Paraquat lung injury in rabbits*

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ABSTRACT An aerosol model for the study of paraquat (PQ) toxicity was developed using a 134 litre chamber and an ultrasonic nebuliser. Three groups of New Zealand white rabbits weighing 2-3 kg were studied. Group I (n = 6) was exposed to 10 g PQ/100 ml double-distilled water (DDW), Group II (n = 24) was exposed to 200 mg PQ/100 ml DDW and a control group (n = 6) was exposed to 100 ml DDW. In a second experiment ten animals (Group III) were exposed to 10 mg PQ/100 ml DDW over a three-month period together with a control group (n = 5). Group I animals died with extensive haemorrhagic pneumonitis 38 hours after the last challenge. Most animals in Group II surviving more than three exposures had a significant reduction ($p < 0.001$) in arterial oxygen tension (PaO_2) and an increase ($p < 0.001$) in the alveolar-arterial O_2 gradient. Specific compliance decreased ($p < 0.005$) and functional residual capacity and breathing frequency increased ($p < 0.05$). Tissue PQ values showed even pulmonary distribution, with evidence of PQ accumulation after repeated inhalation. The lungs showed focal interstitial fibrosis, interstitial thickening, proliferation of macrophages in the alveoli, epithelioid changes in the interstitium, Type II cell hyperplasia, and foci of acute inflammation with consolidation. Controls and Group III animals were normal. This indicates that repeated inhalation of paraquat aerosol induces dose-related interstitial pneumonitis and fibrosis in rabbits.

Paraquat (PQ) has been responsible for many deaths from accidental or intentional poisoning, and its toxic manifestations have been the subject of human and experimental studies (Clark *et al.*, 1966; Gage, 1968; Smith and Heath, 1974; Smith *et al.*, 1974; Fairshir and Wilson, 1975). Paraquat is usually applied for agricultural use as an aerosol. Previous investigators have not found any lung damage in experimental animals exposed to levels of paraquat aerosol similar to those encountered by herbicide sprayers (Gage, 1968).

The purpose of the present study was to determine the dose-related toxicity of paraquat aerosol on the lungs of rabbits. Both acute and chronic changes were examined in pathological and physiological studies. In the chronic experiments serial assessment of pulmonary function was accomplished by

measuring functional residual capacity (FRC), lung compliance (C_L), breathing frequency (f_b), and arterial oxygen tension (PaO_2).

Materials and methods

Thirty-six male New Zealand white rabbits approximately six months old and averaging 2.5 kg in weight were housed in separate cages in a well ventilated room. The animals were provided with Rabbit RationTM (Yoder Feeds) and free access to water throughout the experiment. They were individually weighed and numbered, and randomly divided into three groups, designated control (n = 6), Group I (n = 6), and Group II (n = 24). The control animals received 100 ml double-distilled water (DDW) delivered by a DeVilbiss DEV No. 65 ultrasonic nebuliser (particle mean diameter 4 μm) (Mercer *et al.*, 1968) for a two-hour period, while Group I animals were exposed to 10 g PQ (methyl violagen hydrate, Aldrich) in 100 ml DDW, and Group II animals received 200 mg PQ in 100 ml DDW. The exposures were carried

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out in a 134 litre plexiglas sealed glove box with a constant air flow of 5 l/min provided by a vacuum pump. Air leaving the chamber was passed through a water trap to collect any residual paraquat. All exposures were carried out under a vented hood. Group I animals received only one two-hour exposure. Group II and control groups each received five weekly two-hour exposures with six animals in the chamber for each exposure.

The effect of 10 mg PQ/100 ml DDW given weekly was studied in a second experiment on ten animals (Group III), together with a control group ($n = 5$) in the manner described above, over a three-month period (Table 1). Ten animals were exposed solely for tissue level evaluations, and details of these exposures are given below.

Table 1 *Experimental groups*

Group	Number of animals	Total PQ in aerosol/exposure into 134 l chamber over a 2h period (mg)	Number of weekly 2 h exposures
I	6	10000	1
II	24	200	5*
III	10	10	13
C ₁	6	none	5
C ₂	5	none	13

*Some Group II animals were exposed less than five times (see results section).

DOSE CALCULATION

Doses were calculated taking into account previously noted lethal doses (Clark *et al.*, 1966). Group I was to receive a lethal dose and Groups II and III were to receive less than 10% of a lethal dose. Dosing was calculated by a method based on chamber volume, rabbit tidal volume (V_T), mg PQ/exposure [PQ] and f_b , according to the following formula: dose inhaled per animal = $V_T \times f_b \times [PQ]/\text{chamber volume}$. This method assumed maximal retention of inhaled material and 100% delivery of PQ solution in the form of respirable particles. It was estimated that oral intake would be at least twice the amount inhaled. Assuming inhaled and oral dosage, the intake of PQ/exposure/animal for animals from Groups I, II, and III would be 270 mg, 5.4 mg, and 0.3 mg respectively.

The source of paraquat was methyl violagen hydrate powder which was kept at -20°C in a desiccator. Solutions used were prepared on the day of a challenge and kept at 4°C until nebulisation.

PHYSIOLOGICAL STUDIES

Blood PaO_2 , PaCO_2 , and pH were measured at weekly intervals during the course of the experiments. Blood was obtained in a glass syringe from

the central ear artery after loosely restraining the rabbit in a sheet, and was subsequently analysed using an IL 313 or IL 113 Blood Gas Analyzer (Instrumentation Laboratories) which had been calibrated less than four hours previously. Barometric pressure (P_B) was measured at the time of blood gas analysis. The alveolar-arterial O_2 gradient (ΔAaPO_2) was calculated for each sample using the alveolar air equation. The fraction of inspired oxygen ($F_{\text{I}\text{O}_2}$) was 0.209, P_B was measured, and the respiratory quotient (R) was assumed to be 1 (Murray, 1974). ΔAaPO_2 was studied to balance out variant breathing patterns which affect alveolar CO_2 and thereby alveolar O_2 . P_B variance was also taken into account with this measurement.

Lung C_l , FRC, f_b , and specific compliance were measured in randomly selected animals during the course of the experiment (Davidson *et al.*, 1966). Serial measurements were made on surviving animals. Animals were weighed and injected with 0.15 ml/kg Innovar VetTM (Pitman-Moore, Inc.). After 20 min they were placed supine with limbs extended by elastic bands and upper incisors retracted. The jaws were held firmly in the horizontal plane and the pharynx sprayed with less than 1 ml of 1% lidocaine. A paediatric laryngoscope fitted with a No. 1 Miller blade (Foregger Co., Inc.) was guided carefully past the tongue. The blade was passed over the long soft palate, past the paired anterior tonsils and lifted with firm tension just beyond the epiglottis (Weisbrath *et al.*, 1974). The glottis and vocal cords were exposed and a 1 mm diameter flexible steel wire was introduced between the cords. A Cole (Air Products) endotracheal tube adapted with a 9 cm length of PE 330 (Intramedic, ID 2.921 mm, OD 3.733 mm) was placed over the wire and slid into the trachea. The wire was then removed. A 30 cm length of saline-filled PE 330 with a bevelled end and two side holes was passed to 20 cm and the animal was placed prone in a restrainer box within the plethysmograph (Figure 1). The oro-oesophageal and endotracheal tubes were connected to ports in the plethysmograph.

The plethysmograph was a 50 litre plexiglass box with two side ports and one top port. The latter had a removable lid which gave an airtight seal when fastened. The top was connected to a Statham PM 5TC pressure transducer. The tracheal port was connected to one side of a Hewlett-Packard 267B differential pressure transducer and also to a Statham P6 1G pressure transducer. The saline-filled oro-oesophageal tube was connected to the other side of the differential transducer by a saline-filled bubble-free line equipped with a three-way stopcock for flushing. The transducers were connected to Sanborn-Carrier pre-amplifiers (150-1100AS) of a rectilinear

Fig. 1 Rabbit intubated and in plethysmograph.

Sanborn 150 (150-100B) direct-writing four-channel recorder. Phase lag did not occur with this recording system. The transducers were calibrated in cc air and cm water against atmospheric pressure each day before measurements of compliance.

At the beginning of the study the position of the oro-oesophageal tube in the lower third of the thoracic oesophagus was checked by manipulating its position to give maximal pressure deflections during respiration. The lid was then positioned and the box sealed. Baseline measurements of transthoracic pressure, box volume variation, and f_b were obtained. The air port was then clamped and FRC determined by the method of Dubois *et al.* (1956). The box was re-opened and one ear of the rabbit was shaved, exposing the lateral ear vein. One mg/kg of gallamine triethiodide (Davis and Geck) was given intravenously, and the animal was ventilated with a Harvard Apparatus Respirator (model No. 661) at an f_b of 30 min^{-1} , V_T of 30 ml, and FiO_2 of 30% from an Inspiron Disposable Nebulizer (CM Bard, Inc.). A control strip indicating volume and transthoracic pressure was run to ensure

a common volume history after which successive 5 cc increments of air starting at 5 cc and ending at 60 cc were used to create a static pressure-volume curve. A similar decremental curve was generated. Individual sets of injected volume and the corresponding transthoracic pressure changes were fitted to a least squares regression line. The slope of this line was the passive compliance (C_L).

One-minute ventilation periods were allowed between measurements and the animal's condition was monitored by pupil size, retinal colour and heart rate. Measurements of pressure were made 2 s after volume injections to avoid initial pressure overshoots (Davidson *et al.*, 1966). P_B was measured daily for FRC determinations and converted to centimetres of water. STP corrections were made on volume measurements.

PATHOLOGICAL EXAMINATION

Tissue was prepared for morphometric and pathological examination by killing the animals with 35 mg nembutal sodium/kg body weight (Abbott Labs.). While the heart was still beating synchro-

nously, a 19 gauge needle was inserted into the right ventricle and ice-cold 2.5% glutaraldehyde (Fisher) in 0.1 M Na cacodylate (Fisher) buffer (pH 7.35) infused at pressures no greater than 40 mmHg. The trachea was then isolated and fixative infused at pressures no greater than 20 mmHg until the lungs were completely filled. The specimen was immersed in fixative in a glass jar and stored at 4°C for 24 hours. Sections were then cut from the right apex, right lower lobe and left lower lobe, and placed in buffer. The sections were from similar sites in all animals, and cuts were not selected on the basis of appearance. The sections were sent to the pathology laboratory for staining with haematoxylin and eosin. Lungs in which fibrosis was suspected were sent for reticulum and Masson's trichrome stains (Bowling, 1967).

The remaining lung was removed from fixative and placed in 0.1 M cacodylate buffer, and prepared for scanning electron microscopy as follows: small tissue blocks were dehydrated through a series of alcohols, and were then transferred to liquid nitrogen and freeze fractured (Kessel and Shih, 1974); the tissues were rehydrated and fixed with OsO₄ using a ligand-mediated binding method (Kelley *et al.*, 1973); the tissue was again dehydrated, critical-point dried (Kessel and Shih, 1974), and coated with approximately 100 Å thickness gold applied with a Hummer II Sputter system (Technics, Inc.). Tissues were examined on a Cambridge S4 Stereoscan scanning electron microscope.

TISSUE LEVELS

Tissue paraquat levels were determined by a colorimetric assay (Daniel and Gage, 1966). The single-dose and cumulative effects of 100 mg PQ nebulised in 100 ml over 2 h in the chamber previously described were studied by quantitative analysis of paraquat in the lungs (peripheral, central, basal, and apical tissue), liver, stomach, entire gastrointestinal tract, kidneys, and urine. Two animals were studied at each of three weekly exposures. The animals were killed by air injection at the end of a two-hour exposure. A second group of two animals received 250 mg PQ in 100 ml DDW and was studied immediately following exposure. A third group of two animals received 10 mg/100 ml and PQ levels were measured after a two-hour exposure. Samples were taken from the nebuliser cup at the beginning of exposure and from the solution remaining at the end of two hours in order to determine how much paraquat had been incorporated into the aerosol. In addition two PQ solutions, of 100 mg/100 ml and 250 mg/100 ml, were studied every other day for loss of PQ activity with time when stored at 4°C in the dark. Organs were weighed on removal from

the animal, placed in a sealed container, refrigerated at 4°C, and analysed for paraquat tissue levels during the following week. Cold samples were placed in the cup of an Omni-Mixer (Ivan Sorvall, Inc.), 75 ml of 5% trichloroacetic acid (TCA) (Mallinckrodt) were added, and the tissue was mixed at a setting of 4 for two minutes. The cup contents were divided between two culture tubes and centrifuged for five minutes at 4000 rpm. The supernatant was then transferred to a 150 ml beaker and adjusted to pH 7.0 with potassium carbonate solution. The solution was passed through a 4 ml column of Bio-Rad cation exchange resin under vacuum. The column was then removed from vacuum and eluted with 20 mg of 5 M ammonium chloride, collecting the eluant in two 10 ml aliquots. Two ml of freshly prepared 0.2% sodium dithionite in 1 N sodium hydroxide were added and mixed. Optical density was measured in a Beckman Grating Spectrophotometer at 600 mμ using 10 ml of 5 M ammonium chloride and 2 ml of 0.2% sodium dithionite in the reference cuvette; readings were compared with a standard curve.

Urine and solution samples were initially acidified to pH 1 with 18 N H₂SO₄ and the procedure for organ samples was then followed.

STATISTICAL ANALYSIS

Arterial blood gas and specific compliance values between control and experimental groups were compared using a one-tailed and two-tailed test respectively. Blood gas values were compared between control and experimental groups using a *t* statistic for two means assuming equal variance in the two groups. *P* values were obtained from standard statistical tables (Daniel, 1974). The values of *C_L* were obtained from an IBM 360 computer which also generated a least squares regression line and correlation coefficient. Comparison *t* values were derived from a programmable Hewlett-Packard 25 mini-computer.

Results

CLINICAL MANIFESTATIONS

Group I animals had slow, shallow respirations (40/min) while in the chamber during the initial exposure. They were inactive and inattentive compared with control animals. They appeared to be eating air throughout the second hour of exposure. Within two hours of exposure all animals were cyanotic, dyspnoeic, tachycardic, and had inspiratory and expiratory rhonchi on auscultation. Stridor was noted in two animals. Three animals died within 12 hours of exposure, and the remaining three were dead within 38 hours.

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Group II animals ($n = 24$) and controls showed no evidence of dyspnoea or reduced activity during the first challenge. However, following subsequent weekly challenges some Group II animals did develop air eating, cyanosis, clicking respirations, snuffles (Weisbrath *et al.*, 1974) and tachypnoea. These animals became less active than controls during subsequent exposure periods.

Group III animals were indistinguishable from controls.

PHYSIOLOGY

PaO₂ measurements were made regularly in control and experimental Groups II and III. Comparisons were made against the control group. Table 2 shows mean PaO₂ values and standard deviations for comparison of the control and experimental groups. The PaO₂ values in Group II differed from the control group after exposure to PQ. Δ AaPo₂ results were inverse to changes noted in the PaO₂ studies. After withdrawal from PQ inhalation the mean PaO₂ rose to the control range. PaO₂ and Δ AaPo₂ values in Group III animals were not significantly different from control values. Changes in pH and PaCO₂ were not significant at any time during the test period.

Table 2 PaO₂ (mmHg)

Weeks of challenge	Combined controls	Group II mean ($n = 13$)	Group III mean ($n = 10$)
0	79 $\pm$ 10	NA	73 $\pm$ 7
1	81 $\pm$ 4	66 $\pm$ 17*	75 $\pm$ 8
4	83 $\pm$ 10	47 $\pm$ 10*	80 $\pm$ 13
Recovery values 4 wk after last exposure	77 $\pm$ 13	76 $\pm$ 10	

* $P < 0.05$ in comparison to controls.
NA; Not available.

The pressure-volume studies showed significant differences between nine controls and 16 Group II animals in specific compliance, FRC, and f_b (Table 3). The mean of the specific compliance in Group II was 0.050 compared with a control mean of 0.10 ($P < 0.005$). FRC and f_b were significantly higher in Group II animals ($P < 0.05$). Control and Group III animals showed no statistical differences in these tests.

TISSUE LEVELS

Lung tissue analysis showed even pulmonary distribution with good transport of paraquat to the periphery (Table 4). Accumulation was significant

Table 3 Plethysmographic data

Variable	Controls ($n = 9$)	Group II ($n = 16$)	Group III ($n = 11$)
C _L	2.4 $\pm$ 0.6	2.2 $\pm$ 0.5	2.8 $\pm$ 0.6
Specific C _L	0.10 $\pm$ 0.04	0.05 $\pm$ 0.03**	0.10 $\pm$ 0.04
FRC	28 $\pm$ 14	48 $\pm$ 13*	35 $\pm$ 16
f_b	30 $\pm$ 10	38 $\pm$ 9*	30 $\pm$ 12

* $P < 0.05$ in comparison to controls and Group III.

** $P < 0.005$ in comparison to controls and Group III.

Table 4 Mean lung PQ levels in animals exposed to 100 mg/100 ml DDW with 3 weekly exposures (2 animals killed/exposure)

Lung tissue	1 exposure	2 exposures	3 exposures
Total	28 μ g	33 μ g	53 μ g*
Basal	2.5 μ g/g	3.0 μ g/g	6.9 μ g/g*
Apical	5.1 μ g/g	4.4 μ g/g	5.4 μ g/g
Central	2.8 μ g/g	4.6 μ g/g	7.6 μ g/g*
Peripheral	2.7 μ g/g	5.4 μ g/g	4.4 μ g/g*

* $P < 0.05$ increase in tissue level.

Table 5 Mean organ levels in animals exposed to 100 mg/100 ml DDW with 3 weekly exposures (2 animals killed/exposure)

Tissue	No. of exposures		
	1	2	3
Liver			
Concentration	0.030 μ g/g	0.060 μ g/g	NA
Total tissue level	2.20 μ g	4.10 μ g	NA
Kidney			
Concentration	0.19 μ g/g	0.46 μ g/g	0.80 μ g/g
Total tissue level	3.7 μ g	6.5 μ g	15.0 μ g
Intestines and stomach			
Concentration	0.11 μ g/g	0.70 μ g/g	0.84 μ g/g
Total tissue level	40 μ g	270 μ g	350 μ g

NA; Not available

($P < 0.05$) in the basal, central and peripheral lung samples. The total lung PQ content also rose significantly with repeated exposures over a three-week interval ($P < 0.05$). Levels also increased in the liver, kidney, and gut homogenates (Table 5).

Total lung PQ content was clearly dependent on the initial PQ solution used in the nebuliser. Total lung values of 94 ± 37 , 28 ± 8.5 , and 3.9μ g PQ were found in animals exposed to 250 mg, 100 mg, and 10 mg respectively. The ratio of total lung PQ to total gastrointestinal PQ ranged from 0.12 to 0.33 independent of the amount of PQ incorporated in the aerosol.

No evidence of deterioration was noted in samples repeatedly analysed over a two-week period. Values from these standards varied no more than 5% from known initial concentrations.

Fig. 2 (a) *Normal rabbit lung parenchyma* ($\times 80$; haematoxylin and eosin (H and E)). (b) *Normal parenchyma with a terminal bronchiole and small muscular vessel* ($\times 200$; H and E).

PATHOLOGY

Controls

Lungs of control animals filled easily, and no gross abnormalities were present. Two of the control animals had mild focal epithelial hyperplasia of the small bronchi with infiltration of these areas by a few segmented neutrophils. One animal, which died

spontaneously, had acute bronchiolitis with focal extension into adjacent alveoli. Most of the lung parenchyma in these animals was normal (Figure 2).

Group I

The coats of the Group I animals had yellow discoloration and sparse fur. The lungs of all the

Fig. 3 (a) *Medium-sized artery with perivascular oedema and adjacent intra-alveolar oedema* ($\times 80$; H and E).

Fig. 3 (b) *Lung with oedema, small areas of mild interstitial and subpleural thickening* ($\times 200$; H and E).

animals were markedly congested, and filled slowly with fixative compared with those of the controls. Mediastinal petechiae were seen in one animal. All lung sections showed mild to severe degrees of congestion and oedema. Perivascular collections of fluid, which separated the vessels from the sur-

rounding tissue, were prominent in five animals. Four of six animals had acute focal bronchitis, which had progressed in two animals into early focal pneumonia. Other frequent findings were intra-alveolar haemorrhage and areas in which small bullae had formed (Figure 3).

Fig. 3 (c) Lung with intra-alveolar oedema, widened septae with an increase in the number of type II cells ($\times 500$; H and E).

Fig. 4 Fixative-filled Group II lung on left compared with control lung on right.

Group II

Group II animals had yellow discoloration of the fur. The mucous membranes around the eyes were inflamed unilaterally in two animals. Most of these animals had pleural scarring, most prominent in the lower lobes. The only exceptions were five animals which died or were killed 1-3 wk after the first exposure. Two of these animals died during compliance measurements, and one died with empyema and pleuritis. The other two animals had no gross or microscopic pulmonary abnormalities and no cause of death was determined. Figure 4 shows a typical Group II lung next to a control lung.

Six animals died spontaneously 4-5 d after their third exposure. Their lungs showed focal haemorrhage, oedema, and atelectasis with varying degrees of pneumonitis. There were multiple small areas in which the residual alveoli were lined with cuboidal pneumocytes, filled with macrophages, and associated with thickened alveolar septae. The changes were most prominent in areas of organisation and repair. Several animals also had a narrow rim of subpleural oedema and haemorrhage. Thirteen Group II animals received five PQ exposures. These animals were divided into three groups which were killed 1, 4.5, and 7 wk after the final exposure, with the exception of one animal which died spontaneously with suppurative pneumonia. All four animals killed 1 wk after the final PQ exposure had many alveoli lined with prominent pneumocytes, and

broad areas of interstitial fibrosis with increased reticulin, collagen, and inflammatory cells in residual air spaces. Scanning electron micrographs showed epithelial hyperplasia and pleural scarring (Figure 5). The animals killed 4.5 wk after the final PQ exposure had lungs varying from near normal to those with areas of interstitial fibrosis, proliferation of pneumocytes and inflammation. There were small bands of fibrous tissue which extended to the pleural surface and correlated with the tenting seen on macroscopic examination (Figure 6). One of these animals showed marked cystic changes.

A walled-off abscess cavity was seen in one animal. The group killed 7 wk after the last exposure had predominantly normal lungs with a mild increase in periseptal fibrous tissue, and a few small areas with residual proliferative epithelial changes.

Group III

These animals were indistinguishable from controls.

Discussion

Lung injury has been produced in a number of animal species after parenteral administration of paraquat. The rat and mouse have been studied extensively (Clark *et al.*, 1966; Kimbrough and Gaines, 1970; Vijayarathnam and Corrin, 1971; Smith *et al.*, 1974). Aerosol PQ administration has been reported to cause acute toxicity in laboratory

Fig. 5 (a) Interstitial pneumonitis with interstitial fibrosis and alveolar cell hyperplasia ($\times 80$; H and E).

Fig. 5 (b) Increased interstitial reticulum ($\times 200$; reticulum stain).

Fig. 5 (c) Epithelial hyperplasia and proliferation with fibrosis and inflammation ($\times 500$; H and E).

animals, but there are no previous reports of chronic fibrotic lung disease in animals exposed to PQ spray (Gage, 1968). Some authors note species differences in sensitivity to PQ; the rabbit has been considered to be insensitive compared with the rat (Clark *et al.*, 1966; Gage, 1968; Butler and Kleinerman, 1971).

The cellular and subcellular toxic mechanisms are still unknown. PQ is thought to facilitate superoxide, peroxide, and hydroxyl radical formation. These agents have been implicated in biological tissue injury (Fisher *et al.*, 1973; Fridovich, 1974; Rhodes *et al.*, 1976). Paraquat injury is also associated with a

Fig. 5 (d) Scanning electron micrograph showing prominent alveolar lining cells with a surface macrophage ($\times 854$).

loss of epithelial surfactant resulting in atelectasis (Fisher *et al.*, 1975; Manktelow, 1975).

Pathological changes are dose-dependent, although variation in individual response has been noted (Clark *et al.*, 1966; Smith *et al.*, 1974). High intravenous doses of PQ produce pulmonary haemorrhage, oedema, atelectasis and, occasionally, hyaline membranes in rats and mice. Death has been ascribed to pulmonary insufficiency on the basis of symptoms, signs, and pathological examination (Clark *et al.*, 1966; Smith *et al.*, 1974; Manktelow, 1975). Humans and laboratory animals surviving oral or parenteral sublethal dosing have developed interstitial pneumonitis and fibrosis with parenchymal gland-like proliferation and hyperplasia of granular pneumocytes (Smith *et al.*, 1974; Fairshir and Wilson, 1975). Smith *et al.*

(1974) contend that the process begins in intra-alveolar rather than interstitial foci. This view has not been confirmed. Kimbrough and Gaines (1970) saw a direct effect of small amounts of PQ given intratracheally in the rat, resulting in local fibrosis. In our study, PQ aerosol has produced lesions in rabbit lungs similar to those described in rats and mice given parenteral toxin. Compliance abnormalities in Group II indicated the development of restrictive disease (Turner-Warwick, 1974; Hance and Crystal, 1975; Gibson and Pride, 1976). The increases in FRC and f_b have been noted previously in PQ lung injury and were reversed by bilateral cervical vagotomy in rats (Vizek *et al.*, 1975). Large subpleural cysts represent emphysematous changes seen early in the injury and may account in part for the increased FRC. This air trapping may

Fig. 6 Lung with cystic spaces. Fine fibrous bands extend to the pleural surface with overlying pleural tenting ($\times 30$; H and E).

occur as a result of bronchiolar compression by inflammatory tissue. However, this was not found on pathological examination, and the increased FRC remains a perplexing problem.

The role of infection acting synergistically with alveolar lining cell injury in the pathogenesis of paraquat-induced pulmonary fibrosis in rabbits remains to be investigated. Repeated intubation, anaesthesia, artificial ventilation, or inhalation of contaminated aerosol may have contributed to infection, but cultures from these sources showed no growth.

Tissue levels were similar to those found in rats, and accumulation was again noted (Gage, 1968; Bus *et al.*, 1975; Murray and Bivson, 1975). Paraquat solution was stable when stored at 4°C in the dark over a two-week period. However, we did not assess the stability of PQ in an organ homogenate for a similar period. Levels found may underestimate the amount of PQ present, because PQ metabolites do not affect light absorbance.

Pathological studies showed that the spectrum of disease previously noted was dependent on dose and animal sensitivity. Lesions were focal, frequently subpleural, and interstitial. Areas of fibrosis were seen in foci of cellular inflammation.

The total dose was a result of inhalation and ingestion, as noted in tissue level studies. The relative contributions of these routes will be difficult to assess until organ-specific cellular and subcellular patterns

of injury are elucidated. However, both have been shown to result in tissue damage (Clark *et al.*, 1966; Kimbrough and Gaines, 1970). Tissue and urine levels allow comparison with previous studies by Gage (1968). The issue of relative contributions could be clarified by studying intubated animals breathing aerosol through a one-way valve.

Disease progression was not noted after halting PQ challenge. PaO_2 values improved and pathological findings in Group II animals assessed two months after their fifth exposure were minimal. This may represent selection of resistant animals or the development of effective detoxication mechanisms. Clark *et al.* (1966) and Smith *et al.* (1974) both noted progression of disease after stopping PQ challenge, but neither author studied serial physiological changes.

The model of paraquat lung damage may be useful in the study of the pathogenesis of pulmonary fibrosis. It is easy to set up, needs only low doses of paraquat, thereby reducing the hazard to laboratory workers, and results in fibrotic changes after a short challenge period. Studies of ultrastructural and biochemical changes associated with PQ fibrinogenesis are needed to understand this lesion (Hance and Crystal, 1975).

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