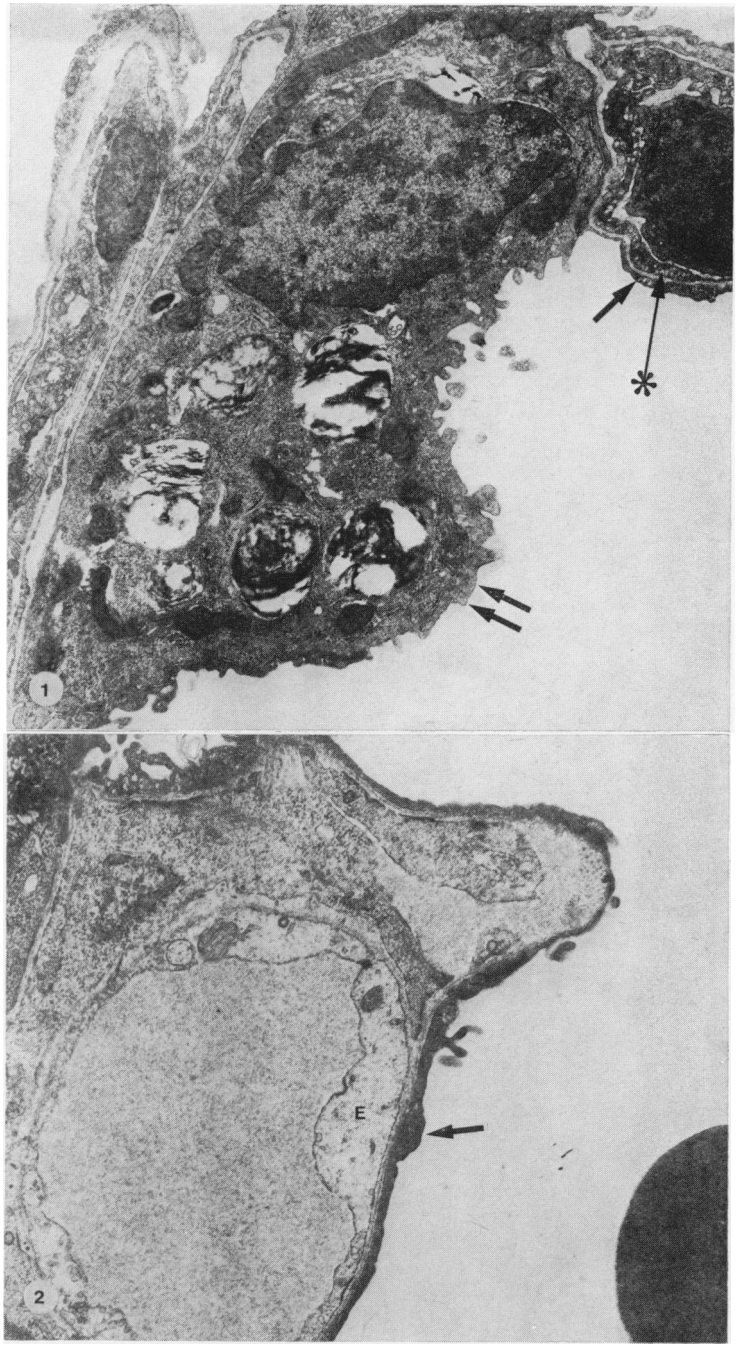

FIG. 1.—Alveolar tissue from a control rat showing normal Type I cell (arrow) endothelial cell (*) and Type II cell (double arrow), × 10,290.

FIG. 2.—Alveolar tissues from a rat exposed to 85% oxygen for 4 days showing swollen less electron-dense endothelial cell (E) and normal Type I cell (arrow). × 13,290.

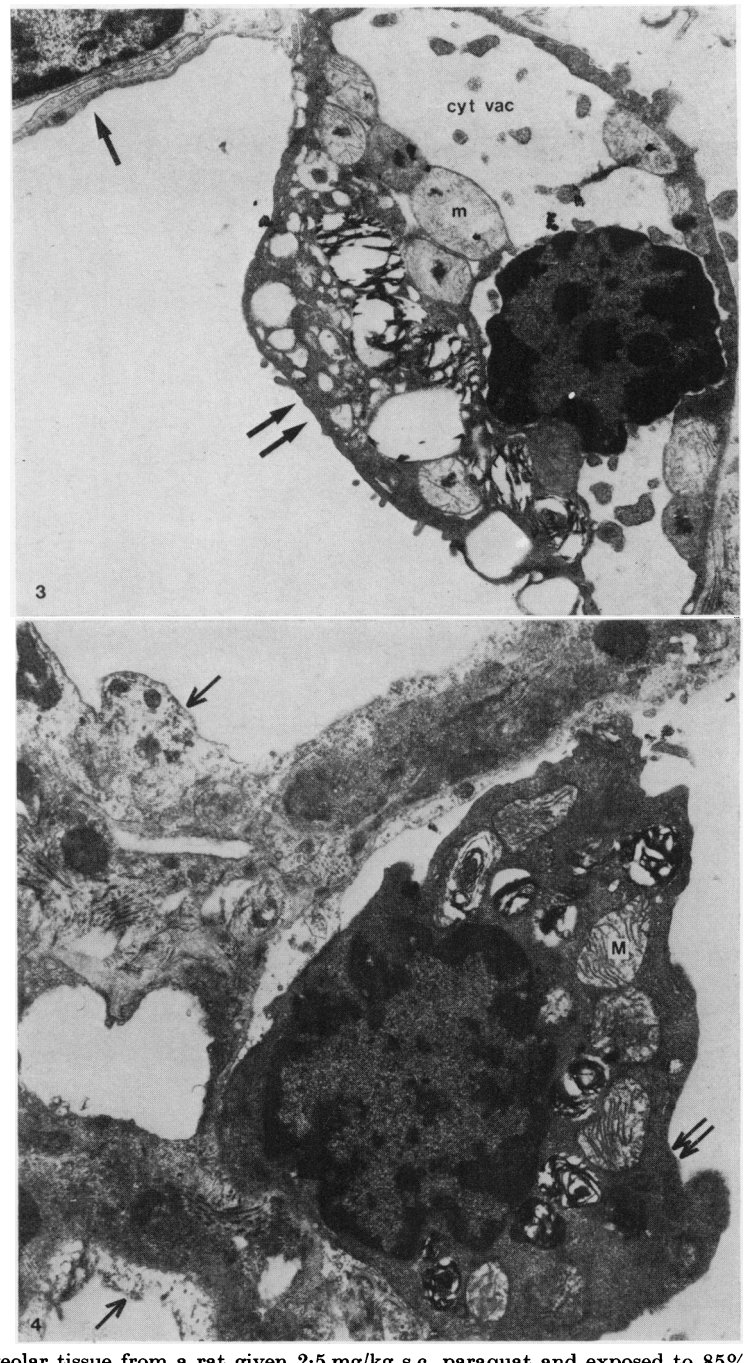

FIG. 3.—Alveolar tissue from a rat given 2·5 mg/kg s.c. paraquat and exposed to 85% oxygen for 24 h, showing a normal Type I cell (arrow), mitochondrial swelling (M) and cytoplasmic vacuolation (CYT VAC) in a Type II cell (double arrow). × 6640.

FIG. 4.—Alveolar tissue from a rat given 20 mg/kg s.c. paraquat and exposed to air for 24 h, showing pale swollen Type I cells (arrow) and mitochondrial swelling (M) in Type II cells (double arrow). × 9860.

(s.c.) and 85% oxygen exposure there was extensive lung damage by 24 h after dosing. The earliest detectable change was seen at 4 h after dosing, when there was perivascular oedema. This became more marked between 16 and 24 h after dosing, when there was perivascular oedema, alveolar oedema and haemorrhage. Lung cells appeared normal 2 and 4 h after dosing, but by 8 h the alveolar Type II cell frequently had swollen mitochondria, dilated endoplasmic reticulum and dilated nuclear membranes. By 24 h the Type II cell was the most severely affected cell type (Fig. 3), and the Type I and endothelial cells were only slightly affected, sometimes appearing swollen.

After 20 mg paraquat/kg (s.c.) and 8 or 24 h air exposure, lungs showed mild inflammatory perivascular oedema when examined under the light microscope. Using the electron microscope slight mitochondrial swelling was detectable in some Type II cells by 8 h. Type I cells sometimes appeared very electron-dense 8 h after dosing, but by 24 h they had become swollen and less electron-dense, with mitochondrial swelling. The endothelial cells were also occasionally swollen and less electron-dense at this time. Type II cells frequently had swollen mitochondria, condensed inclusion bodies and lipid had accumulated in the cytoplasm (Fig. 4).

After 20 mg paraquat/kg (s.c.) and 2 and 4 h 85% oxygen exposure there was haemorrhage and perivascular oedema in the lung when studied under the light microscope. Ultrastructurally, Type II cell mitochondria were swollen, microvilli were lost and inclusion bodies were condensed at 2 and 4 h after dosing. Both the Type I cells and the endothelial cells were less electron-dense than those in control lungs. The Type I cell was ultimately (12 h after dosing) the most severely affected cell type (Fig. 5). These animals did not survive for more than 16 h (Table I).

When animals were dosed with 20 mg diquat/kg (s.c.) and placed in air there was no lung damage at the light-microscopy level, but by electron microscopy there was slight mitochondrial swelling in Type II cells and Clara cells after 24 h. Endothelial cells were occasionally less electron-dense than those from control animals. After 20 mg diquat/kg (s.c.) and exposure to 85% oxygen there was perivascular oedema at 2 and 4 h after dosing. By 8 and 16 h this oedema was extensive, and there was evidence of haemorrhage into the alveoli.

Eight hours after dosing, electron microscopic studies showed that there was minimal damage to the alveolar epithelial cells and to the endothelial cells. By 16 h, the Type II cells were markedly affected. Mitochondria were swollen, inclusion bodies were disrupted and microvilli were lost. The Type I cells, the endothelial cells and some Clara cells were less electron dense than those in control lungs (Fig. 6).

After 10 mg diquat/kg (s.c.) and exposure to 85% oxygen there was perivascular and interstitial odema by 24 h after dosing. Electron-microscopy studies revealed that some endothelial cells were swollen and disrupted. The alveolar epithelial cells were normal.

The extent of damage to alveolar epithelial cells, endothelial cells and the extent of alveolar oedema was graded for each treatment, using a subjective grading scheme. In this scheme extensive necrosis and/or loss of a cell type or types from the alveolar wall was graded as severe damage (+ + +), marked mitochondrial swelling, membrane disruption, vacuolation and swelling of cells was graded as moderate damage (+ +), similar but less severe changes as mild damage (+) and minor changes such as loss of normal cellular electron density and slight mitochondrial swelling as minimal damage ($\pm$). These results are shown in Table III. It was concluded from the results of the morphology studies that the extent of lung cell damage seen after dosing with paraquat (2·5 mg/kg) combined with 85% oxygen was generally similar to that seen after paraquat (20 mg/kg) combined with air, although the specific pattern of cell damage was slightly different.

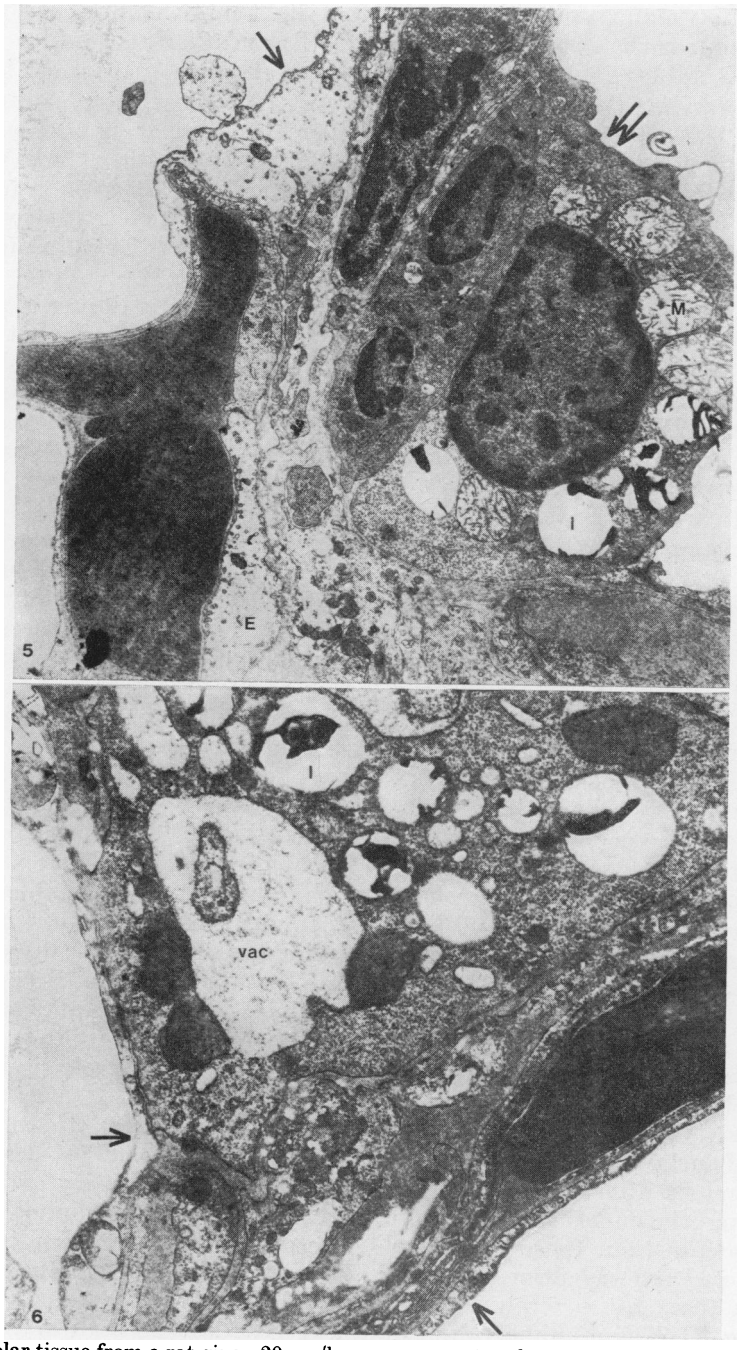

FIG. 5.—Alveolar tissue from a rat given 20 mg/kg s.c. paraquat and exposed to 85% oxygen for 12 h, showing severe swelling and paleness of Type I cells (arrow) and endothelial cells (E), mitochondrial swelling (M) and disruption of inclusion bodies (I) in Type II cell (double arrow). × 6640.

FIG. 6.—Alveolar tissue from a rat given 20 mg/kg s.c. diquat and exposed to 85% oxygen for 16 h, showing slight paleness of Type I cells (arrow), paleness, disruption of inclusion bodies (I) and intracellular vacuolation with oedema (VAC) in a Type II cell. × 13,290.

TABLE III.—*Pattern of lung pathology*

Treatment[a]	Air exposed: Type II cells	Type I cells	Endothelial cells	Lung oedema	85% oxygen exposed: Type II cells	Type I cells	Endothelial cells	Lung oedema
2·5 mg/kg paraquat	0	0	0	0	+++	±	±	++
20 mg/kg paraquat	++	++	+	±	[b]++	[b]+++	[b]+	[b]++
20 mg/kg diquat	±	±	±	0	[c]++	[c]±	[c]±	[c]+++
10 mg/kg diquat	0	0	0	0	0	0	+	+

[a] Rats (5 per treatment) were dosed s.c. with paraquat or diquat and exposed to air or 85% oxygen for 24 h before killing in halothane. The lungs were examined by electron microscopy.

[b] Rats given paraquat (20 mg/kg) and placed in 85% oxygen do not survive to 24 h after dosing. Pattern of lung pathology described is at 12 h after dosing.

[c] Rats given diquat (20 mg/kg) and placed in 85% oxygen do not all survive to 24 h after dosing. Pattern of lung pathology described is at 16 h after dosing.

The extent of damage to the lung is denoted as follows:—+++ severe, ++ moderate, + mild, ± minimal, 0 normal.

^{14}C-*paraquat accumulation by lung slices*

Paraquat accumulation in lung slices prepared from rats 2 h after dosing with 20 mg paraquat/kg (s.c.) and exposure to air or 85% oxygen was not significantly different from controls (Table IV). However, 8 h after dosing, the accumulation of ^{14}C-paraquat was slightly reduced (Table IV). By 24 h after dosing with 20 mg paraquat/kg and air exposure, or 5 mg paraquat/kg and 85% oxygen exposure, ^{14}C-paraquat accumulation was reduced to 20% of control (Fig. 7). Thus, the dose of paraquat needed to reduce paraquat accumulation was much lower in the 85% oxygen-exposed rats compared with the air-exposed rats.

TABLE IV.—*Effect of paraquat combined with air or 85% oxygen exposure on ^{14}C-paraquat accumulation*

Treatment[a]	Paraquat uptake % of control: Air	85% oxygen
2 h after dosing		
Controls	100 ± 5·1 (5)[b]	NM
20 mg/kg	121·9 ± 6·4 (5)[c]	107·1 ± 8·5 (5)
8 h after dosing		
Controls	100 ± 10·6 (10)	117·7 ± 7·2 (5)[c]
2·5 mg/kg	NM	92·7 ± 7·6 (5)
5 mg/kg	NM	94·4 ± 5·7 (5)
10 mg/kg	77·3 ± 10·2 (5)[c]	77·7 ± 9·1 (5)[c]
15 mg/kg	84·7 ± 4·7 (5)[c]	NM
20 mg/kg	61·3 ± 16·6 (5)[c]	NM

[a] Rats were dosed s.c. with various concentrations of paraquat and exposed to air or 85% oxygen for 2 or 8 h. Lung slices were prepared (0·6 mm thick) and incubated in the presence of 10 μM paraquat for 1 h.

[b] Results are expressed as percentage of air exposed controls. Mean ± s.e. (5 animals per determination). Control values of the slice/medium ratio were 5·17 ± 0·55 (10).

[c] Significantly different from control ($P < 0·05$). NM = not measured.

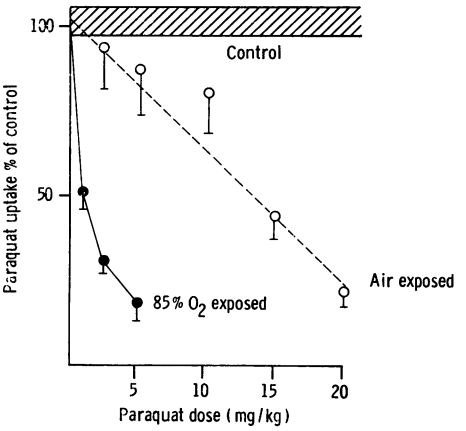

FIG. 7.—Relationship between ^{14}C-paraquat uptake into rat lung slices and paraquat dose 24 h after dosing and exposure to air or 85% oxygen. Lung slices were prepared (0·6 mm thick) and incubated in the presence of 10 μM paraquat for 1 h. Results are expressed as a percentage of air-exposed controls. Mean ± s.e. (5 animals per determination). Control values of the slice/medium ratio were 5·75 ± 0·26.

The ability of lung slices to accumulate paraquat was not significantly affected at any time after dosing with 20 mg diquat/kg and exposure to air (Fig. 8). However, when combined with exposure to 85% oxygen there was a progressive reduction at 8 and 24 h in paraquat accumulation which was slightly less than that seen with

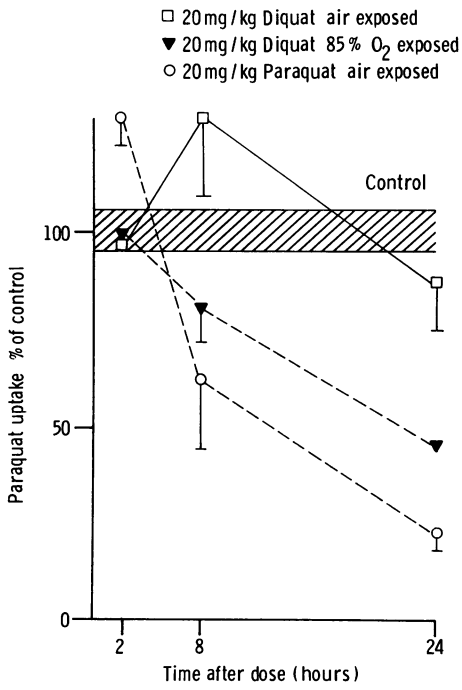

FIG. 8.—Relationship between ^{14}C-paraquat uptake into rat lung slices and time after paraquat or diquat dosing and exposure to air or 85% oxygen. Lung slices were prepared (0·6 mm thick) and incubated in the presence of 10 μM paraquat for 1 h. Results are expressed as a percentage of air-exposed controls. Mean ± s.e. (5 animals per determination). Control values of the slice/medium ratio were 5·17 to 5·75.

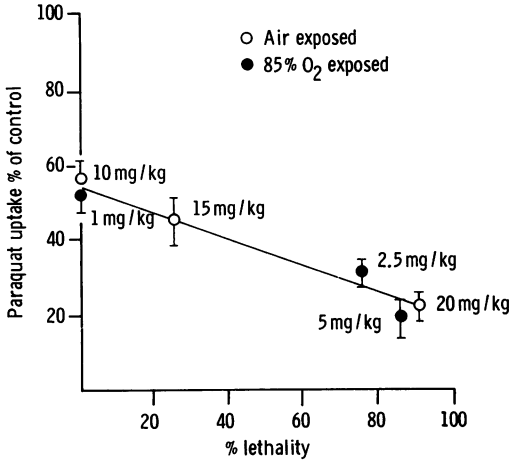

FIG. 9.—Relationship between the percentage lethality of a range of paraquat doses and exposure to air or 85% oxygen and the change in ^{14}C-paraquat uptake in lung slices taken from rats at 24 h after these treatments. Percentage lethality: Rats were dosed with various concentrations of paraquat (20 rats per experiment) and then exposed to air or 85% oxygen. Deaths were recorded daily until 10 days after dosing. Paraquat uptake: Lung slices were prepared (0·6 mm thick) from rats dosed s.c. with paraquat and exposed to air or 85% oxygen for 24 h. Slices were incubated in the presence of 10 μM paraquat for 1 h. Results are presented as mean ± s.e. (5 animals or more per determination).

20 mg paraquat/kg and air exposure (Fig. 8).

The degree of reduction of paraquat accumulation 24 h after paraquat dosing and air or 85% oxygen exposure was directly related to the percentage lethality of that treatment (Fig. 9).

DISCUSSION

Using a lethal dose of paraquat to rats (LD_{100}) Fisher *et al.* (1973) have demonstrated that time to death is shorter during 100% oxygen exposure (itself a lethal treatment, LD_{100}; Haugaard, 1968) compared with the time to death during air exposure. Furthermore, Douze and Van Heijst (1977) and Kehrer *et al.* (1979) have shown that paraquat toxicity in rats is enhanced by exposure to 40, 60, 80 and 100% oxygen. We have shown that s.c. doses of paraquat that were non-lethal during air exposure (*e.g.* 2·5 mg/kg, Table I) became lethal during 85% oxygen exposure. Estimations of LD_{50} values show that paraquat toxicity is enhanced approximately 10-fold during 85% oxygen exposure, whereas diquat toxicity is enhanced approximately 2-fold. These data extend the findings of Fisher *et al.* (1973). Douze and Van Heijst (1977) and Kehrer *et al.* (1979) and lend support to their conclusion that the use of breathing mixtures supplemented with oxygen is contraindicated in human cases of paraquat poisoning.

Since it has been demonstrated (Sharp, Ottolenghi and Posner, 1972) that the toxicity of paraquat is related to its concentration in the lung, it was possible

that the cause of enhanced paraquat or diquat toxicity was due to an increase in concentration of either bipyridyl in the lung. However, this was found not to be the case (Table II). The lung paraquat concentration was lower (by 24 h—Table II) in oxygen-exposed animals compared with rats given the same dose of paraquat (2·5 and 5 mg/kg) but exposed to air. This increased loss of paraquat from the lung was probably due to the development of lung cell damage as has previously been suggested (Wyatt *et al.*, 1981).

Previous studies of enhanced paraquat toxicity by oxygen exposure (Fisher *et al.*, 1973; Douze and Van Heijst, 1977; Kehrer *et al.*, 1979) have not characterized the pathogenesis of lung damage, and also the cell types affected. It was possible therefore that the cause of this enhanced toxicity was the additive effects of alveolar epithelial-cell damage by paraquat, and capillary endothelial-cell damage by oxygen. In these studies we have shown that this was not the case since it was the alveolar epithelial cells (primarily Type II cells) which were damaged by the combination of paraquat and 85% oxygen, and not the capillary endothelial cells (Fig. 5, Table III). In order to validate this finding, we also characterized the morphological effects of paraquat or oxygen when given alone (see Figs. 2 and 4, and Table III). In agreement with previous studies, the alveolar epithelial Type I and II cells were the first cells damaged by paraquat (Vijeyaratnam and Corrin, 1971; Smith and Heath, 1974; Sykes *et al.*, 1977) whereas after oxygen poisoning the capillary endothelial cells were primarily affected (Kistler *et al.*, 1967; Schaffner *et al.*, 1967; Kapanci *et al.*, 1969; Weibel, 1971). The enhancement of damage to the alveolar epithelial cells reported here indicates that it is oxygen which enhances the toxicity of paraquat in the lung, and not paraquat enhancing oxygen toxicity as was suggested by Fisher *et al.* (1973). Furthermore, since low concentrations of paraquat (2·5 mg/kg) and oxygen, or high concentrations of diquat (20 mg/kg) and oxygen have the same effects in the lung (damage to the alveolar Type II cells, Table III) it appears that the interaction between these bipyridyls and oxygen occurs primarily in the alveolar Type II cells.

Although the extent of alveolar epithelial cell damage was similar 24 h after dosing with paraquat combined with air (20 mg/kg) or 85% oxygen exposure (2·5 mg/kg) (treatments that killed a similar proportion of animals—see Table I) there was only very slight lung damage after dosing with diquat combined with air (20 mg/kg) or 85% oxygen exposure (10 mg/kg). Thus, in agreement with Witschi *et al.* (1977), although treatment with diquat caused some lung damage, this did not appear to be involved in the cause of death of these animals. However, lung damage did appear to be involved in the cause of death of rats given 20 mg diquat/kg and exposed to 85% oxygen. These findings are consistent with those of Kehrer *et al.* (1979).

It has previously been shown that the accumulation of paraquat into lung slices taken from lungs with damaged Type I and Type II alveolar epithelial cells is reduced (Smith *et al.*, 1976; Sykes *et al.*, 1977). We have confirmed these findings in our studies. Paraquat accumulation into lung slices was reduced at 24 h after dosing, when the alveolar epithelial cells were damaged either by paraquat or by paraquat or diquat in combination with 85% oxygen exposure (Figs 7 and 8). Furthermore, the degree of reduction in paraquat accumulation was directly related to the lethality of the treatment (Fig. 9). Since paraquat accumulation was not reduced 2 h after dosing (Table IV) when there was no lung damage, and when the lung paraquat concentrations were highest (Table II), this indicates that the paraquat present in the lung does not directly reduce paraquat accumulation. These results suggest that (1) paraquat is at least in part actively accumulated by the alveolar epithelial cells, and that (2) the measurement of paraquat accumula-

tion *in vitro* can be used as a quantitative estimate of the degree of alveolar epithelial cell damage.

We have concluded that (1) the toxic effects of paraquat to the alveolar epithelial cells of the lung are markedly enhanced when paraquat-treated rats are exposed to 85% oxygen, and (2) the combination of low concentrations of paraquat (2·5 mg/kg) and 85% oxygen or high concentrations of diquat (20 mg/kg) and 85% oxygen damages the Type II alveolar epithelial cells.

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